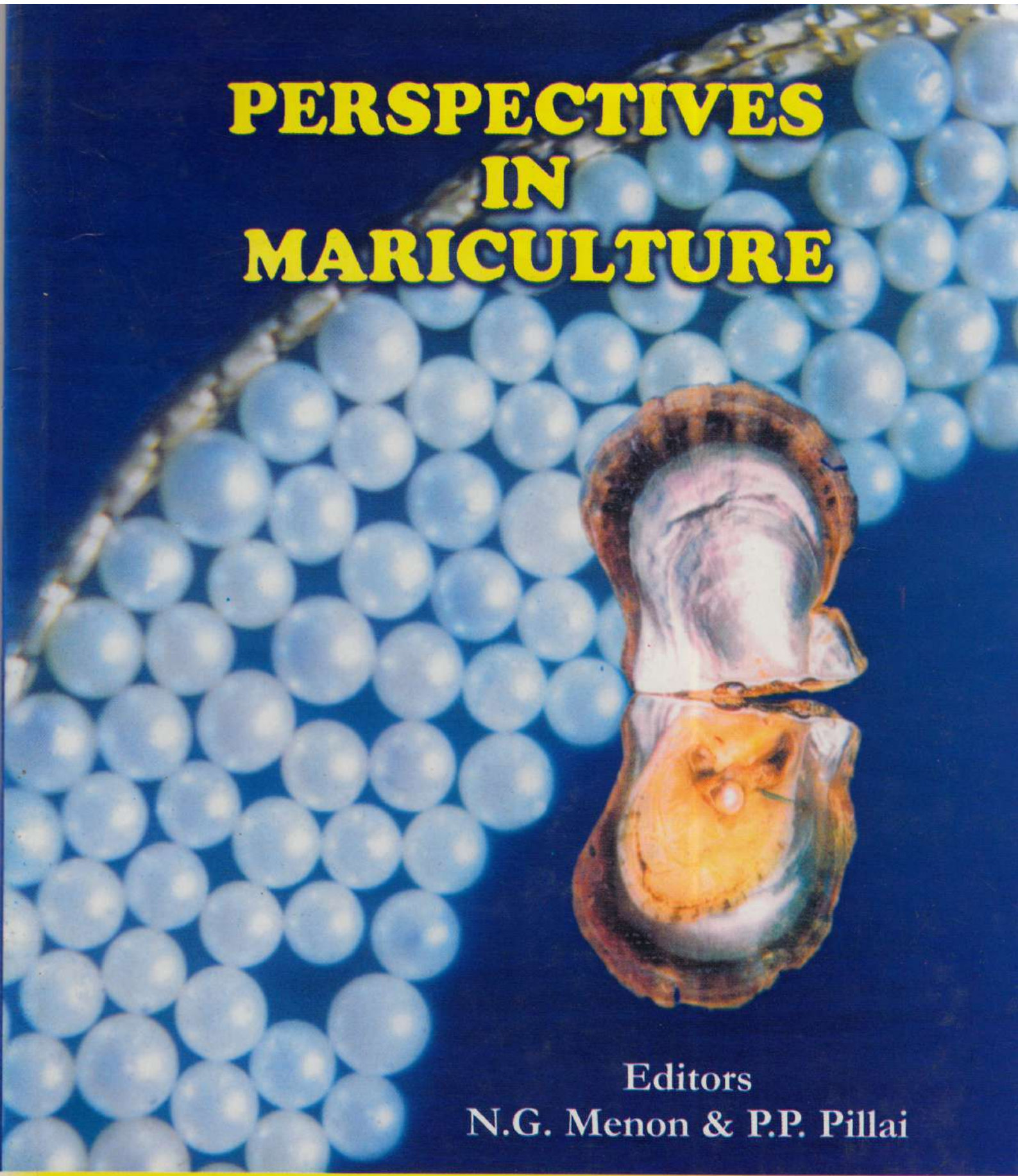
PERSPECTIVES IN MARICULTURE



Editors
N.G. Menon & P.P. Pillai

The Marine Biological Association of India
2001

PERSPECTIVES IN MARICULTURE

Editors

N. G. Menon and P. P. Pillai

Central Marine Fisheries Research Institute, Cochin

The Marine Biological Association of India

Post Box No. 1604, Tatapuram P.O.,

Cochin - 682014

2001

Based on contributions derived from the MBAI organised symposium on 'Ecofriendly mariculture technology package - an update' at Mandapam Regional Centre of CMFRI during April 2000 and co-sponsored by Ministry of Agriculture, Government of India; Indian Council of Agricultural Research; Marine Products Export Development Authority; Tata Chemicals Ltd.; ITAP (P) Ltd. and NEOSPARK Drugs and Chemicals Pvt. Ltd.

Published by : The President, The Marine Biological Association of India, Cochin - 682014

Citation Styles

For entire volume : N. G. Menon and P.P. Pillai (Eds.) 2001, *Perspectives in Mariculture*, The Marine Biological Association of India, Cochin, pp.464

For individual article : K.O. Isaac, 2001. Mariculture in India - an opportunity as vast as the sea. In: *Perspectives in Mariculture*, N.G. Menon and P.P. Pillai (Eds.), The Marine Biological Association of India, Cochin : 265-272

Cover design : Dr. N.G. Menon

The editors thank Shri K. Balachandran for the proof corrections and Shri P. Raghavan for the B/W Photographs.

Price : **Rs. 1000/-**

Printed at Cherrys Printers, L.F.C. Road, Kaloor, Cochin-17 Ph. 530681

PERSPECTIVES IN MARICULTURE

डा० के गोपकुमार
उप महानिदेशक (मत्स्य)
Dr. K. GOPAKUMAR
Deputy Director General (Fisheries)

भारतीय कृषि अनुसंधान परिषद
कृषि भवन, डा० राजेन्द्र प्रसाद रोड, नई दिल्ली-110001
Indian Council of Agricultural Research
Krishi Bhawan, Dr. Rajendra Prasad Road,
New Delhi - 110 001

FOREWORD

The natural living resources of the Indian seas are rich, diverse and are distributed in a variety of ecosystems/habitats ranging from the estuaries, saline lagoons, low lying saline inundated marshes, intertidal and subtidal belt to beyond shallow coastal shelf waters and have immense value and use to humanity. There has been an uninterrupted hunt for the flora and fauna in most of the maritime countries to quench human hunger as well as curiosity. Slowly but steadily the irrational anthropogenic destruction coupled with several other activities like dumping of pollutants, land reclamation, destruction of mangroves have ultimately caused environmental maladies, resource depletion, habitat degradations and even in some cases denudations. Much of these have taken place in the easily accessible coastal waters, fragile sensitive ecosystems and have badly hit the more vulnerable and wanted target, the charismatic species together with an array of ill fated bycatch of the juveniles of the wanted species and a wide spectrum of low-value-high-volume bottom biota forming part of the marine food. This being the global marine fisheries scenario, very promptly and timely the UN interfered through the Biodiversity Convention while the FAO proposed the Code of Conduct for Responsible Fishing. Accordingly, thus there is a global awareness on the need for protection and conservation of marine biodiversity and sustainable fisheries. India has also made considerable efforts to save her marine environment/ecosystems and biodiversity through enactment of laws, rules and regulations under various Acts. On the other hand, our domestic and export trade needs are ever increasing, economic growth is inevitable, and we need to go fast in the globalization and liberalization track. In order to keep pace with the fast track economic growth, population growth, national nutritional requirements, we have to seek ways and means to enhance the marine living wealth and to increase

Phone : Off. 3382713, 3388991 **Extn.** 454; **Fax :** 00-91-11-3387293/3382713; **Resi.:** 4670621
Telex : 031-62249 ICAR IN. **E.Mail :** kgopa@icar.delhi.nic.in:kgopa@yahoo.com **Gram :** AGRISEC

production and utilization either through sustainable coastal fisheries management, deep sea and oceanic fisheries ventures or through the domestication of coastal edible seaweed and animal species in all viable habitats. The resource enhancement through aquaculture has already made tremendous growth in the freshwater sector, while the same in saline medium, though perceptible, has yet to become commercialized, chiefly for want of entrepreneurship and policy changes. There is also need for eco-friendly viable standardized technology package of practices for seaweeds, shellfishes and finfishes. There has been growing concerns about the impacts of mariculture on biodiversity, environment, culture systems and their carrying capacity. Accordingly the legislative, economic and market forces of many aquaculture developing countries have already started persuading the producers to minimize such impacts through better management practices in site selection, husbandry, effluent management and biomanipulation of environment.

The Marine Biological Association of India has been always in the forefront to consider subjects of topical importance and bring together all researchers in the field through organizing workshops/ symposia. It is welcome to note that the Association is bringing out a book entitled **"Perspectives in Mariculture"** by drawing observations, inferences and recommendations discussed, deliberated and concluded in the symposium on **"Ecofriendly mariculture technology packages - An update"** conducted by the Association at Mandapam Regional Centre of CMFRI during April 2000. The ideas generated would further help the researchers to upgrade and refine the various technologies and practices. Our experience in mariculture and the lessons learnt from elsewhere, I hope, would usher our scientists to select the correct path to sustainable mariculture with due consideration to not only the techno-economic feasibility but also the socio-cultural setup prevailing at each region. A mission-oriented and integrated approach by all user agencies and stakeholders is the need of the hour to spread the message of responsible wild hunt and sustainable mariculture. This book presents a cross section of a host of multidisciplinary subjects through 39 papers and I am more than certain that it would form a valuable information base for not only students and researchers but also for farmers, entrepreneurs, research managers and policy planners.

[K. Gopakumar]

PREFACE

In recent years, the marine capture fisheries of India has been registering slow rate of growth of around 4%. The production has reached the threshold limit in the last one decade, a situation similar to that seen in global marine capture fisheries scenario. About 50% of the country's total marine fish production is from the territorial sea which is highly stressed owing to the impacts from many anthropogenic activities. Our capture fisheries thus face many challenging problems related to sustaining the coastal production, conflicting interest groups that play in the sector, conservation *versus* development issues. These problems are further aggravated by the increasing number of fishers and fish consumers, increased internal and external demand for fish and fishery products, technological upgradation and innovations in harvesting, higher *per capita* requirement of 11 kg / year against the present 6 kg / year, highly targeted exploitation of high value charismatic species and a disproportionate technology creep. One of the answers or viable options to the above problems is the domestication of wild coastal species of shellfishes / finfishes, under scientific farming regimes, in the brackish water / shallow coastal areas. While the world aquaculture registers an annual growth rate of about 10%, our mariculture development is quite unimpressive with a production about 3% of the total marine harvest or about 6% of total aquaculture production. Similarly the index of biodiversity utilization for aquaculture is far below even though we are one of the mega biodiversities of the globe. This seems to be not due to the lack of mariculture technologies, but to the reluctance of our entrepreneurs in entering this new venture and longer waiting period and lack of plans and policies, while the seas still promise higher catches often as a result of irrational and damaging fishing pressures in the coastal waters.

The CMFR Institute and other fisheries institutions and maritime universities have been constantly developing, improving and upgrading ecofriendly and techno - econo viable mariculture technologies to suit the Indian needs and situations. Slowly but progressively these outputs have percolated to the coastal, traditional or marginal fishermen and farmers. They are convinced of the usefulness of the technologies. The demonstration programmes, farmers meet and extension activities through various other media have also helped the end users to imbibe and practice fish farming and contribute to a continuous feed back to the technologists. Also, there are apprehensions on commercialization of intensive mariculture and its impacts on the environment, causes for pollution, disease etc. and its sustainability in supplementing marine capture fisheries.

The Marine Biological Association of India has been promoting interaction between scientists and technologists and user groups through national and international symposia on themes of topical importance and through its publications. The MBAI strongly felt that research results on mariculture technologies and their implications on the ecosystem, communities and economics are widely dispersed and poorly documented. Therefore, in order to bring together all workers in mariculture research and to facilitate discussions on research results and plan further work, the Association organized a symposium on 'Ecofriendly Mariculture Technology Packages - An Update' at the Mandapam Regional Centre of CMFRI during 25-26 April, 2000. The research results discussed were synthesized into research papers and and are presented in this book under the title 'Perspectives in Mariculture'.

I take this opportunity to thank all reseach contributors and symposium organizing committees. I am grateful to the co-sponsors the Ministry of Agriculture, Government of India, Indian Council of Agricultural Research, Marine Products Export Development Authority, Tata Chemicals Ltd., Indian Tropical Agro Products (P) Ltd. and NEOSPARK Drugs and Chemicals Pvt. Ltd. I hope that this book would serve a wide range of clients, students, researchers and policy planners in their efforts to popularize and spread mariculture and thereby help to ensure food security and to uplift the quality of life of our fishers and fish farmers.

Cochin
August 2001

MOHAN JOSEPH MODAYIL
President, MBAI

CONTENTS

—x—

Evolution of eco-friendly coastal aquaculture/mariculture technologies

M.N. Kutty,
'Prasadam', Puthur,
Palakkad - 678001, Kerala

ABSTRACT

Global aquaculture production from marine waters, which accounts for 54% of total production, increased from 6.86 million metric tonnes in 1987 to 18.51 million mt in 1996, registering an increase of 270% over the decade. India's marine/coastal aquaculture production is almost restricted to shrimps, as the production of marine finfishes, molluscs and sea weeds are negligible. An index of Biodiversity Utilization for Aquaculture (BUA) calculated for India is quite low (0.13) when compared to the highest (0.51) for Taiwan and Korea (RoK).

India's coastal aquaculture technologies for marine organisms, such as shrimps, crabs, lobsters, mussels, edible oyster, pearl oyster,

sea bass, mullet and milkfish, Gracilaria *and holothurians are yet to spread out. Serious efforts are needed to develop more applicable eco-friendly technologies and improved extension system to propagate them, and perhaps also through transfer of technologies from our Asian neighbours, who have proven expertise in specific areas. It is important, however, to overview and ensure that all the existing and newly introduced technologies are suitably modified, so that they are environment friendly and socially acceptable.*

More recent researches have shown that improved management practices can ensure pollution-free and disease-free culture systems. It is also necessary that besides technological considerations, environmental and socio-economic considerations according to laid-out plans and policies, and effected through discussion and dialogue among all the stakeholders of the aquaculture ventures, should take place, sufficiently early as a part of pre-siting / siting exercise. This should involve also the various sectoral interests in the coastal zone so that aquaculture development would blend in harmony with the other concomitant sectoral developments of the eco-system primarily and the region, as is envisaged in an integrated plan for coastal area development.

Introduction

The aquaculture/mariculture production from marine waters has increased from 6,863,270 metric tonnes (mt) (51% of the total aquaculture production) in 1987, valued at 10.95 billion US$ to 18,609,269 mt (54% of total production) in 1996, valued at 25.80 billion US$, showing an increase of 270% in quantity and 236% in value, over the decade, on the basis of production of all farmed aquatic organisms (FAO, 1998). Eco-friendly aquaculture/mariculture systems, are the present need as a stage has reached in aquaculture development where the impacts of some aquaculture systems have affected and/or will soon affect, if unchecked, the ecology/environment of the aquaculture sites. Besides, these ecological impacts would be accentuated by the socio-economic impacts of the culture activities (Lin, 1989; FAO/NACA 1995; Kutty, 1997, 1998, 1999a). It is largely true that this situation is reflective presently of mainly shrimp culture, around the tropical and semi-tropical belt of the world, but mainly of Asia and the Lain American countries.

The fear of the collapse of shrimp culture began with that of Taiwan (Lin, 1989), followed by partial collapses of farmed shrimp production in China, Indonesia, Thailand and Philippines among other countries (FAO/ NACA, 1995). The collapse of shrimp culture was owing mainly to the lack of understanding of the impacts of the lucrative shrimp farming and its over-exploitation and abuse of the sensitive coastal environment/ eco-system and also the socio-economics of the communities around the farms. This has given rise to the different types of reactions, at one end the puzzled farmer/entrepreneur trying to re-establish the collapsed or collapsing system, avoiding environmental degradation, which caused bursts of uncontrolled diseases in his site, and at other end, serious criticism of the environmentalists, social workers and other vested interests. The latter eventually, in the case of India, lead to the intervention of the Supreme Court India (1996). The SCI banned semi-intensive and intensive aquaculture in the 500m belt along the coasts of India. Virtually this left out only the least producing system, the traditional and extensive, of coastal aquaculture to be continued. Obviously this system is sustainable, but would it be able to produce adequate quantities of shrimp and fish, for nutritional needs of country, and also the needs of an expanding aquaculture sector, bringing considerable economic benefits to the country - to the farmers and the community - and also poverty alleviation through employment and other benefits to the people?

It is paradoxical, that despite some initial collapses, countries like Thailand and China have reacted differently to the above described situation - in that subsequent to initial environmental and socio-economic problems, they have developed new culture technologies to overcome the previous lapses. Thailand has come to an understanding of the problems and has come to set a code of conduct for shrimp culture (Tookvinas *et al.*, 1999 as advised in FAO, 1997) and their shrimp production still maintained at a high level (211,100 mt in 1997, in spite of a small decline a few years ago, FAO, 1999), as world's top producer of farmed shrimp, which ultimately paved the way for an eco-friendly shrimp culture system on a sustainable basis.

The sustainability of aquaculture has been discussed in various

national and global fora, the latest being NACA/FAO aquaculture of the Third Millennium Meeting at Bangkok (NACA/FAO, 2000, resulting in the "Bangkok Declaration on Aquaculture Development"). Other references on discussions on sustainable aquaculture can be seen in Kutty (1999a, 1999b). I have pointed out here a most serious problem facing aquaculture, shrimp culture in particular, owing to its topical and chronological importance, at the outset itself, but salient aspects of eco-friendly and sustainable coastal aquaculture will be discussed further below on the basis of this and other experiences in the relatively short history of aquaculture.

In the present discussion coastal aquaculture and mariculture will be treated together for convenience, even though one can make some distinctions. Coastal aquaculture has been defined to include all land and water based culture systems in brackish and marine waters in a two kilometre belt in the continental shelf off the coastline, and also in the low lying areas beyond the tidal zone (Nunes and Parsons, 1998). In this context it is interesting to cite Barg (1992), who states: "The coastal area is an interface between land and sea, which extends inland and seaward to a variable extent. The term "coastal area" refers to a geographic space, which has not been defined as a zone. Defining boundaries of a coastal "zone" in a given area ("Zoning") will depend on political, administrative, ecological and pragmatic considerations. "Zoning", i.e., the process of defining the boundaries of a coastal area to be developed and managed, is an essential component of Integrated Coastal Area Management (ICAM)". We shall refer to this again, but as it is the definition of coastal aquaculture would include culture in marine waters as well, which would be restricted to the near shore area. There are only a few examples of real offshore aquaculture, even though one could expect that culture of Atlantic salmon, tunas and a few other new entrants could be the first candidates in this respect. It must be pointed out that in several of these cases, as for the tunas and other scombroids, and even in several groupers, the life cycle has not been closed - the culture being mainly dependent on wild seeds. For the real expansion of the culture of such species it is important as a primary requirement that further studies

should be immediately made to close the complete life cycle of the species. This is one of the recommendations of the Aquaculture for the Third Millennium Meeting (NACA/FAO, 2000). More discussion on the species used in aquaculture will follow.

Mariculture, which can be referred to, as culture aquatic denizens in marine waters exclusively, would be different from brackishwater aquaculture, the latter having shares from inland and marine species. The FAO production statistics takes care of this distinction in providing split-up figures for the two components.

The present paper sequentially presents below details of marine aquaculture production - global, country-wise and species group-wise, including as referred to above, a full list of species, categorised by family/order of finfishes, crustaceans, molluscs, seaweeds, and also their respective production figures for 1994 and 1996. We shall also discuss the diversification of aquaculture - the species diversity involved, with examples from some selected countries including India. Some of the major techniques of coastal aquaculture/mariculture have been pointed out and lastly evolution of eco-friendly culture technologies for achieving sustainable production of farmed aquatic organisms discussed.

Some specific examples of attempts to towards sustainable aquaculture incorporating a holistic approach to coastal area development, especially with reference to shrimp farming will be discussed. Much of the descriptions/discussion, which follow are from a global context, except for a few instances such as bio-diversity utilisation in aquaculture, but the experiences/lessons learned from other countries, in a global context are highly pertinent to India, as well as to other developing countries, which have a high stake in aquaculture for food and nutrition, poverty alleviation and other socio-economic advantages. Much of these could accrue from aquaculture development integrated within itself, and with other sectoral activities in the coastal region (Barg, 1992; FAO - Code of conduct on responsible fisheries, 1995; FAO, 1997; NACA/FAO, 2000).

Coastal aquaculture/mariculture production

Global production

The salient aspect of recent increase in production from marine waters has been referred to initially herein. Table 1A gives the details of aquaculture production of all aquatic organisms from marine and inland waters and their proportional changes from 1987 to 1996. These indicate that the percentage increase in production from marine waters is higher (270%) than for inland waters (236%). The relative share of production from marine waters showed that it increased from 51% in 1987 to 54% in 1996.

Table 1A. Global aquaculture production (all aquatic organisms) (in mt.) in marine and inland waters

(Source : FAO, 1998a)

Year	1987 (% of total)	1996 (% of total)	% increase 1987-1996
Marine	6,863, 270	18,509,269	270
	(50.9)	(54.3)	
Inland	6,617,161	15,606,980	236
	(49.1)	(44.7)	
Total	13,480,431	34,116,249	257
		(100)	

Table IB gives the corresponding production for fish and shellfish only (excluding seaweeds and some non-edible species) over the same period. It must be noted that the proportion of marine production is lower here, the main reason being the exclusion of seaweeds. The treatment of statistical information on aquaculture production is being refined by the FAO (FAO, 1999), and different treatments of data obtained by FAO, is reflected herein. Thus Table IC is still different, presenting the same information, but categorised on the basis of environment (aquatic medium) of the species. Here production from brackish water has two components (taken from marine and inland sources), as explained in the Table itself.

Table 1B. Global aquaculture production (fish and shellfish only) (in mt.) in marine and inland waters

(Source : FAO, 1998a)

Year	1987 (% of total)	1996 (% of total)	% increase 1987-1996
Marine	4,018,872 (37.8)	10,772,979 (40.8)	268
Inland	6,616,315 (62.2)	15,606,604(59.2)	235
Total	10,635,187 (100)	26,384,583 (100)	248

Table 1C. Global aquaculture production (in mt.) in 1996 marine and inland waters

(Source : FAO, 1998a)

Environment	All aquatic organisam (% of total)	Fish and shellifish only (% of total)
Marine	17,450,358 (51.1)	9,733,992 (36.9)
Freshwater	15,082,601, (44.2)	15,082, 225 (57.2)
Brackish water	1,583,790* (4.1)	1,568, 366* (5.9)
Total	34,116, 246 (100)	26,384,583 (100)

Leading aquaculture producing regions and countries

Out of a global total production of 32.1 million mt in 1996, Asia accounted for most of it (91,1%), trailed by a wide margin by Europe (4,7%), N. America (1.8%), S. America (1.5%), Africa, Oceania and other countries (former USSR), producing 0.3% in each case (FAO, 1998).

On the basis of overall aquaculture production of fish and shellfish in 1996 (FAO, 1998) the ten leading producer countries are China (17,714,570 mt, accounting for 61% of the global total), India (1,768,422 mt, 7.1%), Japan (829,354 mt, 3%), Indonesia (672,130 mt, 2.5%), Thailand (509,656 mt, 1.9%), USA (393, 331 mt, 1.5%), Bangladesh (390,088 mt, 1.5%), Ro Korea (358,003 mt, 1.4%), Philippines (342,543 mt, 1.3%) and Norway (324,678 mt, 1.2%). An overview of total aquaculture production is needed to understand the relative shares of different categories in production. According to FAO, these comprise seven categories: freshwater fishes, diadromous fishes, marine fishes, crustaceans (all marine, except for a fraction of freshwater crustaceans as shown in the

descriptive tables), molluscs (again mostly marine), aquatic plants (almost all marine/ and other aquatic organisms.

On the basis of the magnitude of production of the major categories of framed organisms the leading producer countries have been ranked (Table 2). Here it can be seen that Japan, China and Indonesia are leading producers of farmed finfishes, but it can be seen that the total production under this category is much less than those of the other categories (see also Table 3). China is the lead producer of crustaceans, molluscs and seaweeds (and also freshwater fishes). As reported in FAO statistics for 1996, India's coastal farming concerns shrimps only (this is discussed further herein) and India is listed as fourth in the list under crustaceans.

Table 2. Leading producer contries of marine fishes, crustaceans, molluscs and aquatic plants (seaweeds) in 1996.

(Source : FAO, 1998a)

Quantity in Mt.

Order of production	Country		(% of total country production)
Marine fishes			
1.	Japan	-	247,827 (18.4)
2.	China	-	240,592 (1.0)
3.	Indonesia	-	113,000 (1.4)
Crustaceans			
1.	China	-	236,309 (1.0)
2.	Thailand	-	230,832 (47.3)
3.	Indonesia	-	157,710 (20.2)
4.	India	-	87,527 (4.95)
5.	USA	-	22,430 (57)
Molluscs			
1.	China	-	6,406,595 (27.7)
2.	Japan	-	490,072 (36.3)
3.	France	-	218,178 (76.4)
4.	USA	-	98,183 (25.0)

Seaweeds			
1.	China	-	5,419,950 (23.4)
2.	Philippines	-	631,387 (65)
3.	Ro Korea	-	539,995 (60)
4.	Japan	-	520,051 (39)
5.	DPR Korea	-	381,000 (78)

Table 3. Global aquaculture production of marine and brackishwater species/groups

(Source:FAO, 1998a; for details of individual species production for 1994 and 1996.)

Main Groups/ Species		Production in 1996(mt)
Diadromous Fishes		
Sturgeons, paddle fishes	-	1077
River eels	-	216,646
Salmon, trouts, smelts	-	1,072,478
Miscellaneous diadromous fishes (eg:milkfish, seabass)	-	380,396
Sub total	-	1,670,597
Marine Fishes		
Flounders, halibuts, soles	-	198
Redfishes, basses, congers	-	221,504
Jacks, mullets, sauries	-	193,230
Tunas, bonitos, billfishes	-	2,090
Miscellaneous marine fishes	-	192,268
Sub total	-	609,290
Marine Crustaceans		
Sea spiders, crabs	-	119,137
Shrimps, prawns	-	914,706
Lobsters, spiny rock lobsters	-	62
Miscellaneous marine crustaceans (eg:*Artemia*)	-	20,269
Sub total*	-	1,054,174
*(excludes fresh water crustaceans	-	92,630)

Molluscs		
Gastropods		
Gastropods		
Abalones, winkles, conchs	-	3,349
Bivalves		
Oysters	-	3,067,316
Mussels	-	1,179,045
Scallops/pectens	-	1,275,958
Clams, cockles, arkshells	-	1,777,543
Cephalopods		
Squids, cuttlefishes, octopuses	-	1
Miscellaneous molluscs	-	1,196,023
Sub total*	-	8,499,235
*(excludes fresh water mollusca-11,821)		
Seasquirts and other tunicates	-	12,672
Miscellaneous aquatic invertebrates	-	13,550
Seaweeds/other acquatic plants		
Brown seaweeds	-	4,583,690
Red seaweeds	-	1,680,733
Green seaweeds	-	47,673
Miscellaneous aquatic plants	-	1,419,370
Sub total	-	7.731,466

Global farmed shrimp production

Global shrimp culture production for the period 1988 to 1997, taken from FAO (1999) is given in Table 4. It increased from 576,453 mt in 1988 to 941, 814 mt in 1997, but the peak of 101, 583 mt is reached in 1995, showing a plateau for the period 1995-1997. This indicates a slowing down of farmed shrimp production as also seen in shrimp culture production in India (Table 5), owing to recent catastrophes in shrimp culture as described herein elsewhere.

Table 4. Global shrimp culture production (t) for 1996

(Source: FAO, 1999)

Year	1988	1989	1990	1991	1992
Produce	576,453	620,502	671,997	832,678	889,678
Year	1993	1994	1995	1996	1997
Produce	847,697	690,385	951,593	949,301	941,814

Table 5. Shrimp culture production and area under culture in India for the years 1993-94 to 1998-99

(Source:MPEDA, Cochin)

Year	1993-94	1994-95	1995-96	1996-97	1997-98	1998-99
Area (ha)	82,540	100,700	118,983	135,582	141,591	135,007
Production (mt)	62,000	82,850	70,573	70,686	66,868	82,634
Production (Kg/ha)	751	823	593	521	470	618

Farmed shrimp production in India

In the case of India, according to MPEDA (Table 5), the farmed shrimp production was 62,000 mt in 1993-94 and 82,000 mt in 1998-99, but there was a slump in between, the production going down as low as 66,868 mt in 1997-98, when the hectarage under culture was maximum (141,591, ha). The unit area (ha) production also decreased from 823 kg/ha in 1993-94 to 618 kg/ha in 1988-99, but the lowest again was in 1997-98. We can read the exciting history of shrimp culture in India in these figures. The initial drop and slowing down was due to environmental degradation and diseases, as elsewhere in Asia, but the recent decrease in unit area production is due to the increase in the hectarage especially in extensive culture, and closing down of the semi-intensive farmers and major operators from the scene, owing possibly to the SCI intervention, as much as the lack of faith in shrimp culture when failure struck, after trying to extract the maximum from the farms, deviating from good management practices. But there is no need for despair as

good management practices (GMP) can be expected to usher in sustainability to the troubled aquaculture system.

Taxonomic groups and species

Global aquaculture production of marine and brackishwater species groups falling under the major categories of diadromous fishes, marine fishes, marine crustaceans, marine molluscs and seaweeds for 1996, is given in Table 3. This gives a fair idea of the spectrum of farmed marine and brackish water animals involved in coastal aquaculture. Out of the total number of 229 cultured salt water tolerant and marine species, 91 are finfishes, 43 crustaceans, 81 molluscs and 14 seaweeds, all of them do not qualify as species under commercial culture. As Garibaldi (1996) pointed out the bulk of production is accounted for by a handful of species, but the others are not to be ignored as new technologies are being developed - the emergence of salmonids in Norwegian and Chilean aquaculture (having developed open sea cages stocking over a million salmon fry in each cage) and tuna culture in Australia are good examples of recent triumphs in mariculture.

Species adaptations to salinity

Eventhough the exclusively freshwater species are not considered here, species which can tolerate and live in the three environments, as exemplified by several diadromous species, like the salmonids, mullets and milkfish among teleosts, which are the typical euryhaline species are relevant in this context. In addition there are the stenohaline species whose salinity tolerance is limited. Among the salt tolerant species there are fresh water species which move into the brackish waters (*Macrobrachium* spp among crustaceans) and also teleosts (several tilapias). Most of these have ion-osmoregulatory capacities, but those with no capacity regulate body salt concentrations conform to the salinity of the ambient medium, and as well known are referred to as osmo-conformers (e.g., crabs, *Eriocheir* spp.).

An aspect which is important in aquaculture is the energy expenditure of the cultured species in different salt media. The regulators spent

energy to keep the body fluid concentration constant (close to 10 ppt salinity in teleosts) by pumping in or out chloride ions through their gills and also by effecting permeability changes in the gill membrane through acclimation/adaptations of aquatic organisms to effect energy saving of the growing organism in the culture set-up, as this can certainly cut costs on feed, which is the costliest input in higher intensity aquaculture.

Aquaculture diversity

It can be seen that maximum exploitation of diversity of aquatic organisms, covering all seven categories has been effected by only two (China and Japan) of the eight countries. India and Bangladesh exploit only two categories - freshwater fishes and crustaceans (mostly shrimps), as per the FAO production data for 1996. There can be disparities in the FAO reporting system, as it is known that India does have aquaculture production of molluscs - especially mussels in the southwest coast but not reflected in the FAO production data. Also India is still in the process of developing and commercialising aquaculture of other marine organisms, even though technologies are available (Devaraj, 1999). It would be noted several species known to be cultured and some under experimental culture in India and other countries, which are listed in FAO (1998a), but are in the stages of being recruited into aquaculture.

An idea of biodiversity utilisation for aquaculture (BUA) can be known from the number of species used by the country for aquaculture. A crude index for BUA has been calculated to standardise comparison on a global basis (Kutty, 1999 a). The values of the BUA along with the details of the number of species and categories utilised and total production for selected countries, and the method of calculation of BUA are given in Table 6. Even though there are limitations in the use the BUA, owing to geographical and country differences, it is felt that the index enables macro comparisons at global level and also accommodates the increase in numbers of species, which will be utilised in future.

It comes out clearly that India has not utilised its biodiversity ad-

equately as India uses only 13 species and has only a BUA of 0.13, while maximum utilised (51 species and BUA of 0.50 by Taiwan and Ro Korea) are quite high.

Table 6. Country-wise details of aquaculture production, numbers of major categories and species used and calculated Biodiversity Utilization for Aquaculture (BUA) in seleccted producer countries (Based on data obtained for 1996, taken from FAO (1998a) (from Kutty, 1999a)

Country	Aquaculture Production in 1996(mt)	No.of major categories utilised [b]	No.of species utilised [c]	Crude Biodiversity Utilisation Index [d]
Korea (Rok)	896,998[a]	7	51	0.50
Taiwan	272,209	7	51	0.50
France	285,721	6	45	0.44
Thailand	509,656	6	35	0.34
Japan	1,349,405	7	33	0.32
Spain	233,833	5	32	0.31
China	23,134,52	7	29	0.28
USA	393,331	5	28	0.27
Philippines	342,678	6	27	0.26
Indonesia	780,130	5	23	0.23
Australia	26,323	5	30	0.29
Chile	323,115	4	15	0.15
India	1,768,422	3	13	0.13
Norway	324,543	3	8	0.08

a) 60% sea weed/aquatic plants

b) Maximum number of categories is 7, namely, fresh water fishes, diadiomous fishes, marine fishes, crustaceans, molluscs, other aquatic animals and aquatic plants

c) Number of species recruited for aquaculture.

d) A Crude Biodiversity Utilization Index is calculated by dividing the number of species utilized by the specific country, by twice the maximum recorded for any country, eg.for India, BUA, is estimated as 0.13, ie.13/51x2,51 being the highest number utilized (Korea, RoK/Taiwan)

Aquaculture systems

The different culture systems such as the coastal ponds, cage and pen culture and the raft/rack and pole and line culture for the different sea farming species along with species names in selected countries in Asia are shown in Table 7. Salient aspects of these culture systems with reference to eco-friendly coastal quaculture are pointed in following section.

Table 7. Major seamfarming species and their culture technologies in selected countries in Asia (Based on NACA, (1991); Taw, 1994 FAO, 1998)

Culture type	Farming technology (Country)	Species-Scientific name(local name) Countries
1. FINFISHES		
Grouper culture	Floating net cages/refts (HK) Hong Kong (HK)	*Epinephphelus akaara* (Red spotted grouper)
	Floating net cages/ponds (MAL)	*Epinephphelus akaara* (Yellow grouper)
		Epinephphelus tauvina (Greasy grouper)
		Epinephphelus chlorostigma (Brown)
	Hong Kong, Malaysia	*Epinephphelus suillus* (Spotted grouper)
		Epinephphelus malabaricus (Strip spotted grouper) Malaysia
Seabass culture	Floating net cages (HK)	*Lates calcarifer* (Giant sea perch)
		Floating net cages/ponds (MAL)
Seabream culture	Floating net cages (HK)	*Chysophrys major* (Silver seabream)
		Rhabdosargus sarba (Goldlined seabream)
	Hong Kong	
		Mylio berda (White seabream)
		Mylio latus (Yellow finned seabream)
		Mylio macrocephalus (Black seabream)
Snapper culture	Floating net cages (HK, MAL)	*Lutjanus russelli* (Russell's snapper)
		Lutjanus argentimaculatus (Mangrove red snapper)
	Hong Kong, Malaysia	*Lutjanus johni* (Golden snapper)

2. CRUSTACEANS

Crab culture	Floating wooden bamboo (MAL, VIE)	*Scylla serrata* (Mud crab) cages with plastic/Styrofoam drum as floats/earthen ponds
Shrimp culture	Earthen ponds-India and several other countries in Asia India, Thailand	*Penaeus indicus* (Indian whita shrimp) *Penaeus* merguiensis (Banana shrimp) *Penaeus monodon* (Giant tiger shrimp) *Penaeus* semisulcatus (Green tiger shrimp)

3. MULLUSCS

Cockle culture	Bottom culture/mud flats (MAL)	*Anadara granosa* (Blood cockle)
Mussel culture	Suspended (Raft/stack) bottom culture (MAL)	*Perna viridis* (Green musel)
Oyster culture	Bottom culture (HK), Suspended (raft and long line) Malaysia, Philippines bottom rack (MAL) Raft (SRL, IND) Hong Kong, China Sri Lanka, India (IND)	*Crassostrea belcheri* (Large oyster) *Crassostrea iredalei* (Philippine cupped oyster) *Crassostrea gigas* (Pacifis cupped oyster)
	Suspended raft & stake / Vietnam, China floating and fixed rafts (MYA) Bottom and suspended (raft & stake) Myanmar (MYA) (VIE)	*Crassostrea rivularis* (Chinese large oyster) *Ostrea folium* (Flat oyster) *Saccoatrea cucullata* (Rock cupped)
Pearl Oyster culture	Suspended raft, net cages (VIE, IND)	*Pinctada formosa* *Pinctada foucata (Japanese pearl oyster)* *Pinctada margaritifers (Black-lippearl* oyster)
	Vietnam	*Pinctada maxima (Silver-lip pearl* oyster)
	Vietnam	*Pteria penguin* (Wing oyster)

4. SEAWEEDS		
Red algae culture	*Eucheuma* - bottom method (nylon between stakes; semi-raft and raft method-stretched nylon mono-filament nets) (MAL) *Gracilaria*-pond culture (net fixed off bottom); open sea long line floating system (SRI, MYA); pond culture (tidal ponds) (poly culture) (VIE); fixed bottom monoline, floating raft monoline, mangrove pond (PHIL)	*Eucheuma cottoni* *Eucheuma spinosum* *Gracilaria* edulis *Gracilaria verrucosa* Gracilaria firma *Gracilaria changi* *Gracilaria heteroclada* *Gracilaria fastigata* *Gracilaria tenuistipitata*

The Regional Seafarming Development and Demonstration Project (RAS/86/024 and RAS/90/002) under FAO/NACA brought together member countries including India in Asia which had committed programmes, in seafarming developments, conducted country based specialised training and workshops on various farming systems and activities through regional co-operation and TCDC. This resulted in transfer of various coastal aquaculture and mariculture technologies to participating countries from expertise donor countries, such as sea-bass culture (Thailand), marine cage culture (Singapore, Hong Kong), seaweed culture (China and Philippines) and pearl culture (India).

As advised by an FAO/NACA mission (Butler *et al*, 1989) a series of seafarming atlases involving 12 countries in the region, namely India, China, Hong Kong, Indonesia, Korea Rep., Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Thailand and Vietnam, were produced (Regional Seafarming Resources Atlas - Vol. I (China, India, Indonesia, Korea Rep., Philippines, Singapore and Thailand, released by RAS/86/024 in 1990, and Vol. II (Hong Kong, Malaysia, Myanmar, Sri Lanka and Vietnam), released by RAS/90002 in 1991).

Evolution of eco-friendly coastal aquaculture/mariculture systems

Environmental and socio-economic impacts of the different culture systems are discussed separately below.

Impacts of pen and cage culture: Pen and cage culture has developed more prominently in the temperate region lately, than in the tropical region, especially owing to the expansion of Atlantic salmon culture in Norway, and also salmonid culture in Chile. It is opined that if the intensity of cage culture is increased, the problems of coastal aquaculture in the tropics, similar to those faced by shrimp culture (Nunes and Parsons, 1998), would increase if not managed properly. Since carnivorous species are usually used they need high protein feeds, and so waste generation can be high. The amount of wasted feed (uneaten feed and unabsorbed nutrients) is higher in cage culture than in shrimp culture. In the temperate region it is estimated that waste generation is in the range of 10-15kg P and 75-95 kg N per year per tonne of fish produced (Enell and Lof, 1983, in Nunes and Parsons, 1998).

It is also feared that feeds given in powder form by cage operators in the tropics would lead to serious feed losses and water quality deterioration around the cages. Nunes and Parsons (1998) list the following effects of waste accumulation in the vicinity of cages, namely, reduction in redox potential, increase in C and N in the sediments, generation of hydrogen sulphide and methane, increase in oxygen consumption by the sediment, biological changes in macrobentic communities around the cages, starting with growth of sulphur bacteria, followed by reduction in the biomass of macrofauna such as crustaceans and molluscs, and eventually dominance of low oxygen tolerant species (eg. capellid polychaetes). These impacts, though limited to 30-100m around the cage site, can at times be significant.

The release of nutrients, N and P can be considerably reduced by improving the quality of the feeds in Atlantic salmon cages, as evidenced by feeding low FCR feeds: which has a very positive effect on the environment and the carrying capacity of the water body (Kutty, 1995).

Polyculture of finfish and holothurians in net pens: An interesting experiment in polyculture of Pacific salmon and holothurians has been reported by Ahlgren (1998). Pacific Salmon - pink *(Oncorhynchus gorbuscha)* and chum (*O.keta*) - one million fry each kept in 18-m diameter circular floating net pens ("Norwegian style") off Alaskan coast, developed problems of net-web fouling and so one hundred red sea cucumbers *(Parastichopus californicus)* were placed individually along the webbing of three selected pens. After six weeks the area of clean webs in the experimental and control pens were measured by counting the number of quarter m^2 quadrants with clean or fouled mesh. In four replicates, Ahlgrcn(1998) observed that the percentage of cleared surface area was 54-68% in the test pens, while in control pens it was 0%, effectively providing that red sea-cucumbers could be cultured successfully in combination with salmon fry. The sea cucumbers assimilated amino acids and other organic matter 2-3 times more efficiently than in their natural environment. This is an example of a positive impact through use of polyculture in net cages.

Impacts of shrimp culture

Potential impacts: Tookvinas *et al.* (2000) from their experience in Thailand list the following as potential impacts of shrimp farms:

- conversion of mangroves and other coastal wetlands to ponds;
- nutrient enrichment and eutrophication of coastal wetland by pond effluents;
- discharge of potentially toxic and bio-accumulative chemicals into natural ecosystems;
- sedimentation in coastal waters because of erosion from ponds and other earthen structure;
- salinisation of freshwater sources by pond effluents and seepage;
- reduction in bio-diversity of coastal eco-system caused by water pollution, sedimentation and toxicity of effluents;
- introduction of non-native species or new shrimp diseases into coastal waters;

- competition with activities for natural resources, and land use disputes.

Problem of aquaculture waste accumulation/disposal from shrimp ponds: The aquaculture waste from shrimp farms is mainly composed of uneaten feed (15-20% of feed given) and faecal wastes (20-25% of feed given), the animal retaining the balance for its growth, maintenance and metabolised waste (excretion). Primavera (1998) observes in the case of shrimp 15% of the feed given is not consumed, 20% egested, 48% spent in maintenance, excretion and ecdysis, and 17% is harvested.

As in the case of other aquaculture systems the faecal and excretory waste and uneaten feed are the source of nutrients, N, P & C, released into the pond water. N and P waste vary considerably with several factors such as the feed quality, feeding patterns and also the environmental variables. The quality of nutrients released changes with the intensity of culture. Gavine and Phillips (1994) estimated that an intensive shrimp farm generates 43% more N and 98% more P waste than a semi-intensive farm. The excess nutrients released, in cases where flushing is inadequate, accumulate in the pond bottom and cause water quality degradation, auto-pollution, and cause algal blooms, and mortality of stocked shrimps. The poor water quality also makes them vulnerable to diseases. So in the process of intensification of the culture activity, excess stocking and feeding, namely, result in algal crashes, and diseases. The wastes/nutrients released from the ponds cause problem, unless the release water is treated and regulated. It is claimed that effluent disposal into oligotrophic waters have caused in some cases, more production of fish and shellfish in the coastal waters, as reported in Thailand, but by and large the effluents disposed cause more damage, as proven in many cases. The serious consequences of bad pond management leading to collapse of farmed ponds and the ecosystem has already been referred to.

Impacts of bivalve culture

Even though the bivalves do not need any artifical feed, as they feed mainly filtered natural food, they can cause similar problems as in other

coastal aquaculture systems (cage and pen) by deposition of organic wastes and faecal matter, causing the same chain of events in water quality deterioration and unwelcome biological changes around the oyster/mussel racks/rafts. As effective filter feeders an individual mussel is reported to filter 2.5 litres of water per day and a rope of mussels over 90,000 litres/day. Filter feeding bivalves retain a very high percentage (35-40%) of seston ingested (Barg, 1992) and it is estimated that a typical oyster rack holding 420,000 oysters would produce 16mt of faecal matter in one season (9 months) (see Nunes and Parsons, 1998). Thus the positive side of using bivalves as biofilters in treatment ponds is to a large extent offset by the negative influence of loading wastes in the environment.

Towards eco-friendly and sustainable coastal aquaculture/mariculture technologies.

Reduction of wastes from aquaculture systems

Reduction of waste from aquaculture installations is the most serious problem to be tackled for evolving eco-friendly aquaculture systems. The following methods (based on Phillips, 1995; Nunes & Parsons, 1998, New, 1999) would help resolve some of the issues involved in coastal aquaculture/ mariculture:

- Using sedimentation and oxidation tanks for treatment of aquaculture effluents.
- Use of biofilters - polyculture of filter feeding fish, oysters, mussels and nutrient absorbing seaweeds.
- Use of mangroves as natural biofilters, adjacent to land-based farms - serving as buffer zones, removing nutrients and organic matter from the effluents.
- Improved management strategies.
- Cage site rotation, allows sediment recovery through natural dispersal and disintegration of wastes.

- Introduction of floating pellets released from cage bottom, which rise/float slowly to surface.
- Using a funnel shaped catchment device and submersible pumps and mixers to collect and disperse organic wastes.
- Use of closed recirculation systems eliminating or reducing discharge of pond effluents to adjacent waters.
- Use of a flow-through system, high flow, circular self-cleaning culture ponds and full treatment ponds for inflow and outflow (effluent) waters to and from rearing ponds

Approaches to sustainable coastal aquaculture

There are several approaches to sustainable aquaculture. The details of some of these are discussed below.

Traditional/extensive culture: The easiest and simplest is the time honoured system of traditional extensive aquaculture, where intensity of applied technology is low, characterised by very low production, stocking and, correctly, without any fertilisation or supplementary feeding. But often increased stocking and fertilisation have crept into the extensive system (improved extensive) practised now in India, though feed may not be applied for increasing the intensity of culture. To begin with this culture was based on natural or auto-stocking, without recourse to stocking of any seed, and hence here a naturally developed polyculture system prevailed. As recognised by the SCI in its ruling this kind of original extensive polyculture would do no harm to the environment and would not also cause any socio-economic problems and hence sustainable. But as pointed out earlier, adopting this method of aquaculture covering all the coastline of India, will seal the fate of aquaculture, *doomed to a very low production for perpetuity,* as pointed out already. Therefore unless for very sensitive environmental and socio-economic constraints, it would be nonadvisable to leave large tracts of potential aquaculture area to simulate the natural ecosystem, and hence low output, especially at this time, when the demand for animal protein (fish protein), to combat malnutrition and need for food/nutritional security is very high.

New approaches: The way out of this muddle is to develop sustainable aquaculture through new approaches and we should also initiate action on realistic policies, plans and regulations, which would protect the interests of all stakeholders in aquaculture. Two new approaches to sustainable aquaculture have been identified: one is the recirculation of water, almost coming to a 'closed system' and the other is the 'open system' of intensive aquaculture as demonstrated in the case of shrimp culture, called the 'third generation culture technology' by New (1999), as exemplified by dry tropical full sea water shrimp farm, set up in the Red Sea Coast of Saudi Arabia (New, 1999).

'Third generation' flow through system: 'Zero pollution' has been reported in this case, as the effluent discharged in the flow through system has almost the same high quality as that of the pumped up water inflow. As can be seen from the details (Fig. 1) the investment and skills needed for construction and maintenance of this third generation farm is high, but the high cost of shrimp *(Penaeus monodon,* supplemented very little by *P. indicus)* makes the farm highly profitable - the sale of one year's harvest alone has covered most of the inception costs of the farm. The nuances of the technology involved is indicated as much as possible in Fig. 1. Basically the success on the enterprise described according to New (1999) is built around a few elements, namely:

1. Siting in a dry tropical area (in this case 'sapkha' of alluvial mud flats).
2. Water management hydraulics, bio-tech adaptations - buffer ponds and treatment ponds occupy a total of over 50% of water surface in the system.
3. Pumping large amounts of high quality natural seawater, to a large upstream reservoir - buffer pond, to let it flow by gravity to rearing ponds, large treatment (depuration) ponds, through mangroves to sea.
4. Development of controlled algal bloom through fertilisation to develop green water in the buffer pond for avoiding algal blooms later in the rearing ponds.

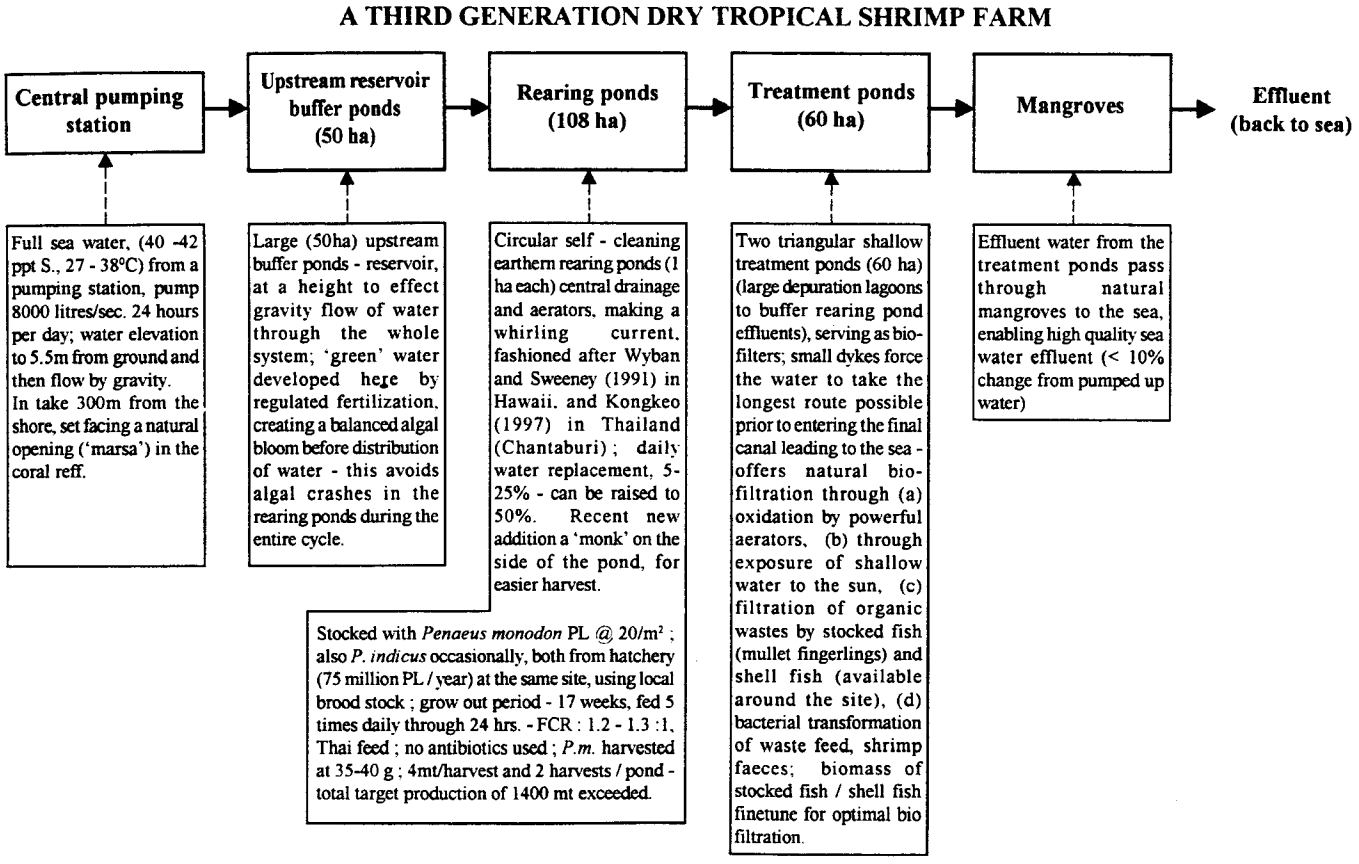


Fig.1. Schematic diagram showing successivel levels of water use/treatment in a 'zero pollution' system - Red Sea Shrimp Farms managed by "Group Consultatif Internationa" in a dry tropical (desert) site ('sapkha') - alluvial mud flats in Red Sea coast of Saudi Arabia (based on New, 1999) (thick arrows indicate flow of water and broken arrows indicate structural/functional details)

5. Having circular central drainage, self-cleaning (aerators making a whirling current) after Hawaiian (Wyban and Sweeney, 1989) and Thailand (Kongkeo, 1997) experiences.

6. Non-use of antibiotics in the culture ponds.

7. Complete dependence on indigenous seeds - development of breeding local species - *P. monodon, P. japonicus and P. semisulcatus.*

8. Having large shallow treatment ponds (27% of total surface area) where depuration takes place through extensive culture of filter-feeding fish (mullets) and shellfish, bacterial disintegration of faecal/excretion wastes, and sunlight enhancing the photosynthesis and oxidative function of the effluent from the shrimp ponds.

9. Further cleaning through natural biofilter - mangrove.

10. Release to sea, high quality effluent sea water, with only about 10% deviation of quality from that of pumped-up inflow water.

Recirculation/closed systems of land based farms: Thailand pioneered the use of closed systems for shrimp culture when harassed by shrimp diseases entering through inflow water from the estuary/sea. The principle is to store water in large reservoir ponds and strip it of all pathogens and use the clean water to feed the culture ponds. The effluent water is not released out to the natural environment, but is collected in treatment ponds, where filter-feeding fish and shellfish, as well as seaweeds are cultured so that the effluent water is cleaned of debris and effluent nutrients, ready for reuse. This is the ideal system, but in all cases this is not possible as there is need for a large portion of the area kept aside for recycling the water and there is always the need for some water to let and some new water taken. The Thai farmers and administrators are aware of this and have hence formulated a code of conduct for shrimp farming, which has already been referred to. The experience of the 'third generation shrimp farm', which use a flow through system (New, 1999), described above in water treatment will be of interest to the recirculation system exponents as well.

An example of a closely related system referred to as Intensive-Ex-

tensive System (Avanimech, 1998) will again be of much value in adapting new systems. Use of "Intensive-Extensive System (IES)" in trials in Negev desert (Israel), which have now become commercial, involves a group of smaller (500 ha) intensive ponds located beside one or more large earthern ponds (5000 ha), that are used for extensive fish culture and water treatment through culture of algae and bacteria.

An intensive pond can hold a fish biomass of 10 to 100 kg (say 1ha pond) per m^2, i.e. 100 to 1000 mt/ha, needing a high quantity of feed, at 2% biomass, it would be $2kg/m^2$ or 20 mt/ha. This pond is connected to the larger extensive pond.

Both high and low (or zero) discharge intensive ponds are used. The high discharge ponds have a daily water exchange rate of 500%; thus each 1000 ha pond, need a treatment pond of 5000 ha. The ratio of 1:5 was found to be too low, for the nutrients released. The production of inorganic N in intensive ponds was 0.7 kg N/mt fish and so with 100 mt fish in pond it would be 70 kg N ha/day, which was found to be too much for the treatment ponds to handle. So one has to increase the proportional size of the treatment pond, which would add considerably to the expenses, which was already high. Also it was found that the treatment pond is not an infinite sink; bottom sediment has to be cleared every 2-3 years.

In low density pond daily water exchange rate is 3-20%, where the feed loss was not much, as the uneaten feed is reused by fish, amounting to feeding twice, once as fresh ingestion by fish, second by bacterial ingestion, resulting in low FCR. This is a very significant observation. The production costs are still high and so very high cost fish can only be grown economically in the set up under the conditions. This study gives much insight into the closed system aquaculture, which will be of value to those who are working in this subject area. It is interesting to note that both in the presently described recirculation system and in the flow through system there are several similarities especially in the treat-

ment of water and the large water surface area of the treatment ponds. In both indeed high technology and high investment is needed, suggesting that the environmental and social costs of intensive pond culture is quite high, and perhaps by intuition the developing countries are inclined or forced to choose less intensive systems. But it is heartening that both systems could be sustainable, the main difference will be the economics of production; the investor has to pay more for high intensity production for meeting the costs of producing high quality effluents either for reuse or for letting into the natural ecosystem (environmental and social costs).

Integration of coastal aquaculture with halophyte crops: Brown and Glenn (1999) have been studying edible halophyte (such as *Salicornia bigelowi*) recycling shrimp effluents. The integrated system they have suggested is detailed in Fig.2. Halophytes such as *S. bigelowi* have multiple uses. While some are edible for man, they can be used as forage sheep (and goats) and some also yield oil seeds, from which high quality edible oil can be obtained. Brown and Glenn(1999) propose growing halophytes using shrimp pond effluents for irrigation - abandoned or unused shrimp farms can also be used for growing halophytes. The outflow from the halophyte plots will be hypersaline and so organisms such as *Artemia* could be cultured in this media and the final effluents taken to salt pans for extracting salt. The suggested integration may specially be applicable along southern Tamilnadu coastal areas in places like Mandapam and Tuticorin, where abandoned/unused shrimp ponds, salt pans and *Artemia salina* growing naturally are available. So the proposed system may be worthy of emulation to add to the productivity of such arid/semi-arid coastal areas and improve the socio-economics of the rural communities involved through integrated coastal aquaculture systems.

Conclusions

As evident from the present analysis, compared to other Asian countries Indian aquaculture is among the least diversified. Indian coastline

and EEZ are much longer/higher than those of any other country in Asia. We have to develop these vast areas to produce more through coastal aquaculture and mariculture. Attempts to diversify Indian coastal aquaculture, by recruiting more coastal and marine species to commercial aquaculture. We certainly do not lack the technology development skills, but we should also transfer technology and skills in new ventures if we lack them and adapt them to our conditions. Perhaps we should study the market situations well in our efforts to commercialise new technologies, or we should take steps to create new market avenues for the new aquaculture products. A review of production per unit length (km) of coast line and/or unit area (Km^2) of EEZ, showed that except for shrimps we are in lowest rung, even small Asian countries doing much better in the production of marine commodities such as molluscs, seaweeds and finfishes (Kutty, 1995a).

While considerable information on coastal/marine resources is available on a country wise and regional basis, in India and other Asian countries for evolving an effective coastal zone management planning (Barg, 1992) involving coastal aquaculture, there is further need for assessing the land, water, economic and human resources available for development (Kapetsky, 1987; Kapetsky *et al.* 1987). With the availability of specialised computer software it would be advisable to use the GIS (Geographical Information System) for coastal resource planning, with special reference to aquaculture (Kapetsky, 1987; Kapetsky et al. 1987). Appropriate zones in the coastal region could be identified for development of coastal aquaculture systems as done recently in Sri Lanka (FAO, 1997b, 1998b). With the background of the recent catastrophes in coastal aquaculture (collapses of shrimp culture) in Asia it is important that environmental and all other sectoral considerations including socio-economic issues, are paramount in bringing about an integrated coastal management plan, where sustainable aquaculture has its due share (Barg, 1992; Kutty, 1998; ACIR-MOFI, 1999). For inventory and monitoring

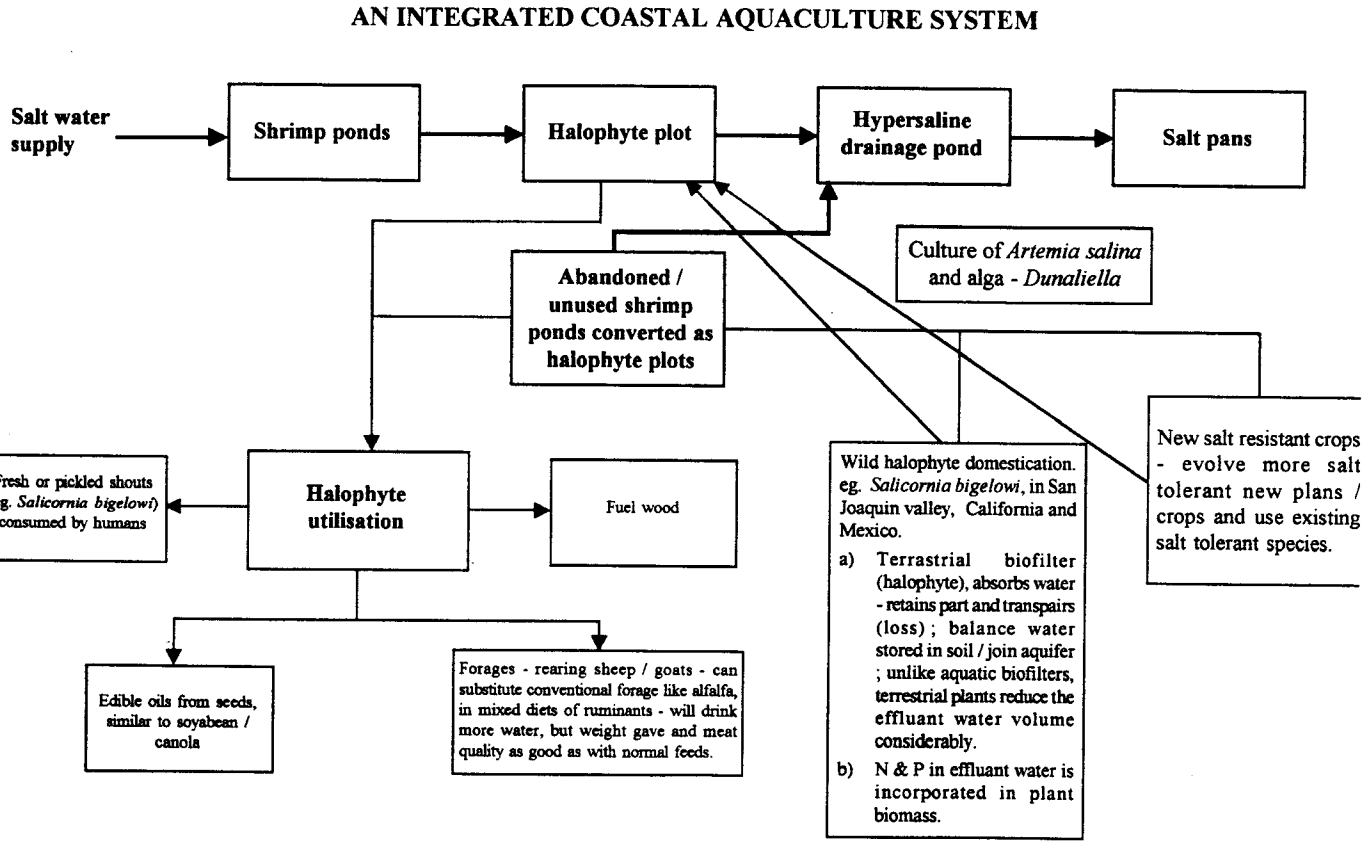


Fig. 2. Schematic Diagram showing proposed use of halophytes in integrated coastal aquaculture (shrimp - halophyte/ salt resistant crops/*Artemia* / *Dunaliella* / salt production integration (Based on Brown & Glenn (1999)} (Thick arrows indicated flow of water and thin arrows explain linkages)

of shrimp farms SAR satellite data and detailed image analysis as done recently in Sri Lanka (Travaglia *et al*, 1999) would also be especially helpful, in solving issues already existing and evolving more eco-friendly coastal aquaculture / mariculture systems.

India's coastal aquaculture technologies are yet to spread out, as already indicated and apparently Indian scientists are in different stages in developing technologies for various marine organisms, such as shrimps, crabs and lobsters among crustaceans, mussels, edible oyster and pearl oyster, among molluscs, sea bass, mullet and milkfish among finfishes, *Gracilaria* among sea weeds and also holothurians (Devaraj, 1999). While some of the technologies are known for decades or longer (shrimps, oysters, *Gracilaria*), some are in the threshold of entering the arena (groupers, holothurians).

Serious efforts are needed to boost our coastal aquaculture production, developing more applicable eco-friendly technologies and improved extension system to propagate them, and perhaps also through transfer of technologies from our Asian neighbours, who have proven expertise in specific areas, and as already indicated, it is important, however, to overview and ensure that all the existing and newly introduced technologies are suitably modified, if not already in an acceptable form, so that they are environment friendly and socially acceptable. For it has been recognized now that it is not the lack of technologies, which causes problems, but it is the impacts, environmental and socio-economic, of applied technologies, often in the absence of plans and policies, which undermines the process of aquaculture development at all levels. Coastal shrimp culture in India and elsewhere has indeed brought the problems into focus, but it is noteworthy that in no country other than India the preventive steps taken at national level are so drastic.

It is becoming obvious that culture systems, which are environmentally and socio-economically unsound, are not sustainable. More

recent researches have shown that improved management practices can ensure pollution-free and also disease-free culture systems. It is also necessary that besides technological considerations, environmental and socio-economic considerations according to laid-out plans and policies, and effected through discussion and dialogue among all the stakeholders of the aquaculture ventures, should take place, sufficiently early as a part of pre-siting / siting exercise. This should involve also the various sectoral interests in the coastal zone so that aquaculture development would blend in harmony with the other concomitant sectoral developments in the coastal zone, as is envisaged in an integrated plan for coastal area development (ICAM). While integration at the biological/technological level such as by adoption of polyculture through new experimentation and trials would usher in more sustainability, intersectoral integration needs paramount consideration as well with involvement of the local populace. Experience and expertise in the countries in the region can be pooled together in this context through specialized development agencies such as FAO and NACA to adapt and evolve more eco-friendly and sustainable mariculture/coastal aquaculture systems.

In developing eco-friendly coastal aquaculture systems built into an integrated coastal management plan for India CMFRI should take the leadership in integrating coastal aquaculture and mariculture with activities within the fisheries sector itself and also with the other sectors such as forestry, tourism and other interests (FAO, 1995; 1997a). There are some very novel and refreshing developments as detailed above, but still the tasks are many in finding ways for developing these and sharing of experiences of other Asian countries with similar concerns, as pointed out already, will be very helpful.

References

ACIAR-MOFI. 1999. Mixed shrimp farming – mangrove forestry models in the Mekong

Delta – Termination Report, part A – Main Report and Executive Summary. ACIAR-MOFI Projects FIS/94/12 Australia Institute of Marine Science, RIA No. 2, Vietnam, NACA, Bangkok. 33p.

Ahlgren, M.O. 1998. Consumption and assimilation of salmon net-pen fouling debris by the red sea cucumber *Parastichopus californicus* : Implication for polyculture. *Journal of the World Aquaculture Society.* **29** : 133–139.

Avanimelach, Y. 1998. Minimal discharge from intensive fish ponds. *World Aquaculture* 29(1): 32–37.

Barg, U.C. 1992. Guidelines for the promotion of environmental management of coastal aquaculture development (based on a review of selected experiences and concepts). FAO *Fisheries Technical Paper* No. **328**, FAO, Rome, 122p.

Brown, J.J. and E.P. Glenn 1999. Management of saline aquaculture effluent through the production of halophytic crops. *World Aquaculture*, **30(4)**: 44-49.

FAO, 1995. Code of conduct for responsible Fisheries FAO, Rome, 41p.

FAO, 1997a. Aquaculture Development – FAO *Technical Guidelines for responsible Fisheries* No. **5**, Rome, 40p.

FAO, 1997b. Method of establishing coastal aquaculture zones in Sri Lanka. Based on the work of C.L. Angell, *Field Document* No. **1**, TCP/SRL/6712(A), FAO, Bangkok.

FAO, 1998a. Aquaculture Production Statistics 1987–1996. FAO *Fisheries Circular* No. **815**, *Rev.***10**, FAO, Rome, 197p.

FAO, 1998b. Coastal Aquaculture zoning in Sri Lanka, Technical Co-operation Programme, Aquaculture Development. Based on the work of Charles L. Angell, FAO, Bangkok.

FAO, 1999. Aquaculutre Production Statistics 1988–1997. FAO *Fisheries Circular* No. **815**, *Rev* **11** Rome.

FAO/NACA, 1995. Regional study and workshop on environmental assessment and management of aquaculture development (TCP/RAS/2253). NACA Environment and Aquaculture development Series No. 1. 492p. NACA, Bangkok.

FAO/NACA, 1996. Regional study and workshop on the taxonomy, ecology and processing of economically important seaweeds. NACA Environment and Aquaculture Development Seris No.3. 341p. NACA, Bangkok.

Garibaldi, L. 1996. List of animal species used in aquaculture. *FAO Fisheries Circular No.* 914 FIRI/C914. FAO, Rome.

Gavine, F & M.J.Phillips. 1994. Review of integrated farming of shrimp. NACA, Bangkok.

Goyard, E., J. Patrois, J.M. Peignon, V. Vanaa, R. Dufour, and E. Bedier. 1999. IFREMER' shrimp genetics program. *The Advocate.* **2(6)**:26-28.

Kapetsky, J.M. 1987. Satellite remote sensing to locate and inventory small water bodies for fisheries management and aquaculture development in Zimbabwe. *CIFA Occas. Pap.* 14 : 11P.

Kapetsky., J.M. L. Mcgregor, H. Nanne 1987. A geographical information system and remote sensing to plan for aquaculture development : FAO UNEP / GRID Co-operative Study in Costa Rica. FAO *Fisheries Technical Paper* 287 : 5/p.

Kongkeo, H. 1997. Intensive shrimp farming systems in Indonesia, Philippines, Taiwan and Thailand. *Aquaculture Research,* **29**: 789 - 796.

Kutty, M.N. 1995. Aquaculture development and sustainability in Southeast Asia, pp.91-108. In: Bagarino, T.U. & Flores, E.C.C. (eds.) *Towards Sustainable Aquaculture in Southeast Asia and Japan.* SEAFDEC Aquaculture Department, Iloilo, Philippines.

Kutty, M.N. 1997. What ails aquaculture? *Aquaculture Asia,* **2**: 8-10.

Kutty, M.N. 1998. Lessons in shrimp culture: an appraisal of problems in shrimp culture raised in the article, "Social impacts of shrimp farming in Nellore district, India". *Aquaculture Asia,* 3: 16-18.

Kutty, M.N. 1999a. Aquaculture development in India from a global perspective. *Current Science,* **76(3)**: 333-341.

Kutty, M.N. 1999b. Aquaculture development and sustainable environment, p. 1-25. In: Sukumaran N.(ed.) *Sustainable Environment.* M.S.University Sri

Paramakalyani Centre for Environment Sciences, Alwarkurichi, Tamil Nadu.

NACA/FAO, 2000. *The Bangkok Declaration on Aquaculture.* Prepared at the NACA/FAO Conference, *Aquaculture in the third Millennium*, Bangkok, Thailand, 25 February 2000.

New, M.B. 1999. Sustainable shrimp farming in the tropics : third generation farming technology. *World Aquaculture.* **30(4)**: 34–43.

Nunes, A.J.P. and G.J. Parsons. 1998. Dynamics of tropical coastal aquaculture systems and the consequences to waste production. *World Aquaculture.* 29(2) : 27–37.

Phillips, M.J. 1995. In: Bagarino, T.U. & Flores, E.C.C. (eds.) *Towards Sustainable Aquaculture in Southeast Asia and Japan.* SEAFDEC Aquaculture Department, Iloilo, Philippines. pp 37-62

Primavera, J.H. 1994. *Infofish international.* **1/94**: 44-49.

Taw, N. 1994. Guide on the farming of seaweed *Gracelaria* species. Seaweed Production Development Project, PHI/89/004. *Field Document.* 17. Department of Aquaculture, BFAR and FAO/UNDP, Manila, Philippines.

Tookwinas, S., S. Dirakkait, W. Prompoj, C.E. Boyd, and R. Shaw. 2000. Thailand develops code of conduct for shrimp farming. *Aquaculture Asia*, **5(1)**: 25–28.

Travaglia, C, J.M. Kapetsky and G. Profeti. 1999. Inventory and monitoring of shrimp farms in Sri Lanka by ERS SAR data. *Environmental and Natural Resources Working Paper* No. 1, FAO, Rome, 34p.

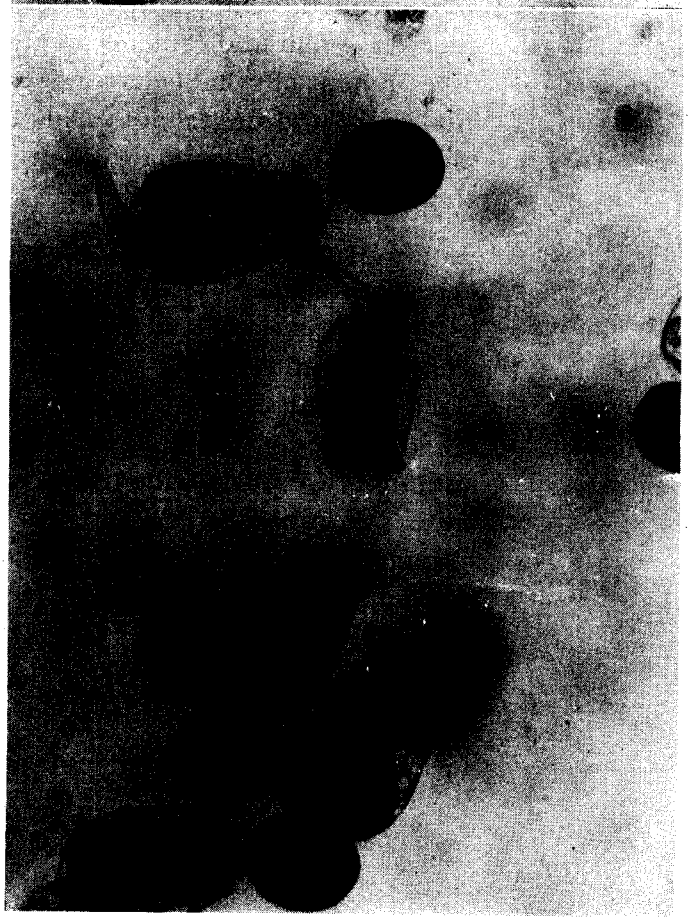
SCI. 1996. Judgement of the Supreme Court of India in the case related to Aquaculture, December, 1996, 44p.

Wyban J.A. and J.N. Sweeney. 1989. Intensive grow-out trials in a circular pond, *Aquaculture*, **75**:215-216.

Rotifer as live feed for larviculture of marine fishes - a research review

G. Gopakumar[1] and
V. Jayaprakas[2]
[1]*VRC of C.M.F.R.I., Vizhinjam, Trivandrum – 695 521*

[2]*Department of Aquatic Biology and Fisheries, University of Kerala, Trivandrum– 695 007*



ABSTRACT

The most critical factor in the commercial farming of fish and shellfish is the dependable availability of healthy fry produced in hatcheries. Most of the fishes having aquaculture potential have larvae with very limited yolk reserves and the transition stage from endogenous to exogenous feeding is very critical, often resulting in mass mortalities. The recent success in the consistent supply of many marine and brackish water fish seeds can be attributed to the mass production technologies of high quality live feeds. Research has resulted in the worldwide use of the brackish water rotifers Brachionus plicatilis *and* B.

rotundiformis *as successful diets for the developing larvae. This paper reviews the present status of rotifer research on the different aspects of rotifer culture such as strain selection, mass culture techniques, nutritional quality of rotifer feed and the general requirements for mass culture namely dissolved oxygen, pH, temperature, salinity and unionized ammonia. The salient aspects of rotifer biology relevant to the culture of rotifers and the role of rotifers in the ecosystem are reviewed. The recent research findings on monitoring, harvesting, storage, enrichment, feeding strategies and problems associated with rotifer culture are also reviewed in this paper.*

Introduction

In the last few decades, considerable progress has been achieved in the industrial farming of several species of fish and shellfish. It is well established that the most critical factor in the commercial farming of fish and shellfish is the dependable availability of healthy fry produced in hatcheries. Inconsistent supply of seed was the major constraint for the development of aquaculture of many marine and brackishwater fish and shellfish. One of the major reasons for the improvements in the success of fish and crustacean culture in the 1970s and 1980s was the development of reliable hatchery techniques for the mass production of quality fry and fingerlings. This breakthrough was achieved mainly by the production of adequate quantities of high quality live feeds (Sorgeloos and Leger, 1992)

Most marine fish with aquaculture potential have larvae with limited yolk reserves, small mouths and primitive digestive systems. Nutrition at this early larval stage is very critical and live food production techniques and feeding strategies need to be developed before commercial level production. Over the past few decades adequate feeding strategies have been developed for several fish and crustacean larvae, resulting in the world-wide use of various species of microalgae (approximate size range 50-200μm), the rotifers *Brachionus plicatilis* and *B. rotundiformis* (50-200μm) and the brineshrimp *Artemia* (420-8000μm) (Lubzens, 1987; Lubzens *et al.*, 1989; Dhert and Sorgeloos, 1994; Lavens *et al.*, 1995).

Rotifers

Rotifers are aquatic microscopic invertebrates comprising about 2000 species of unsegmented, bilaterally symmetrical pseudocoelomates. They are commonly referred to as 'wheel animalcules' as their disc like anterior end (corona) bears resemblance to a pair of revolving wheels due to the synchronized beating of their coronal cilia. The rotifers show worldwide distribution and the majority of them inhabit freshwater and some genera also occur in brackishwater and marine habitats. They exhibit fascinating strategies of reproduction, population dynamics, spatial and vertical distribution and survival. Many species are notable for their ecotypic and cyclomorphic variations.

The length range of rotifers is generally 100-1000μm, although the largest species may surpass 2000μm. The body is elongated or saccate, sometimes cylindrical, worm like, or even spherical. The majority of rotifers in natural conditions are females. Males are definitely known for relatively few species; they are much smaller than females, degenerate and seldom live for more than two or three days. In general the body consists of three regions- the head including corona, body or trunk and foot. The head is not well delimited and carries several organs- the corona, the mouth opening and several sensory organs and appendages. The ciliated corona serves as locomotory and food collecting organ. The foot extends from the body ventrally or more commonly terminally.

Internal organization

Rotifers possess a spacious pseudocoelom and muscles, nerves, digestive, reproductive and protonephridian organs are found within this cavity. Respiratory and circulatory systems are absent.

The muscular system consists of both smooth and striated muscles occurring in small bands of longitudinal and circular fibres inserted at various points on the integument or between the integument and the viscera. Contractions of these muscles cause a swift beat of appendages resulting in a rapid explosive movement ('jump'). The nervous system of rotifers is simple consisting of a large cerebral ganglion which is located dorsally below the corona. A paired protonephridial system, comprising of two parallel tubules and flame cells maintain the internal osmotic

pressure and remove toxic metabolites and nitrogenous wastes, mainly ammonia.

Gonads are paired in class Bdelloidea and class Seisonidea. In bdelloid males are entirely unknown and reproduction is always asexual. In the third class Monogononta, only one gonad is present. Males in this class have not been found for a large number of species, eventhough it is generally assumed that all the monogononts are capable of producing males under proper conditions. The appearance of males is usually limited to a few days or a week in one reproductive season and during the rest of the time reproduction is parthenogenetic. The reproductive organs of female rotifers consist of ovary, vitellarium and follicular layer (Amsellem and Ricci, 1982). At birth the total number of oocytes are already present in the ovary. Male rotifers are always smaller than females. Usually the digestive organs of males are rudimentary or entirely absent. The single testis is large and saccate with about 50 mature sperms floating freely within. A ciliated vas deferens leads from the testis to the penis, and one or rarely two prostate glands discharge into it. Rotifers are usually oviparous, they release their eggs outside the body where the embryo develop. Many planktonic rotifers carry their eggs attached to the mother by a thin thread (*Brachionus*, *Polyarthra*), others attach them to a substratum (*Asplanchnopus*, *Epiphanes*) or release them into the plankton (*Notholca*, *Ploesoma*).

The class Seisonidea reproduces exclusively bisexually, class Bdelloidea reproduces entirely by asexual parthenogenesis and class Monogononta reproduces by a mixture of these two extremes- cyclical parthenogenesis (Fig. 1). Parthenogenesis dominates the monogonont life cycle where reproduction occurs in the absence of males (amictic phase). Under certain environmental conditions (temperature, crowding, food quality and quantity changes) males may be produced and sexual reproduction takes place (mictic phase). Mictic and amictic females are morphologically indistinguishable. Amictic females are diploid and produce diploid (amictic) eggs. They develop mitotically into females (Birky and Gilbert, 1971; Gilbert, 1983). Sexual (mictic) reproduction can be initiated concurrently with amictic egg production in any season, in response to certain environmental factors which are poorly understood.

Environmental stimulus for mixis has been described for a few species of *Asplanchna*, *Brachionus* and *Notommata* (Gilbert, 1980; Pourriot and Snell, 1983; Snell and Boyer, 1988). Dietary tocopherol (Vitamin E) controls the shift from amictic to mictic reproduction in most *Asplanchana* species (Gilbert, 1977). Population density is widely attributed as a stimulus for mictic female production in *Brachionus* (Gilbert, 1977; Pourriot and Snell, 1983, Snell and Boyer, 1988; Carmona *et al.*, 1994). Genetic factors also play an important role in determining sensitivity of strains to mictic stimuli (Snell and Hoff, 1985; Lubzens *et al.*, 1985).

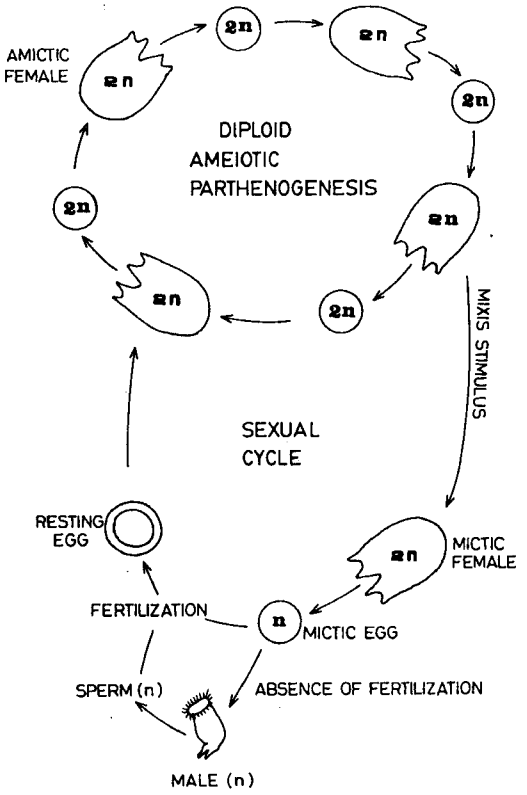


Fig. 1. Life Cycle of a Brachionid

When sufficient intensity of mictic stimulus has been received, amictic females begin producing mictic as well as amictic daughters. The proportion of mictic daughters and duration of their production depends on the strength of the mictic stimulus (Snell and Boyer, 1988). Mictic females produce male eggs which are smaller and more numerous than amictic eggs. A male must inseminate a mictic female within four hours of birth for fertilization (Snell and Childress, 1987). Fertilized mictic female produces a diapausing embryo called a resting egg. Mictic females produce haploid eggs through meiosis. If unfertilized these develop into haploid males. Males live only for 2-5 days because they do not feed (Snell, 1977, King and Miracle, 1980).

Resting eggs are diploid and possess thick, often sculptured walls that are characteristic of the species. This dormant stage is very resistant to harsh environmental conditions (Gilbert, 1974) and may be dispersed over wider areas by the wind, water and migrating animals like water birds. After a period of dormancy resting eggs respond to species specific environmental conditions and hatch releasing diploid amictic females that enter into the asexual phase of the life cycle. The stimuli that induce hatching include change in light, temperature and salinity (Pourriot and Snell, 1983). Formation of resting eggs promotes survival and dispersal and can be considered adaptive in unpredictable environments. Resting eggs have been known to hatch even after twenty years of dormancy (Nipkow, 1961). Because of this capacity for extended dormancy, resting eggs could accumulate in sediments. In the studies conducted at limited sites, the density of resting eggs ranged from 100 to 400 eggs/cm^2 (Snell *et al.*, 1983). These workers found that the highest densities occurred on the sediment surface. Appearance of floating resting eggs of *B. plicatilis* is also reported (Hagiwara, 1996).

Planktonic rotifers swim constantly. Swimming is accomplished with the aid of coronal cilia and may or may not be combined with the creation of feeding currents. Swimming speed is crucial in food acquisition and mate finding. Speed is temperature dependent and varies with the age of the planktonic species. All free swimming non-predatory and sessile species use the coronal cilia to create a water current passing by

the mouth where food selection may take place or all particles of an appropriate size class can be ingested non-selectively. *Brachionus* move small food particles into the buccal region through a process of filter or suspension feeding.

In many aquatic food webs, rotifers serve as important species indicating the extent of exposure to toxicants and quantifying toxic effects. Using rotifer models, toxicity is being investigated on several levels from communities to molecular basis. Changes in the structure of rotifer assemblages are being used as indicators of water quality.

The saline rotifers – *B. plicatilis* and *B. rotundiformis*

B. plicatilis, an euryhaline rotifer, is an important and essential food source in the early part of the commercial rearing of many marine fish and a few shrimp species. Two morphotypes – S (small) and L (large) are distinguished based on morphological and physiological differences (Fu *et al.*, 1991a,b; Rumengan *et al.*, 1991; Fu *et al.*, 1993). These strains could be selectively employed for feeding fish larvae depending on the mouth size of the larvae. The two strains coexist in wild stock, with one of the strains becoming dominant due to environmental conditions, particularly water temperature (Fukusho, 1989a,b). Recent studies on morphology, karyotype, genetics including allozyme constitution and reproductive behaviour of 'S' and 'L' type *B. plicatilis* showed that these types are best treated as different species. A reexamination of existing available names revealed *B. plicatilis* O.F. Muller 1786 and *B. rotundiformis* Tschugunoff 1921 as the correct names for the 'L' and 'S' type respectively (Segers, 1995, Gomez and Serra, 1995a,b; Hagiwara *et al.*, 1995; Natesan *et al.*, 1996). There are various geographical strains, cyclomorphic forms and ecotypes of these two types in the different habitats.

B. plicatilis can reproduce either by mictic or amictic reproduction. The mictic patterns of *B. plicatilis* was recently investigated by Gomez and Serra (1995b). Aquaculturists promote only amictic reproduction because the rate of amictic reproduction is faster than mictic reproduction; males which are only produced during mixis are inferior nutritionally due to the lack of a functional digestive system and the onset of mixis can cause culture collapse (Meragelman *et al.*', 1985). A

model evaluating the contribution of environmental factors to the production of resting eggs in *B. plicatilis* was developed by Lubzens *et al.* (1993). Pozuelo and Lubian (1993) reported that mixis is a strain dependent component of general reproduction response. However, some clones are known to be exclusively amictic (Meragelman *et al.*, 1985). Depending on conditions, an amictic female may produce 20 or more eggs during her seven to ten day of life time (Hoff and Snell, 1989). She carries all her eggs attached to the posterior portion of her body until they hatch. S and L type *B. plicatilis* have an identical pattern of fecundity (Hirayama and Rumengan, 1993). Some life history characteristics of *B. plicatilis* that were fed a variety of algal diets were investigated by Korstad *et al.* (1989a,b). Carmona *et al.* (1993) reported that mixis can be initiated in *B. plicatilis* by pre-conditioning of culture medium by crowding.

B. plicatilis and B. rotundiformis as live feed

B. plicatilis and *B. rotundiformis* cultures have now become an indispensable aspect of many marine finfish hatcheries. *B. plicatilis* and *B. rotundiformis* are excellent first feeds for fish larvae due to their small size, ability to be cultured at high densities, high reproductive rate with parthenogenetic mode of reproduction, slow swimming speed and habit of staying suspended in the water column, ability to tolerate wide range of salinities, ability to be easily enriched with fatty acids, antibiotics etc. and hence can be used to transfer these substances to the larvae. There are other rotifer species that possess some or all these characteristics, but *B. plicatilis* and *B. rotundiformis* are widely used in mariculture because they are able to thrive in a wide range of salinities.

Culture of B. plicatilis and B. rotundiformis

Takashi Ito (Ito 1955, 1957a,b, 1960) was the first to discover that *B. plicatilis* is an excellent food for the larvae of the marine fish *Placoglossus altivelis*. By 1970s *B. plicatilis* has become widely accepted as the best food in the early stages of larval rearing of marine fin fish. The method to produce the rotifer by feeding marine *Chlorella* was developed, but the quantity generated was not sufficient to meet the entire demand for feeding larvae and juveniles. In the 1970s baker's yeast was

introduced as a food organism for rotifers and the foundation of the current mass production system utilizing a combined feeding programme of marine microalgae and baker's yeast was established.

Now rotifers are mass-produced in hatcheries all over the world. Theilacker and Mc Master (1971) found that *B. plicatilis* was an excellent food source for larval anchovies (*Engraulis mordax*). Arnold and Holt (1991) while describing the various methods for the culture of *B. plicatilis* in Texas emphasized the importance of keeping culture containers and water clean, controlling contaminants such as ciliates and bacteria, harvesting daily to maintain the culture in growth phase and adding some algae daily. Orhun *et al.*, (1991) described a practical approach to high density production of *B. plicatilis* at California using two low cost tanks, partial automation of harvesting, water exchanges and waste removal. Hirayama and Satuito (1991) recommended that nutritional improvement of baker's yeast for growth of *B. plicatilis* can be done by rearing yeast in a medium rich in organic nutrients which can incorporate essential lipids into the cells. The selection of optimum phytoplankton species for rotifer culture during cold and warm seasons and their nutritional value for marine finfish larvae was investigated by Hur (1991). While suggesting improvements in the design of mass culture system of *B. plicatilis*, Snell (1991) recommended three thrust areas for research – (a) the identification of culture instability and sudden crashes (b) role of nitrogen excretion and unionized ammonia toxicity and (c) early detection of stress in mass cultures by monitoring reproductive traits, swimming ability and enzyme inhibition.

Strain selection : *B. plicatilis* is widely distributed in brackishwater ponds and lakes. There are lot of strain variations. The reproductive rate, size, optimum culture conditions and frequency of mixis vary among different strains (Lubzens *et al.*, 1989; Lubzens, 1987; Fukusho 1989a; Meragelman *et al.*, 1985; James and Abu Rezeq, 1989c; Fushimi, 1989; Mustahal *et al.*, 1991). In a culture system amictic reproductive rate should be maximized while frequency of mixis should be minimized. Size is an important factor because different target species and developmental stages within species have different optimum food sizes. Great care should be taken when selecting a strain to be cultured, since different strains may perform better at different temperatures and salinities, local conditions as well as those required by the target species.

Mass culture methods

A wide variety of culture systems have been employed which can be categorized into four basic methods viz., batch culture, semi-continuous culture, feedback culture and continuous culture (Lubzens, 1987).

Batch culture: In this method, all the rotifers are harvested when the density of rotifers reaches the desired level. It can be done in outdoor or indoor tanks. Fukusho (1989a) describes a typical Japanese hatchery using 100 m^3 tanks for rotifer culture and a diet of microalgae, usually *Nannochloropsis* and baker's yeast. After a growth period of a few weeks, rotifer densities of about 100/ml are reached and the mass culture is then harvested for several days. Six 100 m^3 tanks hold a standing crop of rotifers from which 1-2 billion can be harvested daily. Using this system, 600 m^3 of water must be managed to produce about one billion rotifers per day. Batch culture of rotifers is the most reliable method but also the least efficient in terms of labour and facilities needed to culture a given number of rotifers (Trotta, 1981; Fushimi, 1989).

Semi-continuous culture: Here, a given population is allowed to grow until it reaches certain population density. Then it is partially harvested and fresh medium is added. The growth and harvest procedures are repeated several times before the water quality mandates that the tank be drained and cleaned. This method can also be practiced in both indoor the outdoor tanks. According the Lubzens (1987) semi-continuous rotifer culture employs vessels ranging from a few hundred liters to 200,000 liters. Relatively high densities can be obtained in the smaller volume cultures. Build up of waste products like uneaten food and contamination are the problems in semi-continuous systems. This makes them less reliable than batch cultures.

Feedback culture: Accumulation of high levels of unused food and excretory products occurs in high density rotifer cultures. These are removed into a decomposer tank. The decomposed matter is employed as fertilizer for algal cultures, which are used to feed rotifer cultures (Hirata, 1979).

Continuous culture: These are delicately balanced systems in which the culture organisms are harvested continually and receive constant

nutrient replenishment. Continuous cultures are the most efficient ways to produce a consistent supply of high quality algae and rotifers. Since continuous culture apparatuses must be maintained under strictly defined conditions, they are always closed and indoors. This limits their size and may add to the cost of operations. The most advanced design of continuous culture is the chemostat which has been applied to aquaculture by James and Abu-Rezeq (1989a,b). In this approach, algae and yeast are supplied continuously at a predetermined rate. The culture is diluted by a certain volume each day and this volume is harvested to obtain rotifer biomass. Production from 1 m^3 chemostat is sufficient to meet the rotifer needs of most small and medium sized hatcheries. Chemostat mass cultures have yielded the greatest rotifer biomass production per unit of effort thus far recorded in aquaculture.

General requirements for culture

Nutritional quality and rotifer feeds : The type of feed used for culturing rotifers can have a significant effect on the cost of operations and on the nutritional value of rotifers (Carnic *et al.*, 1993). When choosing a feed one must consider both the requirements of the rotifer as well as the needs of the target species. *B. plicatilis* and *B. rotundiformis* have broad nutritional requirements. These animals ingest many types of feed including bacteria, so long as it is of appropriate particle size. The rotifers require Vitamin B_{12} (Yu *et al.*, 1989; Maruyama and Hirayama, 1993) and Vitamin A (Fukusho, 1989a). In contrast, the fish larvae require long chain 'highly unsaturated fatty acids' (HUFAs) on the n-3 series, mainly 18:3 n-3, 20:5 n-3 and 22:6 n-3 (Watanabe *et al.*, 1983a). Since many marine fishes cannot synthesize these fatty acids, feeds must contain high level HUFAs. The nutritional quality of rotifers is primarily determined by the type of feed given to the rotifers. Other factors to consider when selecting a feed type include the stability of culture required as well as the availability and cost of purchasing or producing the feed.

Many species of algae are used as rotifer diet according to availability under local conditions and the exact nutritional requirements of the rotifers and the target species. The most commonly used species are *Nannochloropsis oculata, Tetraselmis tetrathele, T. suecice, Isochrysis*

galbana and *Chlorella vulgaris* (Hirata, 1989; Hirayama *et al.*, 1989). Species high in n-3 HUFAs such as *Nannochloropsis* spp. are regarded as very good feeds. The main drawback in using phytoplankton is the huge amount of labour, time and facilities that must be developed for producing the large quantities needed to feed rotifers. Alternatively, marine yeast (*Candida* sp.), baker's yeast (*Saccharomyces cerevisiae*) and caked yeast (*Rhodotorula* sp.) have all been successfully used for rearing rotifers. But yeast has no nutritional value and they lack the much needed HUFAs (Walford and Lim, 1992). Problems encountered with the use of yeast include more frequent rotifer culture crashes and poor survival in target species that have high HUFA requirement (Fukusho, 1989a; Hirayama and Funamoto, 1981). The latter problem is solved by giving rotifers a mixed feed of algae and baker's yeast or by feeding rotifers with algae high in HUFAs for a few hours or days prior to harvest. The use of bacteria as feed for *B. plicatilis* is also investigated, which revealed that addition of Vitamin B_{12} producing bacteria can greatly enhance the growth of cultured *B. plicatilis* (Yu *et al.*, 1989). Photosynthetic bacteria along with baker's yeast have also been used to feed semi-continuously cultured rotifers (Fushimi, 1989; Gatesoupe *et al.*, 1989; Fukusho, 1989a). An overall better quality of rotifers cultivated with algae was noted than that cultivated with yeast and oil (Oie *et al.*, 1994). Relatively high reproductive rates were found in three strains of rotifers fed with frozen *Nannochloropsis* biomass (Lubzens *et al.*, 1995). The study revealed that application of frozen *Nannochloropsis* biomass may promote easier management in the production of lipid enriched rotifers. Attempts to culture *B. plicatilis* with microencapsulated diets were also made (Teshima *et al.*, 1981).

The amount of food supplied and the frequency of feeding can also affect the nutritional quality and growth rate of rotifers. Ingestion rates are correlated with the size of the particles offered and their concentration (Fukusho, 1989a). The absolute quantity of feed provided/rotifer/day is the most important parameter. The algal density measured in cells per ml will vary greatly with the cell size of the particular algae being used. As many as 20 million cells per ml of *N. oculata* (2μm) may be required at the start of the culture. Feeding rates are estimated to be 100,000 - 150,000 *N. oculata* cells / rotifer / day. The daily ration will

also depend on temperature (Nagata, 1989) and salinity (Lubzens, 1987). When temperature and algal concentration interacts, a great influence on filtration rate and marginal influence on ingestion rate of *B. plicatilis* was noted (Acosta Jimeno and Perez Enriques, 1995). Feeding frequency is an important factor affecting rotifer quality and growth rate (Meragelman *et al.*, 1985; Lubzens, 1987; Lubzens *et al.*, 1989). Lebedeva and Orienko (1995) found that *B. plicatilis* feeding rate was largely determined by temperature and food concentration. Most of the nutritional value of rotifers comes from their gut contents, partially digested and highly concentrated phytoplankton, yeast, bacteria etc, not from their own tissues. Hence if rotifers are deprived of food prior to harvest, their nutritional quality will be poor.

A recent breakthrough in production of an artificial diet (Culture Selco, Artemia Systems NV, Belgium) which completely replaces algae and at the same time eliminates the need of an extra enrichment period for enhancement of the rotifer's dietary value (Nyonje and Radull, 1991; Lavens *et al.*, 1995). This dry product needs to be suspended in water prior to feeding. Provided it is continuously aerated and cold stored, the food suspension of Culture Selco can be used in automatic feeding for as long as 48 hr. Under the standard feeding protocol developed a doubling time for different rotifer strains was generally obtained every three days. Under optimal conditions, doubling time of the population may be even expected every 24hr (Lavens *et al.*, 1994). Excellent results in using this diet as an alternative to the traditional mixture of algae and yeast were also obtained on a commercial scale in different bream and bass hatcheries (Komis *et al.*, 1991). This artificial diet gives constant and predictable levels of n-3 HUFA enrichment which cannot be reached by any other micro algal enrichment procedures. Rotifers grown on Culture Selco have constant levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of 6 mg per gram DW and 4 mg per gram DW respectively. Recent investigations on gilthead seabream larviculture (Mourente *et al.*, 1993) demonstrated that during first feeding the best growth rate was achieved with a diet of Culture Selco reared rotifers, which combined a high n-3 HUFA content with a high DHA : EPA ratio.

Dissolved Oxygen: The optimum Dissolved Oxygen (DO) required depends on temperature, food type and rotifer density. If microalgae are

used as sole feed, the amount of aeration that must be provided is lower than that if yeast is being used. This is because given sufficient light algae produce oxygen, while yeast and associated bacteria consume it. Fukusho (1989a) reported that at 20 °C both L and S type rotifers consume 7.07×10^{-5} ml oxygen/day. The rate increases to 10.04×10^{-5} ml/day at 25 °C and to 16.48×10^{-5} ml/day at 30 °C. Hirata and Yamasaki (1987) studied the relationship between oxygen consumption and food availability in *B. plicatilis* and found it to range from 1 – 7 ml/ind/hr. and was seen to increase with increased feeding. The relative swimming speed of *B. plicatilis* was decreased when the concentration of oxygen was very low. Rotifers spend more time in very low concentration of oxygen by slowing and by turning more (Reale *et al.*, 1993). Fushimi (1989) states that 60-100 liters of air/min/m^3 must be provided to the yeast fed rotifer cultures of rotifer density 1000 ind/ml. Blowers, air stones, airlift pumps and PVC piping into which holes have been drilled can be used for aeration.

Light: Rotifers are cultured indoors either with constant or part time illumination. Hoff and Snell (1989) recommended a light : dark cycle of 18 : 6 hours . According to Fukusho (1989a) for *B. plicatilis* the beneficial effect of light may be indirect by stimulating growth of photosynthetic bacteria and microalgae in the rearing tanks.

pH: *B. plicatilis* tolerates a wide range of pH (5-10), the optimum pH range for culture is reported to be 5-9 by Fukusho (1989a) and 7.5 to 8.5 by Hoff and Snell (1989). The optimum may vary depending on the type of feed (Furukawa and Hidaka, 1973). Yu and Hirayama (1986) stated that pH indirectly influences rotifer population by its effect on the amount of unionized ammonia in the culture water. Fushimi (1989) reports an example of rotifer mass culture in which the pH was maintained at 8.0 to 8.2 with hydrochloric acid and sodium hydroxide.

Temperature: The optimum temperature will depend on the strain being cultured (Snell and Carrillo, 1984, Fukusho, 1989a). Theilacker and Mc Master (1971) stated that maximum reproduction occurred between 30 and 34 °C. In general the recommended temperature is between 20 and 30 °C.

Salinity: *B. plicatilis* and *B. rotundiformis* are known for their wide range of salinity tolerance. According to Hoff and Snell (1989) salinities rang-

ing from 1-60 ppt may be tolerated by *B. plicatilis*, but 10-20ppt will give the best growth. Salinity may have a large effect on reproductive rate. Different strains and clones have different salinity optima (Lubzens, 1987). A culturist must also take into consideration the salinity at which the target species will be grown. For example, rotifers cultured at 20 ppt should be acclimatized for a day at 30 ppt before being fed to fish larvae in 40ppt seawater (Lubzens, 1987). Otherwise rotifers will be stressed and they stop swimming. Rotifer filtration rates may also vary with salinity and are reduced at high salinities. James and Abu Rezeq (1990) found that n-3 HUFA content in L-type *B. plicatilis* was highest for those cultured in 30 ppt sea water while 15-20 ppt water was correlated with higher n-3 HUFA content in S-type rotifers tested. Lorica lengths for both the strains were significantly greater at 5 ppt than at 30 ppt. Rapid shifts in salinity and temperature may result in immobilized, non-swimming rotifers. Only slight changes in mobility were observed when rotifers were exposed to changes in temperature from 20°C –30°C and to an increase in salinity from 20×10^{-3} to 30×10^{-3}. When salinity was reduced to 15×10^{-3} and 5×10^{-3} the proportion of mobile rotifers was considerably reduced (Oeie and Olsen, 1993).

Unionized ammonia: Hirata and Nagata (1982) showed that *B. plicatilis* raised on *N. oculata* excrete ammonia, urea and phosphates. Yu and Hirayama (1986) found that unionized ammonia levels can be one of the restrictive factors affecting increase of the rotifers in mass production. Hoff and Snell (1989) recommend that free ammonia concentration should not exceed 1 mg/litre.

Filtration of culture water: Debris that accumulates during high density culture of rotifers can be detrimental both to the health of the rotifers and to the larvae that feed on rotifers (Fushimi, 1989). Removing this debris can enhance water quality in the rotifer and larval rearing tanks and also reduces pathogenic bacteria clogging of nets during harvesting. Fushimi (1989) describes two general types of filtering equipment used in high density batch cultures. In one type a filter is inserted directly into tank – eg. filtering mats which are placed on bottom of the tanks and washed daily. The other consists of a separate filtering tank attached to the main culturing tank.

Monitoring: Parameters such as pH, D.O., temperature, salinity, food

density and ammonia concentration should be monitored and kept within pre-determined levels. Most culturists monitor their rotifers at least once a day. The usual method is to remove a fixed quantity of culture water and observe it under a microscope. The number of rotifers, their activity and the presence of any contaminants like protozoa are noted. Snell *et al.*, (1987) proposed two techniques for determining whether rotifers were under stress. The first is to test the swimming activity of a single rotifer in a 1 ml chamber. A grid with 1mm squares is placed under the chamber and the number of squares entered is recorded for 30 seconds. The second technique is to count the number of eggs carried by each female. Decreases in swimming activity and egg ratios indicated that the rotifers were being stressed. Korsted *et al.*(1995) used swimming speed and egg ratio as predictors of the status of rotifer cultures. Yufera *et al.*(1993) developed a mathematical model in order to obtain a reliable estimate of dry mass of *B. plicatilis* from two single easily determined parameters, the egg female ratio and the mean lorica length.

Harvesting: Harvesting is done by passing the culture water through fine nylon or silk netting. In Japan 80-100μm mesh size net is used to harvest L-type rotifer, 50-70μm mesh size for S-type rotifers. Mechanized means of harvesting are also being tested in Japan (Fushimi, 1989).

Storage: Storage is necessary for maintenance of stock cultures, short term preservation of live harvested rotifers and long term preservations of dead harvested rotifers. Stocks of rotifers could be stored at 3°C for a month without special attention which enabled the recovery of cultures when necessary (Ortega *et al.*, 1995). Alternatively, adult rotifers can be maintained at a low temperature which discourage rapid population growth (Coves *et al.*, 1990). The possibility of cryopreservation is also being studied (Lubzens, 1987); Lubzens *et al.*, 1989; Toledo and Kurokura, 1990; Lubzens *et al.*, 1992). The utility of frozen rotifers for feeding larvae is also investigated (Fontaine and Revera, 1980; Foscarini, 1988).

Enrichment of rotifers

After finding out the importance of n-3 HUFA in live feeds, various methods have been tried to improve the nutritional qualities of rotifers by feeding them with various kinds of microdiets, microencapsulated diets, a new type of baker's yeast and emulsified lipids rich in n-3 HUFA

together with fat soluble vitamins (Watanabe and Ali, 1985). Among them the direct method in which emulsified lipids are used and the indirect method using newly developed yeast are the most popular.

Indirect method : A new kind of yeast has been developed for rotifers to improve upon the nutritional value of rotifers cultured on baker's yeast (Imada *et al.*, 1979). This new type of yeast (n-yeast) was produced by adding fish oil or cuttlefish liver oil as a supplement to the culture medium of baker's yeast, resulting in a high content of lipid and n-3 HUFA. The incorporation of n-3 HUFA from n-yeast reached a maximum around 12 hour of feeding. Rotifers grown on n-yeast had a superior food value for larval fish (Kitajima *et al.*, 1980a, b).

Direct method : The rotifers can also be enriched by n-3 HUFA rich emulsions directly (Ostrowski and Divakaran, 1990). Here the lipids containing n-3 HUFA are homogenised with small amount of raw egg yolk and water and the resulting emulsion is fed directly to the rotifers (Watanabe and Ali, 1985). Rotifers took up lipids very easily and the concentration of n-3 HUFA reached the maximum between six and twelve hours of feeding. This is the easiest means of enriching rotifers, but it can cause clumping of the rotifers and degradation of the larval rearing tank water quality (Hoff and Snell, 1989). Rodriguez *et al.* (1996) investigated the improvement of nutritional value of rotifers by varying the type and concentration of oil and enrichment period. They obtained the highest lipid levels when triglycerols (TAG) were used. Increasing the enrichment period rather than the amount of oil present in the medium was found to be most efficient in increasing the n-3 HUFAs in rotifers. Nichols *et al.*(1996) studied the enrichment of *B. plicatilis* by feeding an Antarctic bacterium containing polyunsaturated fatty acids, The bacterial strain with the ability to produce EPA was shown to be a potential alternative enrichment food for *B. plicatilis*. A simple two step culture using *Chlorella vulgaris* and *Isochrysis galbana* for DHA enrichment was suggested by Takeyama *et al.*(1996).

Problems associated with rotifer culture

Prevention of a 'crash' or rapid production decrease is a vital aspect of rotifer culture. Culture crashes occur frequently in modern hatcheries due to water quality problems, accumulation of waste products and

unionized ammonia. Nutritional deficiencies such as lack of Vitamin B_{12}, other Vitamins or free amino acids (Fyhn, 1989) and toxins produced by bacteria are also attributed as probable causes of culture crashes. Fushimi (1989) reported that crashes are especially common in yeast – fed cultures. Fushimi (1989) suggested that declining water temperatures may sometimes be responsible for sudden population decreases in rotifers.

Feeding *B. plicatilis* to fish larvae

B. plicatilis was successfully employed for the mass raising of commercially important fishes like sole (*Solea solea*) (Howell, 1973), sea bass (*Dicentrarchus labrax*) (Barnabe, 1974), grey mullet (*Mugil cephalus*) (Nash *et al.*, 1974), red seabream (*Pagrus major*)(Fujita, 1979), milkfish (*Chanos chanos*)(Liao *et al.*, 1979; Juario *et al.*, 1984), turbot (*Scophthalmus maximus*) (Kuhlmann *et al.*, 1981; Olsen and Minck, 1983; Witt *et al.*, 1984) flounder (*Paralichthys olivaceus*) (Fukusho *et al.*, 1985) and gilthead seabream (*Sparus aurata*). Rotifers can be used as supplementary feed for penaeid mysids up to PL4 (Kongkeo, 1991). In China, *B. plicatilis* is employed for larval rearing of crabs namely *Charybdis japonica*, *Portunus trituberculatus* and *Scylla serrata* (Chen and Long, 1991).

Lubezens (1987) listed five requirements of *B. plicatilis* for optimization of growth and survival of fish larvae – (a) size (b) distribution and concentration of rotifers in the larval tanks (c) total amount available (d) digestibility and absorption and (e) nutritional quality. Since the size of the prey eaten is a function of larval mouth width, the *B. plicatilis* strain selected should be of the required size suitable for the mouth size of the target larvae. The number of rotifers required depends on the size of the rotifer strain and the duration it is supplied in the fish larval tanks. Generally rotifers are given to the larvae for seven to thirty days after exogenous feeding has begun. It is necessary to supply more rotifers than the fish will eat, the number will depend on the predatory ability of the larvae being cultured (Fukusho, 1989a, b). This is because the rotifers must be maintained at a density high enough to allow the fish to feed efficiently (Theilacker and Mc Master, 1971; Hoff and Snell, 1989). But high rotifer concentrations could cause the fish to ingest beyond the limit to assimilate (Lubzens *et al.* 1995). In turbot larvae (*Scopthalmus maximus*) it was found that the highest feeding rate was obtained with 10 rotifer ml^{-1} on 4^{th} and 5^{th} days (Olmedo *et al.*; 1995).

Disease control of microbial infections through live food in larviculture of fish and shellfish is also getting relevance today. Presently these infections are treated or prevented by dissolving high doses of broad spectrum antibiotics in the culture water. The major constraint of this method is the uncontrolled use of high quantities of expensive drugs and concern over their subsequent discharge into the environment and possible development of resistant bacteria (Brown, 1989). The oral delivery of the drug can be achieved through live feed. It has recently been demonstrated on a laboratory scale that live food enrichment technique through bioencapsulation in *Brachionus* and *Artemia* may be an excellent tool for the prophylactic and therapeutic treatment of larvae with drugs as well as vaccines (Verpract *et al.*, 1992).

The Indian scenario

The Indian scenario of rotifer research in general and of *B. plicatilis* and *B. rotundiformis* in particular is still in is its infancy. Almost the entire quantum of work done on rotifers in India is pertaining to taxonomy and ecology of freshwater rotifers, mainly from North India. Sharma (1991) gave a detailed resume of the present state of Indian work on rotifers. Sharma and Michael (1980) gave a synopsis of taxonomic studies on Indian rotifers. Our knowledge on freshwater rotifers from Kerala is mainly due to Nayar and Nair (1969) and Nair (1972). The information on brackishwater rotifers of Kerala are mainly due to the works on general brackishwater plankton ecology by Abdual Aziz (1978), Nair *et al.*, (1984), Nair *et al.*, (1985), Shibu (1991), Bijoy (1991), Harikrihsnan (1993), Bijoy and Abdul Aziz (1994), Anuradha (1996) and George (1996).

The only experimental studies done on rotifers in India are the investigations on the production of mictic and amictic females in *B. patulus* and the combined effect of food and temperature on life table parameters and population dynamics of *B. patulus* (Rao and Sarma, 1986; Sarma and Rao, 1991). The information on the culture of *B. plicatilis* from India is restricted to the reports by Muthu (1982), Santhanam and Velayudhan (1991) and Rafiuddin and Neelakantan (1990). Gopakumar (1998) made a detailed study on the brackishwater rotifers of Kerala with special reference of *B. plicatilis*. The ecological studies covered were the community structure and succession of rotifers in relation to environmental parameters, diel variation and distribution pattern of rotifers. The mor-

phometric characterization of five clones of *B. plicatilis* and size composition were reported. The experimental studies were on the reproductive potential of five strains of *B. plicatilis* under different salinities, feed types, feed concentrations and temperatures and on the life table parameters and patterns of growth and multiplication of two strains. Results of different culture methods and larval feeding experiments were also investigated in the study.

Discussion

It is quite evident that larviculture nutrition, particularly first feeding by the early larval stages is the major bottleneck for the industrial upscaling of aquaculture of fish and shellfish. Hence the feeding of larval fish still continues to be an active field of research of vital importance in all aquaculturally advanced nations. It is felt that paucity of information on the local strains of *B. plicatilis* and *B. rotundiformis* which are recognised as indispensable live feeds for marine larviculture is the major bottleneck in the progress of marine finfish hatchery production in India.

Strain variation of *B. plicatilis* and *B. rotundiformis* is one of the major parameters which require detailed investigations. The size as well as optimum conditions for best multiplication will vary in the different strains and it has a direct impact on the larval feeding. An extremely small strain (SS strain) has been isolated and its morphology and reproduction has been examined by aquaculturists.

The reproductive pattern of rotifers in relation to various external stimuli is an area of vital significance. If the factors inducing mictic reproduction are clearly identified, the mass production of resting eggs which can be stored in dry condition for ready use can be achieved. Experimental studies on reproductive potential under varying interacting parameters like salinity, temperature, feed type and feed concentration are crucial for the optimization of mass culture of different strains. The mass culture methods and conditions suited for mass production of different strains have to be evaluated. Investigations on nutritional quality and enrichment studies on cultured rotifers are of paramount significance for successful larviculture programmes. Finally the larval feeding strategies for the different species of fish and shellfish have to be standardized in terms of size of rotifers, concentration of rotifers in the

rearing tanks, nutritional quality and feeding protocols.

Improvements in increasing the quality, quantity and reliability of mass production of local strains of *B. plicatilis* and *B. rotundiformis*, understanding the causes of decline of populations, developing techniques for stable yield at low cost, nutritional improvement by enrichment procedures, manipulation of reproductive strategies, genetic improvement of the strains, cryopreservation and development of feeding protocols for the fish larvae of the different species will substantially enhance the hatchery production of seed of many commercially important fishes of India, having aquaculture potential.

References

Abdul Aziz, P.K. 1978. *Ecology of the retting grounds in the backwater systems of Kerala.* Ph.D. thesis, University of Kerala.

Acosta Jimenco, J. and R. Perez Enriquez. 1995. Feeding behaviour of the rotifer *B. plicatilis* related to temperature and microalgal concentration. *Rev. Invest. Mar.*, **16**: 23-28.

Amsellem, J. and C. Ricci. 1982. Fine structure of female genital apparatus of *Philodina* (Rotifera, Bdelloidea). *Zoomorphology*, **100**: 89-105.

Anuradha Rammohan. 1996. *Plankton ecology of Kadinamkulam lake-a backwater system of Southern Kerala Coast.* Ph.D. Thesis, Univ. Of Kerala, pp.273.

Arnold, C.R. and G.J. Holt. 1991. Various methods for the culture of the rotifer, *Brachionus plicatilis* in Texas. In: Fulk and Main (Eds.) *Rotifer and Microalgae culture systems-Proceedings of a U.S.-Asian Workshop. Honolulu, Hawaii*, 119-124.

Barnabe, G. 1974. Mass rearing of the bass *Dicentrarchus labrax* L. In: J.H.S. Blaxter (ed.). *The Early Life History of Fish.* Springer-Verlag, Berlin: 749-753.

Bijoy Nandan, S. 1991. *Effect of coconut husk retting on water quality and biota of an aquatic biotope in Kerala.* Ph.D. Thesis, Univ. of Kerala.

Bijoy Nandan, S., and P.K. Abdual Aziz. 1994. Zooplankton of the retting zones in Kadinamkulam estuary, Kerala. *Mahasagar*, **27**(1): 59-65.

Birky, C.W., Jr. and J.J. Gilbert. 1971. Parthenogenesis in rotifers: the control of sexuality and asexuality. *Am. Zool.* **11**: 245-266.

Brown, J. 1989. Antibiotics: their use and abuse in aquaculture. *World Aquaculture*, **20**(2): 34-43.

Carmona, M.J., M. Serra and M.R. Miracle. 1993. Relationships between mixis in *B. plicatilis* and pre-conditioning of culture medium by crowding. *Hydrobiologia*, **255/256**: 145-152.

Carmona, M.J., M. Serra and M.R. Miracle. 1994. Effect of population density and genotype on life history traits in the rotifer, *Brachionus plicatilis*. O.F. Muller. *J. Exp. Mar. Biol. Ecol.*, **182**(2): 223-235.

Carnic, M., J. Sanko-Nijire and B. Skaramuca. 1993. Dietary effects of different feeds on the biochemical composition of the rotifer *Brachionus plicatilis* Muller. *Aquaculture*, **110**(2): 141-150.

Chen, X.Q. and L.J. Long. 1991. Research and production of live feeds in China. In: Fulk and Main (Eds.). *Rotifer and Microalgae culture systems*-Proceedings of a U.S.-Asian Workshop. Honolulu, Hawaii, 186-200.

Coves, D., P. Audiredu and L.I. Nicolas. 1990. Rotifers-Rearing technology. In: G. Barnabe (Ed.) *Aquaculture*. Vol. 1. Ellis Horwood, New York. 232-245.

Dhert, P. and P. Sorgeloos. 1994. Live Feeds in Aquaculture. *Proceedings of the conference INFOFISH-AQUATECH 94 helt on 29-31 August in Sri Lanka.* 209-219.

Fontaine, C.T. and D.B. Revera. 1980. The mass culture of the rotifer, *Brachionus plicatilis* for use as foodstuff in aquaculture. *Proc. World. Maricult. Soc.*, **11**: 211-218.

Foscarini, R. 1988. A review: intensive farming procedure for seabream (*Pagrus major*) in Japan. *Aquaculture*, **72**: 191-246.

Fu, Y., K. Hirayama and Y. Natsukari. 1991a. Morphological differences between two types of the rotifer *Brachionus plicatilis* O.F. Muller. *J. Exp. Mar. Biol. Ecol.*, **151**: 29-41.

Fu, Y., K. Hirayama and Y. Natsukari. 1991b. Genetic divergence between S and L type strains of the rotifer *Brachionus plicatilis* O.F. Muller. *J. Exp. Mar. Biol. Ecol.*, **151**: 43-56.

Fu, Y., A. Hagiwara and K. Hirayama. 1993. Crossing between seven strains of the rotifer *Brachionus plicatilis*. *Nip. Sui. Gak.*, **59**: 2009-2016.

Fujita, S. 1979. Culture of red sea bream. *Pagrus major* and its food. *Spec. Publ. Europ. Maricult. Soc.*, **4**: 183-197.

Fukusho, K. 1989a. Biology and mass production of the Rotifer *Brachionus plicatilis*. *Int. J. Aq. Fish. Technol.*, **1**: 232-240.

Fukusho, K. 1989b. Biology and mass production of the rotifer *Brachionus plicatilis* (2). *Int. J. Aq. Fish. Technol.*, **1**: 292-299.

Fukusho, K., M. Okauchi, H. Tanaka and S.I. Wahyuni. 1985. Food value of rotifer *Brachionus plicatilis* cultured with *Tetraselmis tetrathele* for larvae of a flounder *Paralichthys olivaceus*. *Bull. Natl. Res. Inst. Aquacult.*, **7**: 29-36.

Furukawa, I. and K. Hidaka. 1973. Technical problems encountered in the mass culture of the rotifer using marine yeast as food organisms. *Bull. Plank. Soc. Jpn.* 20: 61-71.

Fushimi, T. 1989. Systematising large-scale culture methods. In: K. Fukusho and K. Hirayama (Eds.) *A Live Feed-the Rotifer Brachionus plicatilis*. Koseisha-Koseikaku, Tokyo. 118-134.

Fyhn, H.J. 1989. First feeding of marine fish larvae: are free aminoacids the source of energy ? *Aquaculture*, **80**: 11-120.

Gatesoupe, F.J., T. Arakawa and T. Watanabe. 1989. The effect of bacterial additives on the production rate and dietary value of rotifers as food for Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, **83**: 39-44.

George Thomas. 1996. *Comparative studies on the ecology of mangrove communities along the backwater system of Kerala*. Ph.D.Thesis, Univ. of Kerala.

Gilbert, J.J. 1974. Dormancy in rotifers. *Trans. Am. Micr. Soc.*, **93**: 490-513.

Gilbert, J.J. 1977. Mictic female production in monogonont rotifers. *Arch. Hydrobiol. Beih.*, **8**: 142-155.

Gilbert, J.J. 1980. Female polymorphisms and sexual reproduction in the rotifer *Asplanchna*: evolution of their relationship and control by dietary tocopherol. *Ann. Nat.*, **116**: 409-431.

Gilbert, J.J. 1983. Rotifera In: K.G. Adiyodi and R.G. Adiyodi (Eds.). *Reproductive biology of invertebrates* Vol. II: Spermatogenesis and Sperm function. John Wiley and Sons, Ltd. New York. 181-193.

Gomez, A. and M. Serra. 1995a. Behavioural reporductive isolation among sympatric strains of *Brachionus plicatilis* Muller, 1786: insights into the status of this taxonomic species. *Hydrobiologia*, **313/314**: 111-119.

Gomez, A. and M. Serra. 1995b. Mictic patters of the rotifer *Brachionus plicatilis* Muller in small ponds. *Hydrobiologia*, **313/314**: 365-371.

Gopakumar, G. 1998. *Studies on brackishwater rotifers of Kerala with specieal reference to Brachionus plicatilis O.F. Muller as live feed for Aquaculture.* Ph.D.Thesis, Univ. of Kerala.

Hagiwara, A. 1996. Appearance of floating resting eggs in the rotifers *B. plicatilis* and *B. rotundiformis*. *Bull. Fac. Fish. Nagasaki. Univ.*, **77**: 111-115.

Hagiwara, A., Jung, Min-Min, T. Sato and K. Hirayama. 1995. Interspecific relations between marine rotifer *Brachionus rotundiformis* and zooplankton species contaminating in the rotifer mass culture tanks. *Fish. Sci.*, **61**(4): 623-627.

Harikrishnan, K. 1993. *Zooplankton ecology of certain aquatic biotypes in Kerala.* Ph.D.thesis, Univ. of Kerala.

Hirata, H. 1979. Rotifer culture in Japan. *Spec. Publ. Eur. Maricult. Soc.*, **4**: 361-375.

Hirata, H. 1989. Feed types and method of feeding. In: K. Fukusho and K. Kitajima (Eds.) *A Live feed-the rotifer, Brachionus plicatilis.* Koseisha-Koseikaku, Tokyo. 73-86.

Hirata, H. and W. Nagata. 1982. Excretion rates and excreted compounds of the rotifer *Brachionus plicatilis* O.F.Muller in culture. *Mem. Fac. Fish., Kagoshima. Univ.*, **31**: 161-174.

Hirata, H. and S. Yamasaki. 1987. Effect of feeding on the respiration rate of the rotifer *Brachionus plicatilis*. *Hydrobiologia*, **147**: 282-288.

Hirayama, K. and H. Funamoto. 1981. Supplementary effect of several nutrients on nutritive deficiency of baker's yeast on population growth of the rotifer *Brachionus plicatilis*. *Bull. Jpn. Soc. Sci. Fish.*, **49**(4): 505-510.

Hirayama, K. and C.G. Satuito. 1991. The nutritional improvement of baker's yeast on the growth of the rotifer *Brachionus plicatilis*. In: Fulk and Main (Eds.) *Rotifer and Microalgae culture systems*-Proceedings of a U.S.-Asian Workshop, Honolulu, Hawaii, 151-162.

Hirayama, K. and I.F.M. Rumengan. 1993. The fecundity patterns of S and L type rotifers of *B. plicatilis*. *Hydrobiologia*, **255/256**: 153-157.

Hirayama, K., I. Maruyama and T. Maeda. 1989. Nutritional effect of freshwater *Chlorella* on growth of the rotifer *Brachionus plicatilis*. *Hydrobiologia*, **186/187**: 39-42.

Hoff, H. and T. W. Snell. 1989. Plankton Culture Manual. 2nd Edition Florida Aqua Farms, Florida. 126.

Howell, B.R. 1973. Marine fish culture in Britain. VIII. A marine rotifer *Brachionus plicatilis* Muller, and the larvae of the mussel *Mytilus edulis L.*, as foods for larval flat fish. *J. cons. Inst. Explor. Mer.*, **35**: 1-6.

Hur, Sung Bum. 1991. The selection of optimum phytoplankton species for rotifer culture during cold and warm seasons and their nutritional value for marine fish larvae. In: Fulk and Main (Eds.) *Rotifer and Microalgae culture systems*-Proceedings of a U.S.-Asian Workshop, Honolulu, Hawaii, 163-173.

Imada, O., Y. Kageyamma, T. Watanabe, C. Kitajima, S. Fujita and Y. Yonge. 1979. Development of a new yeast as a culture medium for living feeds used in the production of fish seed. *Bull. Jpn. Soc. Fish.*, **45**: 955-959.

Ito, T. 1955. Studies on the "*mizukawari*" in eel-culture ponds. I. The feeding activity of *Brachionus plicatilis* on phytoplankton (as a cause of "*mizukawari*"). *Report of Faculty of Fisheries*, **2**(1): 162-167.

Ito, T. 1957a. Chlorine contents and plankton fauna of eel culture ponds on the West Coast of Ise Bay. *Report of Faculty of Fisheries*, **2**(3): 473-501.

Ito, T. 1957b. Studies on the "*mizukawari*" in eel-culture ponds. VI. The relations between the growth of *Brachionus plicatilis* and the quantity of phytoplankton. *Report of Faculty of Fisheries*, **2**(3): 502-516.

Ito, T. 1960. On the culture of mixohaline rotifer *Brachionus plicatilis* O.F. Muller in the sea water. *Report of Faculty of Fisheries*, **3**(3): 708-739.

James, C.M. and T. Abu Rezeq. 1989a. An intensive chemostat culture system for the production of rotifers for aquaculture. *Aquaculture*, **81**: 291-301.

James, C.M. and T. Abu Rezeq. 1989b. Production and nutritional quality of two small-size strains of the rotifer *Brachionus plicatilis* Muller as a basis for mass production. In: G. Shelf and C.J. Soeder (Eds.) *Algae biomass*. Elsevier/North-Holland Biomedical Press, New York. 307-313.

James, C.M. and T. Abu Rezeq. 1990. Efficiency of rotifer chemostats in relation to salinity regimes for producing rotifers for aquaculture. *J. Aqua. Trop.*, **5**: 103-116.

Juario, J.V., M.N. Duray, V.M. Duray, J.F. Nacario and J.M.E. Almendras. 1984. Induced breeding and larval rearing experiments with milkfish *Chanos chanos* (Forskal) in the Phillippines. *Aquaculture*, **36**: 61-70.

King, C.E. and M.R. Miracle. 1980. A perspective on aging in rotifers. *Hydrobiologia*, **73**: 13-19.

Kitajima, C., T. Arakawa, S. Fujita, O. Omada, T. Watanabe and Y. Yonge. 1980a. Dietary value for red seabream with a new type of yeast. *Bull. Jpn. Soc. Sci. Fish.*, **46**: 43-46.

Kitajima, C., M. Yoshida and T. Watanabe. 1980b. Dietary value for ayu *Plecoglossus altevelis* of rotifer *Brachionus plicatilis*, cultured with baker's yeast, *Saccharomyces cerevisiae* supplemented with cuttle fish liver oil. *Bull. Jpn. Soc. Sci. fish.*, **46**: 47-50.

Komis, A., P. Candreva, V. Franicavic, V. Moreau, E. Van Baller, P. Leger and P. Sorgeloos. 1991. Successful application of new combined culture and enrichment diet for the mass cultivation of the rotifer *Brachionus plicatilis* at commercial hatching scale in Monaco, Yugoslavia, France and Thailand. In: *Larvi'91-Fish and Crustacean Larviculture Symposium* (P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier) (Eds.), European Aquaculture Society, Special Publication, 15, Gent, Belgium.

Kongkeo, H. 1991. An overview of live feeds production system design in Thailand. In: Fulk and Main (Eds.) *Rotifer and Microalgae culture systems*-Proceedings of a U.S.-Asian Workshop, Honolulu, Hawaii, 175-186.

Korstad, J., A. Neyts, T. Danielsen, I. Overrein and Y. Olsen. 1995. Use of swimming speed and egg ratio as predictors of the status of rotifer cultures in aquaculture. *Hydrobiologia*, **313/314**: 395-398.

Korstad, 1989b. Life history characteristics of *Brachionus plicatilis* (rotifera) fed different algae. *Hydrobiologia*, **186/187**: 43-50.

Korstad, J., O. Vadstein and Y. Olsen. 1989a. Feeding kinetics of *Brachionus plicatilis* fed *Isochrysis galbana. Hyrobiologia*, **186/187**: 51-57.

Kuhlmann, D., G. Quantz and U. Witt. 1981. Rearing of turbot larvae (*Scophthalmus maximus* L.) on cultured food organisms and post metamorphosis growth on natural and artificial food. *Aquaculture*, **23**: 183-196.

Lavens, P., P. Sorgeloos, P. Dhert and B. Devresse. 1995. Larval foods. In: N.R. Bromage and J.R. Roberts (eds.) *Broodstock management and egg and larval quality*, 373-379.

Lavens, P., P. Dhert, G. Merchie, M. Stael, and P. Sorgeloos. 1994. A standard procedure for the mass production of an artificial diet for rotifers with a high nutritional quality for marine fish larvae. *Proceedings of the Third Asian Fisheries Forum, Singapore.* Asian Fisheries Society. Phillippines (in press).

Lebedeva, L.I. and O.N. Orienko. 1995. Feeding rate of *B. plicatilis* O.F. Muller on two types of food depending on ambient temperature and salinity. *Int. rev. Gesamt. Hydrobiol.*, **80**(1): 77.

Liao, I.C., J.V. Juario, S. Kumagai, H. Nakajima, M. Natividad and P. Buri. 1979. On the induced spawning and larval rearing of the milkfish *Chanos chanos* (Forskal). *Aquaculture* **18**: 75-93.

Lubzens, E. 1987. Raising rotifers for use in aquaculture. *Hydrobiologia*, **147**: 245-255.

Lubzens, E., G. Minkoff and S. Maron. 1985. Salinity dependence of sexual and asexual reproduction in the rotifer *Brachionus plicatilis*. *Mar. Biol.*, **85**: 123-126.

Lubzens, E.. A. Tandler and G. Minkoff. 1989. Rotifers as food for aquaculture. *Hydrobiologia*, **186/187**: 387-400.

Lubzens, E., S. Rothbard and A. Hidani. 1992. Cryopreservation as a tool in aquaculture. *Isr. J. Aqucult. Bamidgets*, **44**(4): 137.

Lubzens, E., Y. Wex, G. Minkoff and F. Adler. 1993. A model evaluating the contribution of environmental factors to the production of resting eggs in the rotifer *B. plicatilis*. *Hydrobiologia*, **255/256**: 127-138.

Lubzens, E., D. Rankevich, G. Kolodny, O. Gibson, A. Cohen and M. Khayat. 1995. Physiological adaptations in the survival of rotifers (*B. plicatilis* O. F. Muller) at low temperatures. *Hydrobiologia*, **313/314**: 175-183.

Lubzens, E., O. Gibson, O. Zmora and A. Sukenik. 1995. Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture. *Aquaculture*, **133**: 295-309.

Maruyama, I. and K. Hirayama. 1993. The culture of the rotifer *Brachionus plicatilis* with *Chlorella vulgaris* containing vitamin B_{12} in its cells. *J. World. Aquacult. Soc.*, **24**(2): 194-198.

Meragelam, E., E. Lubzens and G. Minkoff. 1985. A modular system for Small-scale mass production of the rotifer, *Brachionus plicatilis*. *Israel Journal of Zoology*, **33** : 186-194.

Mourente, G., A. Rodriguez, D.R. Tocher and J.R. Sargent. 1993. Effects of dietary docosa hexaenoic acid (DHA: 22: 6n-3) on lipid and fatty acid composition and growth in gilthead seabream (*Sparus aurata* L.) larvae during first feeding. *Aquaculture*, **112**: 79-98.

Mustahal., S. Yamasaki and H. Hirata. 1991. Salinity adaptability of five dif-

ferent strains of the rotifer *Brachionus plicatilis*. *Bull. Jap. Soc. Sci. Fish.*, **57**(11): 1997-2000.

Muthu, M.S. 1982. Methods of culturing zooplankton. *C.M.F.R.I. Spe. Pub.*, **8**: 119-125.

Nagata, W.D. 1989. Nitrogen flow through a *Brachionus/Chlorella* mass culture system. *Hydrobiologia*, **186/187**: 401-408.

Nair, N.B., M. Arunachalam, P.K. Abdual Aziz, K. Krishnakumar and K. Dharmaraj. 1985. Ecology of Indian estuaries: Distribution and seasonal variation of zooplankton in the Ashtamudi estuary. *Proc. Natn. Acad. Sci. India*, **55B**: 272-289.

Nair, K.K.N. 1972. Sessile rotifers of Kerala. *J. Ker. Acad. Biol.*, **3**(2): 30-36.

Nair, N.B., K. Krishnakumar, M.Arunachalam. P.K. Abdual Aziz and Dharmaraj. 1984. Ecology of Indian estuaies-Studies on the zooplankton ecology of Kadinamkulam backwater. *Proc. Indian. Acad. Sci. (Anim. Sci.)*, **93**(6): 573-584.

Nash, C., C.M. Kuro and S.C. McConnel. 1974. Operational procedures for rearing larvae of grey mullet (*Mugil cephalus* L.). *Aquaculture*, **3**: 15-24.

Natesan Munuswamy, A. Hagiwara, Gopal Murugan, K. Hirayama and H. Dumont. 1996. Structural difference between the resting eggs of *B. plicatilis* and *B. rotundiformis* (Rotifera, Brachionidae): an electron microscopic study. *Hydrobiologia*, **318**: 219-223.

Nayar, C.K.G. and K.K.N. Nair. 1969. A collection of brachionid rotifers from Kerala. *Proc. Indian. Acad. Sci.*, **69B**: 223-233.

Nichols, O.S., P. Hart, P.D. Nichols and T.A. McMeekin. 1996. Enrichment of the rotifer *B. plicatilis* fed an Antartic bacterium containing polyunsaturated fatty acids. *Aqaculture*, **147**(1-2): 115-125.

Nipkow, F. 1961. Die Radertiere in plankton des zurichsee und ihre Entwicklungsphasen-Schweiz Z. *Hydrol.* **23**: 398-461.

Nyonje, B. and J. Radull. 1991. The effects of feeding freshwater *Chlorella*, baker's yeast and Culture Selco on the culture of rotifers (*Brachionus* sp.) In: P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier (Eds.) *Larvi-'91 Spec. Publ. Eur. Aquacult. Soc.*, **15**: 106-109.

Ostrowski, A.C. and S. Divakaran. 1990. Survival and bioconversion of n-3 fatty acids during early development of dolphin fish (*Coryphena hippurus*) larvae fed oil enriched rotifers. *Aquaculture*, **89**: 273-285.

Oeie, G. and Y. Olsen. 1993. Influence of rapid changes in salinity and temperature on the mobility of the rotifer *Brachionus plicatilis*. *Hydrobiologia*, **255/256**: 81-86.

Oie, G., K.I. Reitan and Y. Olsen. 1994. Comparison of rotifer culture quality with yeast plus oil and algal based cultivation diets. *Aquacult. Int.*, **2**(4): 225-238.

Olmedo, M., B. Alvarez-Blaquez and C. Gomez. 1995. Effect of rotifer (*Brachionus plicatilis*) density on the first feeding of turbot larvae (*Scophthalmus maximus* L.) In: Pittman, R.S. Batty, J. Verreth (Eds.) *Mass rearing of juvenile fish, selected papers from a symposium held in Bergen, 21-23 June 1993.* 202.

Olsen, J.O. and F. Minck. 1983.. A technical solution to the mass culturing of larval turbot. *Aqacult. Eng.*, **2**: 1-12.

Orhun Refik, M., S.R. Johnson, D.B. Kent and R.F. Ford. 1991. Practical approach to the high density production of the Rotifer *Brachionus plicatilis*. In: Fulk and Main (Eds.*) Rotifer and Microalgae culture systems*-Proceedings of a U.S.-Asian Workshop, Honolulu, Hawaii. 73-78.

Ortega, A., A. Estevez., F. Linares and D. Carnero. 1995. Changes in the chemical composition of starved rotifers (*Brachionus plicatilis*) kept at ambient and low temperatures for extensive periods. In: Pittman, K., R.S. Betty and J. Verreth (Eds.) *Mass rearing of juvenile fish*-selected papers from a symposium held in Bergen 21-23 June 1993. 112-118.

Pourriot, R. and T.W. Snell. 1983. Resting eggs in rotifers. *Hydrobiologia*, **104**: 213-214.

Pozuelo, M. and L.M. Lubian. 1993. Asexual and sexual reproduction in the rotifer *Brachionus plicatilis* cultured at different salinities. *Hydrobiologia*, **255/256**: 139-143.

Rafiuddin, A.S. and K. Neelakantan. 1990. Production of rotifer, *Brachionus plicatilis* Muller fed with different cell densities of microalgae *Chlorogibba trochisciaeformis* Gieter. *Proc. Indian. Acad. Sci. (Anim. Sci.)*, **99**(6): 519-523.

Rao, T.R. and S.S.S. Sarma. 1986. Demographic parameters of *Brachionus patulus* Muller (Rotifera) exposed to sublethal DDT concentrations at low and high food level. *Hydrobiologia*, **139**: 193-200.

Reale, D., P. Clement and A. Esparcia-Collado. 1993. Influence of the concentration of oxygen on the swimming path *Brachionus plicatilis* O.F. Muller. *Aquaculture*, **40**: 103-108.

Rumengan, I.M.F., H. Kayano and K. Hirayama. 1991. Karyotype of S and L type rotifers *Brachionus plicatilis* O.F. Muller. *J. Exp. Mar. Biol. Ecol.*, **154**: 171-176.

Santanam, R. and P. Velayudhan. 1991. Mass culture of zooplankton for coastal aquaculture. *Fish. Chimes*, **11**(3): 39-40.

Sarma, S.S.S. and T.R. Rao. 1991. The combined effects of food and temperature on the life history parameters of *Brachionus patulus* Muller in relation to food and temperature. *Proc. Indian. Acad. Sci.* (Anim. Sci.) **99**: 335-343.

Segers, H. 1995. Nomenclatural consequences of some recent studies on *Brachionus plicatilis* (Rotifera, Brachionidae). *Hydrobiologia*, **313/314**: 121-122.

Sharma, B.K. 1991. Rotifera In: *Animal Resources of India (Protozoa to Mammalia) State of Art.* Zoological Survey of India, 69-88.

Sharma, B.K. and R.G. Michael. 1980. Synopsis of taxonomic studies on Indian Rotatorica. Hydrobiologia, 73(1-3): 229-236.

Shibu, S. 1991. *Ecology of Paravur lake.* Ph.D. thesis, Univ. of Kerala.

Snell, T.W. 1977. Life span of male rotifers. *Arch. Hydrobiol. Beiheft.*, **8**: 65-66.

Snell, T.W., B.E. Burke and S.D. Messur. 1983. Size and distribution of resting eggs in a natural population of the rotifer *Brachionus plicatilis*. Gulf Research Reports, **7**: 285-288.

Snell, T.W. and K. Carrillo. 1984. Body size variation among strains of the rotifer *Brachionus plicatilis*. *Aquaculture.*, **37**: 359-367.

Snell, T.W. and F.H. Hoff. 1985. The effect of environmental factors on resting egg production in the rotifer *Brachionus plicatilis*. *J. world. Mariculture*, **16**: 484-497.

Snell, T.W. and M.J. Childress. 1987. Aging and loss of fertility in male and female *Brachionus plicatilis* (Rotifera). *Int. J. Invertebrate. Repr. Dev.*, **12**: 103-110.

Snell, T.W., M.J. Childress and E.M. Boyer. 1987. Assessing the status of rotifer mass cultures. *J. World. Aquacult. Soc.*, **18**: 270-277.

Snell, T.W. and E.M. Boyer. 1988. Threshold for mictic female production in the rotifer *Brachionus plicatilis*. *J. Exp. Mar. Biol. Ecol.*, **124**: 73-85.

Snell, T.W. 1991. Improving the design of mass culture systems for the rotifer, *Brachionus plicatilis* In: W. Fulks and K.L. Main (Eds.) *Rotifer and microalgae culture systems. Proceedings of a U.S.-Asia Workshop, Honolulu,* 61-71.

Sorgeloos, P. and P. Leger. 1992. Improved larviculture outputs of marine fish, shrimp and prawn. *J. World. Aqu. Soci.*, **23**: 251-264.

Takeyama, H., K. Iwamoto, S. Hata, H. Takano and T. Matsunaga. 1996. DHA enrichment of rotifers: A simple two step culture using the unicellular algae *Chlorella regularis* and *Isochrysis galbana*. *J. Mar. Biotechnol.*, **3**(4): 244-247.

Teshima, S., A. Kanazawa and M. Sakamoto. 1981. Attempts to culture the rotifers with microencapsulated diets. *Bull. Jap. Soc. Sci. Fish.*, **47**(12): 1575-1578.

Theilacker, G.H. and M.F. McMaster. 1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. *Mar. Biol.*, **10**: 183-188.

Toledo, J.D. and H. Kurokura. 1990. Cryopreservation of the euryhaline rotifer *Brachionus plicatilis* embryos. *Aquaculture*, **91**: 385-394.

Trotta. P. 1981. A simple and inexpensive system for continuous monoxenic mass culture of marine miroalgae. *Aquaculture*, **22**: 383-387.

Verpract, R., M. Chair., P. Leger, H. Nelis, P. Sorgeloos and A.P. De Leenheer.1992. Live food mediated drug delivery as tool for disease treatment in larviculture-the encrihment therapeutics in rotifers and *Artemia* nauplii. *Aquaculture Engineering*, **11**: 133-139.

Walford, J. and T.J. Lim. 1992. High density production of rotifers (*Brachionus plicatilis*) using baker's yeast (*Saccharomyces cerevisiae*) and their n-3 highly unsaturated fatty acid content. *J. Aquacult. Trop.*, **7**(2): 287-30.

Watanabe, T., C. Kitajima and S. Fujita. 1983a. Nutritional values of live organisms used in Japan for mass propagation of fish: A review. *Aquaculture*, **34**: 115-143.

Watanabe, T. and Ali, Syed Ahmad. 1985. Nutritional quality of live food organisms and their enrichment. *C.M.F.R.I. Spe. Publ.*, **43**: pp.26.

Witt, U., G. Quantz and D. Kuhlmann. 1984. Survival and growth of turbot larvae *Scophthalmus maximus* L. reared on different food organisms with special regard to long chain polyunsaturated fatty acids. *Aquacult. Eng.*, 177-190.

Yu, J.P., A. Hino, R. Hirano and K. Hirayama. 1989. Function of bacteria as vitamin B_{12} producers during mass culture of the rotifer *Brachionus plicatilis*. *Nippon Suisan Gakkaishi*, **55**(10): 1799-1806.

Yu. J.P. and K. Hirayama. 1986. The effect of unionised ammonia on the population growth of the rotifer in mass culture. *Bull. Jpn. Soc. Sci. Fish.*, **52**(9): 1509-1513.

Yufera, M., E. Pascual, and J. Guinea, 1993. Factors influencing the biomass of rotifer *Brachionus plicatilis* in culture. *Hydrobiologia*, **255/256**: 159-164.

Brackish water shrimp monoculture: a low input-based ecofriendly approach

R.K. Mohanty and P.N. Patnaik[1]

Water Technology Centre for Eastern Region, Bhubaneswar-16

[1]Orissa State Fisheries

ABSTRACT

Confined brackishwater shrimp farming is a low input-based modified extensive system in the small scale aquaculture sector using rain-fed seasonal ponds (without water exchange and drainage) which almost eliminates the risk of environmental problems. Three confined brackishwater ponds in the Binchanapalli area of Chilka lagoon were used for monoculture of Penaeus monodon *for three consecutive years (1993-1996). The experiment was carried out by drying, ploughing, liming @ 250kg/ha, manuring with RCD @ 50.0t/ha, fertilization (SSP &Urea, 1:1@ 100kg/ha and daily feeding with snail meat (75%) and GOC (25%) @ 25.0%-6.0% of biomass. For all ponds, in 1st, 2nd, and 3rd year, the stocking density was 15625, 20625 and 25625 pcs/ha respectively. The highest gross and net productivity (1283.19) kg and 1274.07 kg/ha/yr) were recorded in pond-2 at a stocking density of 25625 pcs/ha. Growth variation at different stages and from pond to pond were highly significant statistically (P<0.01). Water and soil quality towards the end of each crop was not much degraded as compared to semi-intensive culture. Sediment and waste matter production at the pond bottom was much less being 0.46 cm(avg) in dry condition (46m^3/ha). The culture practice is simple and easy with higher benefit: cost ratio (11.59 at 18% DF).*

Introduction

The "confined pond" culture of penaeid prawns through a simple and low cost technology for easy adoption, by the small/weaker section farmers is a unique pond culture system having no arragements for water exchange during the entire culture period. Monoculture of *Penaeus monodon* in confined brackish water ponds became sucessful for the first time in Orissa, India, in the Chilka lagoon area during 1984 (Monanty, 1985). Under this system, simple rain-fed dugout ponds are well prepared with liming and fertilization before onset of monsoon rains. When the rainwater accumulates, the saline soil bed of the pond influences the freshwater to become brackishwater and the desired level of salinity automatically maintained due to leaching and recharging action of salt present in the soil. Since there is no facilities for water exchange, feeder channel and sluices are not required. Generally low stocking density and application of organic and inorganic manures in lesser quantity are followed. Feeding and manuring are carried out carefully keeping the water volume in mind. Confined ponds are generally shallow and maintained about 120cm depth of water to facilitate raising of two crops per year.

Material and methods

Three brackishwater rainfed confined ponds of 0.16-0.18 ha size, in the Binchanapalli area of Chilka lagoon were used for mono culture of *Penaeus monodon* for three consecutive years (1993-96). The experiment was carried out by drying ploughing, liming @250kg/ha, manuring with RCD@ 5.0t/ha, fertilization (SSP and urea, 1:1) @ 100kg/ha and daily feeding with snail meat (75%) and GOC (25%) @ 25.0-6.0% of biomass. For all the ponds in the 1st, 2nd and 3rd year, the stocking density was 15625, 20625 and 25625 pcs/ha respectively. Periodical water and soil sample analysis was done using standard methods (ALPHA, 1989 and Biswas, 1993). Weekly growth estimation was carried out by sampling prior to feeding. The length-weight relationship and growth estimation was carried out by sampling prior to feeding. The length- weight relationship and growth performance were statistically (ANOVA) analysed.

Results and discussion

Pond no. 1, 2 and 3 were harvested after 100 and 98 days in respect of first and second crops every year, during 1993-96. The gross and net yield, survival rate and specific growth rate of *P.monodon* under different stocking densities are presented in Table 1. Highest average gross and net yield of 641.597 kg/ha/crop and 637.036 kg/ha/crop respectively from pond no.2 under stocking density of 15,625 pcs/ha were obtained. Gross and net yield per hactare per year under three different densities ranged from 508.682 kg and 503.340 kg to 1283.194 kg and 1274.072 kg respectively among three ponds. Pond no.2 demonstrated the highest average gross productivity (557.473 kg/ha/crop) for three densities. By increasing the density from 15,625 to 20,625 pcs/ha and from 20,625 to 25,625 pcs/ha the corresponding average net yield gain for three ponds were 25.81% and 13.5% respectively. Growth rate of *P.monodon* at different growth stages and stocking densities were highly significant (ANOVA, Table.2).

Table 1. Results of monoculture of *Penaeus monodon* in confined brackishwater ponds at Binchanapalli.

Culture results	Pond number, crop number water area and culture period					
	Pond 1 (0.16 ha)		Pond 2 (0.18 ha)		Pond 3 (0.17 ha)	
	1st crop Aug.-Nov.	2nd Crop Dec.-Mar	1st crop Aug.-Nov.	2nd Crop Dec.-Mar.	1st crop Aug.-Nov.	2ndCrop Dec.Mar.
1993-94						
1. Culture 2 duration (days)	100	98	100	98	100	98
2. Average water depth (m)	1.06	0.86	1.12	0.88	1.10	0.83
3. Number stocked	2500	2500	2813	2813	2656	2656
4. Stocking density (No/ha)	15625	15625	15625	15625	15625	15625
5. Average size at stocking TL (mm)	29.27	28.08	29.32	30.26	29.28	29.19
BW (g)	0.189	0.165	0.176	0.230	0.171	0.171
6. Initial stocked biomass (kg)	0.472	0.412	0.495	0.647	0.454	0.454
7. Final harvested biomass (kg)	70.220	60.481	88.790	74.296	47.090	39.386
8. Harvested number	1972	1946	2374	2261	2237	2061

9. Average individual weight (g)	35.61	31.08	37.40	32.86	21.05	19.11
10. Average individual total length (mm)	164.18	157.84	167.22	156.80	142.52	138.11
11. Retrieval (%)	78.88	77.84	84.39	80.37	84.22	77.59
12. Sex ratio (M : F)	1:1.172	1:1.285	1:0.878	1:1.200	1:1.214	1:0.853
13. Grass yield (kg/ha/crop)	438.925	378.006	493.277	412.755	277.000	231.682
14. Net yield (kg/ha/crop)	435.925	375.431	490.527	409.161	274.329	229.011
15. Gross yield per year (kg/ha)	816.881		906.032		508.682	
16. Specific growth rate (SGR)(%)	35.421	31.546	37.224	33.296	20.879	19.325
17. Monthly average increment of body length (mm/30 days)	40.47	39.72	41.37	38.74	33.97	33.34
18. Average count (No/kg)	28	32	27	31	48	53

1994-95

1. Culture 2 duration (days)	100	98	100	98	100	98
2. Average water depth (m)	1.08	0.78	1.12	0.86	1.07	0.80
3. Number stocked	4100	4100	4613	4613	4356	4356
4. Stocking density (No/ha)	25625	25625	25625	25625	25625	25625
5. Average size at stocking TL (mm)	29.11	28.89	29.63	28.21	28.40	28.42
6. Initial stocked biomass (kg)	0.668	0.701	0.895	0.747	0.727	0.719
7. Final harvested biomass (kg)	96.880	90.880	121.785	109.190	64.967	59.425
8. Harvested number	3166	3106	3686	3630	3389	3346
9. Average individual weight (g)	30.60	29.26	33.04	30.08	19.17	17.76
10. Average individual total length (mm)	156.68	152.68	160.62	154.60	137.44	132.40
11. Retrieval (%)	77.22	75.75	79.90	78.69	77.80	76.81
12. Sex ratio (M : F)	1:1.104	1:1.109	1:1.113	1:1.119	1:1.103	1:0.988
13. Grass yield (kg/ha/crop)	605.500	568.000	676.583	606.611	382.159	349.500

14.Net yield (kg/ha/crop)	501.325	563.618	671.611	602.461	377.882	345.329
15.Gross yield per year (kg/ha)	1173.500		1283.194		731.739	
16.Specific growth rate (SGR)(%)	30.44	29.68	32.84	30.53	19.00	17.95
17.Monthly average increment of body length (mm/30 days)	38.57	37.89	39.30	38.69	32.71	31.83
18.Average count (No/kg)	33	34	30	33	52	56

1995-96.

1. Culture 2 duration (days)	100	98	100	98	100	98
2. Average water depth (m)	1.11	0.83	1.13	0.89	1.06	0.82
3. Number stocked	3300	3300	3713	3713	3506	3506
4. Stocking density (No/ha)	20625	20625	20625	20625	20625	20625
5. Average size at stocking TL (mm)	28.10	29.61	29.66	29.18	29.33	28.31
BW (g)	0.168	0.174	0.175	0.170	0.172	0.168
6. Initial stocked biomass (kg)	0.554	0.574	0.650	0.631	0.603	.0.589
7. Final harvested biomass (kg)	87.830	77.498	112.161	95.850	55.948	49.153
8. Harvested number	2614	2556	3133	3080	2796	2609
9. Average individual weight (g)	33.60	30.32	35.80	31.12	20.01	18.84
10.Average individual total length (mm)	160.34	154.68	162.80	155.37	140.30	132.06
11.Retrieval (%)	79.21	77.45	84.35	82.95	79.75	74.41
12.Sex ratio (M : F)	1:1.061	1:1.113	1:1.251	1:0.982	1:1.114	1:0.889
13.Grass yield (kg/ha/crop)	548.937	484.362	623.116	532.500	329.106	289.135
14.Net yield (kg/ha/crop)	545.475	480.775	619.505	528.994	325.560	285.670
15.Gross yield per year (kg/ha)	1033.299		1155.616		618.241	

16.Specific growth rate (SGR)(%)	33.43	30.76	35.62	31.58	19.84	19.05
17.Monthly average increment of body length (mm/30 days)	39.66	38.28	39.63	38.63	33.29	31.76
18.Average count (No/kg)	30	33	28	32	50	53

Note : TL = Total body length; BW - Body weight, M = Male, F = Female

Table 2. ANOVA of growth rates of *Penaeus monodon* at different growth stages.

Source of variation	S.S.	D.F	M.S.	Fcal
Between duration of rearing	0.475	6	0.0791	12.1692 ($P < 0.01$)
Between ponds	0.615	2	0.3075	46.3076 ($P < 0.01$)
Error	0.0784	12	0.0065	--
Total	1.1684	20	--	--

Water and soil quality (Tables 3,4) towards the end of each crop was not much degraded as compared to semi-intensive culture. Sediment and waste matter production at the pond bottom was much less being 0.46 cm (avg) in dry condition (46 m^3/ha). Correlation of prawn yield and certain observed soil quality variables (pH, organic carbon, available nitrogen and soil salinity) were statistically significant ($P<0.05$) during the experiment (Table-5).

All the three ponds had consistently alkaline water showing pH variation from 7.0-8.6. Average monthly values of dissolved oxygen for three ponds were higher in winter months and showed a decreasing trend from February to April and it coincided with increase in water temperature. Monthly average values of inorganic phosphate showed more

or less bi-modal variation. The gross primary productivity values were high during October-November (803-1026 mgC/m^3/6-hour day) and low during December-February (268-308 mgC/m^3/6-h. day). The annual mean gross production was 565.90 mgC/m^3/6-h. day. There was no marked difference in gross production at surface and bottom, since the ponds were shallow. ANOVA of primary productivity showed that the variation between ponds was highly significant ($P<0.001$) and not between months. The benthic population showed seasonal changes and variation between ponds. The monthly average benthic biomass was 11.08, 16.72 and 4.03g/m^2 in pond no.1, 2 and 3 respectively.

Table 3. Seasonal variations in water quality of confined brackishwater ponds at Binchanapalli (averaged from two years observations)

Physico-chemical	Mean values ± SD		
Parameters	Monsoon	Winter	Post-winter
Air temperature (C^0)	33.94 ± 0.88	25.45 ± 2.54	29.67 ± 3.39
Water temperature (C^0)	34.51 ± 0.59	26.47 ± 2.62	29.33 ± 2.46
Turbidity (ppm Si O_2)	88.70 ± 3.94	86.83 ± 4.54	89.67 ± 3.43
pH	8.01 ± 0.08	8.00 ± 0.06	8.07 ± 0.16
Dissolved Oxygen (ppm)	6.64 ± 0.50	5.72 ± 0.29	4.59 ± 0.45
Salinity (ppt)	9.50 ± 0.47	14.31 ± 3.27	27.77 ± 2.88
Total alkalinity (ppm)	67.30 ± 6.51	88.03 ± 9.23	100.20 ± 2.05
Free Carbon Dioxide (ppm)	3.91 ± 1.19	5.84 ± 1.30	8.40 ± 1.82
Nitrate-nitrogen (ppm)	0.047 ± 0.013	0.064 ± 0.01	0.052 ± 0.009
Inorganic phosphate (ppm)	0.07 ± 0.022	0.076 ± 0.017	0.0615 ± 0.025

Table 4. Soil quality (Physico-chemical conditions) of confined brackishwater ponds used for culture of penaeid prawns at Binchanapalli in Ganjam district of Orissa during 1994-95 and 1995-96 (Values averaged for two years) ± S.D.

Sl. No.	Soil quality parameters	Month	Pond1	Pond2	Pond3	Average for 3ponds
1.	Physical composition					
	a) Sand (%)	June	59.78	54.98	54.30	56.35
		December	54.13	50.87	51.34	52.11
		May	54.88	52.83	51.38	53.03
		Av.	56.26 ± 2.50	52.89 ± 1.67	52.34 ± 1.38	53.83 ± 1.87
	b) Silt (%)	June	8.92	13.00	24.86	15.59
		December	9.45	14.01	21.24	14.90
		May	8.90	11.26	16.35	12.17
		Av	9.09 ± 0.25	12.76 ± 1.13	20.82 ± 3.48	14.22 ± 1.47
	c) Clay (%)	June	31.30	32.01	20.83	28.04
		December	36.41	35.12	27.42	32.98
		May	36.21	35.90	32.26	34.79
		Av	34.64 ± 2.36	34.34 ± 1.68	26.84 ± 4.68	31.93 ± 2.85
2.	pH	June	7.41	8.12	6.84	7.45
		December	7.64	8.23	6.64	7.43
		May	7.79	8.25	7.02	7.68
		Av	7.61 ± 0.15	8.20 ± 0.05	6.83 ± 0.15	7.52 ± 0.11
3.	Salinity (Nacl mg/ 100mg)	June	878.03	687.61	415.83	660.49
		December	873.11	685.59	399.37	652.69
		May	902.77	772.70	420.34	698.60
		Av	884.64 ± 12.91	715.30 ± 40.59	411.85 ± 9.01	670.59 ± 19.86
4.	Available nitrogen (Nmg/100g)	June	14.65	17.07	13.86	15.19
		December	16.00	16.88	13.47	15.44
		May	14.45	17.26	13.07	14.92
		Av	15.03 ± 0.68	17.07 ± 0.15	13.46 ± 0.32	15.18 ± 0.21

5.	Available phosphorus (P_2O_5 mg/100g)	June	2.76	2.89	1.38	2.67
		December	2.38	2.65	1.54	2.18
		May	2.57	3.28	1.90	2.58
		Av	2.57 ± 0.155	2.94 ± 0.259	1.60 ± 0.28	2.47 ± 0.21
6.	Organic carbon (%)	June	0.354	0.399	0.244	0.331
		December	0.291	0.382	0.186	0.286
		May	0.248	0.398	0.207	0.284
		Av	0.297±0.043	0.393±.007	0.212±0.023	0.300±0.021

Table 5. Correlation of prawn yield (X_1) and certain observed soil quality variables (X_2) of confined brackishwater pounds.

Sl. No.	Variation (X_2)	D.F.	r	P
1.	pH	11	0.64	< 0.05
2.	Organic carbon(%)	11	0.66	< 0.05
3.	Available phosphorus (mg P/100g)	11	0.33	Not significant
4.	Available nitrogen (mg N/100g)	11	0.69	< 0.01
5.	Soil salinity (mg Nacl/100g)	11	0.67	< 0.05

In this experiment, low organic and inorganic input and low stocking density was the major factor for non-degradation of water and soil quality. This also helps in avoiding water exchange and pond aeration to minimize input and operation cost and makes the culture practice more simple, flexible and eco-friendly. The economic evaluation of mono culture of *P.monodon* in confined brackish water ponds indicated high profitability, where B-C ratio was 11.59 at 18% DF. However, for sustainable development of this farming system avoidance of improper pond preparation, over stocking (>2 pcs/m^2), over manuring, over fertilization (NACA, 1994), over medication and over feeding (Clifford, 1992) are advisable and instead of maximum production maximum profit should be aimed at.

Acknowledgements

We warmly thank Dr.S.K.Mohanty (Ex.Joint Director of Fisheries and Head, World Bank Assisted Fish and Shrimp Culture Project, Orissa) and Prof.M.C.Dash (Chairman, Orissa Pollution Control Board) for reviewing the manuscript.

References.

APHA, 1989. *Standard methods for examination of water and waste water*, 17th edition, American Public Health Association Washington D.C. USA.

Biswas, K.P., 1993. *Fisheries Mannual.* Bhagabat Press and Publisher. Cuttack, India, 436p.

Clifford, H.C. 1992. Marine Shrimp Pond Management. In : Wyban.J.(Edt.) *Proceedings of the special session on shrimp farming.* World aquaculture Society, Baton Rough, LA, USA, 110-137.

Mohanty, S.K. 1985. Development of brackish water prawn farming in Orissa - *Status Report* BFDA (Puri-Ganjam), No-2 : 84p.

NACA, 1994. The Environmental Management of Coastal Aquaculture: An assessment of shrimp culture in southern Thailand. *Final Report*, submitted to the office of Environmental Policy and Planning. NACA, Bangkok, Thailand, 167p.

Studies on the management of biopond system in a semi-intensive shrimp farm

S.K. Mohanty and R.K. Mohanty[1]

At/Po. Sunhat; Dist. Balasore, Orissa-756002.
[1]Water Technology centre for Eastern Region, Bhubaneswar - 16.

ABSTRACT

Preliminary studies on biopond management in a 11.5 ha private semi-intensive shrimp farm at Chandipur coast of Orissa were undertaken during March-July, 1996 to treat discharge water (effluent) from 10 shrimp ponds having total water spread area of 6.8 ha, where stocking of P.monodon, *total feed consumption, days of culture, daily average water exchange and biomass production were 23 pcs/m^2, 33.33t, 124 days, 10.3% (10506m^3) and 24.29t respectively. The four units of biopond system with a total water area of 1.72 ha (25.3% of total culture area) were used for sedimentation of effluent from 6.8 ha shrimp ponds, culture of clams (1.2kg/m^2) mullets (2pcs/m^2) and* Gracilaria *spp. (1.4kg/m^2) and aeration by 4-one hp paddle wheel aerators to reduce pollutants and toxic level of effluent before it was released to the receiving water course. Operation of biopond could reduce the BOD, TSS, total nitrogen, total phosphorus, secchi disc transparency, nitrite, hydrogen sulphide and free ammonia from 7.3 ppm, to 2.25ppm, 162.0 ppm to 37.6ppm, 2.2ppm to 1.02ppm, 0.5ppm to 0.16ppm, 11cm to 48cm, 0.046 ppm to 0.002ppm, 0.25ppm to 0.01ppm and 0.12ppm to 0.002 ppm respectively. Secondary aquaculture production (mullet @ 1.37 t/ha,* Gracilaria *@ 8.43t/ha and clam @4.38/ha) in four months was the spin-off from biopond system.*

Introduction

The economic disastrous events that affected shrimp farms worldover including India during 1987-95 were mostly due to the discharge of untreated effluents from the farms where higher stocking densities, inappropriate feeding/feed use, improper farm design, faulty pond and water management, excessive use of ground water and unplanned farm development concentrating around small estuaries and sea coast were followed. Lack of consideration of the environmental compatibility of farming sites also contributed to ecological problems.

There are two major shrimp farm by-products that contribute to the degradation of water quality and coastal environment. They are: (i) nutrient-laden effluents (which can be a potential cause for nutrient enrichment and eutrophication in natural waters) and (ii) suspended solids containing high levels of organic matter (which may be a potential cause for sedimentation of water systems in the coastal area) which adversely affect the benthic ecosystem of the estuary and coastal waters (Anon, 1994). To resolve these problems in commercial shrimp farming, particularly to reduce the impact of farm effluent on the receiving waters, there is a definite need to develop an appropriate and cost-effective treatment system for shrimp pond effluents. Although such treatment can be split into physical, chemical and biological (Ghosh and Bal, 1995), employment of biological/bio-filtration method is considered to be cost-effective and simple and it is more so in Indian condition where hi-tech treatment system/plant would be a very costly proposition. The biological system needs to be widely tried/experimented in commercial shrimp farms in the country inorder to standardise the biopond management system including secondary aquaculture methods. The present work is a preliminary attempt in this direction.

Material and methods

A 11.5ha creek-based private semi-intensive shrimp farm outside

Coastal Regulation Zone (CRZ) at Chandipur coast of Orissa state was chosen for undertaking the preliminary studies on bio-pond management during March-July, 1996. The farm included 10 nos. growout ponds with total water spread area (WSA) of 6.8 ha and a 4-split reservoir with 2.8ha WSA (41% of pond WSA). Semi-intensive culture of *P.monodon* with average stocking density of 23Pcs/m^2 was carried out in the farm for an average 124 days of culture (DOC). Imported C.P. feed from Thailand (33.33t) was used in all 10 ponds. Average water exchange was 10.3% (10,506m^3). The source water from the tidal creek of river Budhabalanga was filled into the reservoir by booster pumps and was sedimented, chlorinated and dechlorinated before being used in the growout ponds. Four nos. of 1 H.P. paddle wheel aerators were used in each pond of average 0.68ha WSA to increase dissolved oxygen content in the pond water and to reduce sedimentation in the periphery of the pond bottom. Harvesting was done by draining to reduce organic and nutrient load in the waste/drainage water.

The biopond, basing on the available land area in the farm was devided into 4 sections/units having total water spread area (WSA) of 1.72 ha (25.3% of pond WSA). With 1.9 m designed depth of water column in the biopond, the effluent holding capacity was 32,680m^3, which was 3 times the discharge in 48 hours from the ponds. First (0.9ha), second (0.16ha), third (0.16ha) and fourth (0.5ha) sections/units of the biopond were used for sedimentation, clam (*Meretrix*) and mullet (*M.tade* and *L.macrolepis*) culture, seaweed (*Gracilaria*) culture and aeration using 4nos. of 1H.P. paddle wheel aerators respectively. Effluent from 10 growout ponds during water exchange and harvesting enters the sedimentation unit of the biopond. All the 4 sections/units were interconnected with baffles and screens (8mesh/inch). Mullets, clam and *Gracilaria* were stocked @ 2/m^2 (av.size 7.3g.), 190/m^2 (av.size 6.3g) and 1.4kg/m^2 respectively. Clam culture was undertaken using rafts with hanging coir ropes (Vedavyasa Rao, 1995). *Gracilaria* culture was done by using hanging coir ropes from fixed bamboo poles. Mullet and clam culture was

carried out without supplementary feeding and fertilization.

Waste water (effluent) samples from the drainage channel at the entry point into the sedimentation section of the biopond and the samples of treated effluent from the aeration section (4th unit), after 48 hours of entry of effluent into the biopond system, were regularly analysed thoughout the experiment (124 days). pH, salinity, dissolved oxygen and transparency of effluent were determined by using water pH meter (Checker-1, HANNA, USA), salinity-refractometer (S.10, ATAGO, JAPAN), D.O. meter (YSI-55, USA) and secchi disc respectively. Total alkalinity, BOD, COD, total nitrogen, total phosphorus, total suspended solids (TSS), ammonia, nitrite and hydrogen sulphide values were determined by using standard methods (Biswas, 1993; APHA, 1995).

Results and discussion

Physico-chemical parameters (mean value) of intake water filling reservoirs, waste water discharged from growout ponds during culture period, effluent before flowing into the biopnd from main drainage canal and the treated effluent in the aeration unit before being released to the receiving water course (Budhabalanga river) as recorded during the experimental period are presented in Table1. All parameters (except D.O. and pH) in the pond waste water showed increasing values over intake water. Mean values of total suspended solids (TSS), total nitrogen, total phosphorus, ammonia, B.O.D,C.O.D., nitrite and hydrogen sulphide in the treated effluent after aeration (before releasing into the receiving water course) showed significant decrease over untreated effluent by 76.8%,53.6%,68%,98%,69.2% 58.5% 95.6% and 96% respectively, whereas turbidity was decreased by more than 4 times and dissolved oxygen increased by 57.6% in the treated effluent. pH values showed fluctuation. Parameters of treated effluent were well within the standards prescribed by the Department of Agriculture and Cooperation (Ministry of Agriculture), Government of India in 1995 (currently followed by Aquaculture Authority).

Table 1. Physcio-Chemical parameters (Mean Values) of intake and discharge water of shrimp pond and biopond during March-July, 1996.

Parameter	Intake water in the reservoir	Discharge Water from grow out ponds	Untreated effluent at the entry point in the biopond	Treated effluent after aeration before release to the receiving Water course.	Standard for treated effluent prescribed by MOA,[1] GOI[2] in 1995 (now follwed by Aquaculture Authority)
pH	7.85	7.64	7.19	7.88	6-8.5
Turbidity/transparency(cm)	33.75	18.87	11.0	48.00	-
Temperature (°C)	28.10	28.50	30.34	30.62	-
Total Suspended Solids (mg/l)	142.4	176.3	162.0	37.6	100.0
Salinity (ppt)	27.8	29.05	30.32	30.18	-
Dissolved oxygen(mg/l)	5.88	3.58	3.28	5.17	>3.0
Total alkalinity (mg/l)	124.3	138.66	132.72	152.21	-
Total nitrogen (mg/l)	0.42	2.18	2.20	1.02	2.0
Total phosphorus (mg/l)	0.013	0.53	0.50	0.16	0.2
Free ammonia (mg/l)	<0.01	0.14	0.12	0.002	0.5
BOD (mg/l)	1.13	5.87	7.30	2.25	20.0
COD (mg/l)	29.73	76.83	88.1	36.52	75.0
Nitrite (mg/l)	<0.01	0.04	0.046	0.002	-
Hydrogen Sulphide (mg/l)	<0.01	0.21	0.25	0.01	-

1 = Ministry of Agriculture; 2 = Government of India.

Mullets (*M.tade and L. macrolepis*), clam (*Meretrix*) and sea weed (*Gracilaria*) were used for secondary aquaculture in the section II and III of the biopond. The results of secondary aquaculture (spin-off) are presented in Table 2. Mullets, clam and *Gracilaria* registered encouraging yield of 1.37t/ha, 4.38t/ha and 8.43t/ha respectively without additional input cost (except for the stocking materials) in 4 months culture duration, while effectively reducing the concentrations of pollutants by biofiltration and consumption of plankton in the nutrient-rich effluent.

Table 2. Results of secondary aquaculture in biopond

Biopond section/ unit and area(ha)	Species of filter organism/ plant used for aquaculture in biopond.	Size at Stocking (g)	Stocking density	Culture duration (days)	Survival (%)	Size at harvest (g)	Total biomass weight at harvest (kg)	Net biomass (kg)	Yield(t/ ha)
II	I) Mullet	7.3	2Pcs/m^2	124	57.4	132.6	243.5	220.14	1.37
(0.16 ha or									
1600m^2	ii) clam	6.3	190	124	63.3	13.6	2617.2	702	4.38
III			Pcs/m^2						
(0.16 ha or	Seaweed	-	1.4Kg/m^2	124	-	-	3590.0	1350	8.43
1600m^2)	(Gracilaria)								

Treatment of effluents may be required, particularly in intensive aquaculture system (Barg, 1992). In general, pollution potential of shrimp farm effluent is considerably less than that of domestic or industrial waste water (Anon, 1994; Ghosh and Bal, 1995). However, pollution problem often arises because of high volumetric flow rates of effluents, particularly when many shrimp farms are set up and operated in areas with limited water supplies or poor flushing capacity. In such case, semi-intensive and modified extensive shrimp farms do require cost effective and simple system of effluent treatment. Use of sedimentation ponds (Henderson and Bromage, 1988), off-bottom polyculture of bivalves and sea seeds (Ruying and Qingyin, 1992) or shrimp/oyster co-production systems (Wang, 1990) may prove very successful in reducing effects of waste loads from shrimp farm effluents. Kanit and Dusit (1992) found in the laboratory experiment that 340g of *Gracilaria*, 400g of green mussel in 300 liters of shrimp pond waste water reduced BOD level from 5.75 mg/l to 1.5mg/l and zero respectively in 48 hours, while 55% ammoniacal nitrogen was reduced. One gram of oyster (meat weight) could reduce suspended solids from 50-110mg/l to 12-15mg/l (Macintosh and Philips, 1992). An experimental waste water treatment system using biofilters (common mussels and *Gracilaria*) could help reduce the nutrient load of shrimp farm effluent in Malayasia (Enander Mans and Mans Hasselstorm, 1994). These studies in different parts of the world indicate that the biological treatment methods could be found potential for shrimp pond effluents.

Although semi-intensive and modified extensive shrimp culture registered a rapid growth in India in the recent past, cost effective effluent treatment systems in commerical units have not been tried systematically and widely for standardisation, except for using sedimentation ponds in stray cases. The present experiment on biopond management, while presenting useful data on biological treatment of shrimp pond effluent and secondary aquaculture, lend support to the findings and remarks of of the above mentioned authors. This work being preliminary in nature, suggests further studies to workout appropriate ratio of treatment area and growout WSA in relation to specific culture system, right species of finfish, shellfish and sea weeds and standardisation of the process for closed system of water management to make shrimp aquaculture environment-friendly.

Acknowledgement

The authors wish to record their sincere thanks to Dr. M.C. Dash, Chairman, Orissa Pollution Control Board, Bhubaneswar (Ex-Professor of Ecology, Sambalpur University) for his keen interest during the course of the investigation and for critically reviewing the manuscript.

References

Anon, 1994. How to compete with a deteriorated environment in shrimp culture. *C.P.Shrimp* News., 2(6):1

Anon, 1994. The environmental management of coastal aquaculture : An assement of shrimp culture in south Thailand (Final Report),167 pp. *NACA. Bangkok.*

APHA, 1995. Standard Methods for Examination of Water and Waste water(19th.ed.). *American Public Health Association, Inc.*, Washington, DC.

Barg, U.C. 1992. Guidelines for the promotion of environmental management of coastal aquaculture development., *FAO Fish Tech. Pap.* No. **328,** 122pp.

Biswas, K.P. 1993. Fisheries Manual, *Bhagabat Press and Publisher,* Cuttack Orissa (India) 423pp.

Enander Mans and Mans Hasselstorm. 1994. An experimental waste water treatment system for a shrimp farm. *INFOFISH* Int., 4/94:56-61.

Ghosh, T.K. and A.S. Bal. 1995. Enviornmental problem associated with semi intensive shrimp farming and control measures. Souv. *INDAQUA* 95: 91-97. *Marine Products Export Development Authority*, Kochi, (India).

Henderson, J.P. and N.R.Bromage. 1988. Optimising the removal of suspended solids from aqucultural effluents in settlement lakes. *Aquacult. Eng.*, **7**:167-81.

Kanti, C. and T. Dusit. 1992. Use of mussel and *Gracilaria* to treat the effluent water from intesive shrimp pond . *National Institute for Coastal Aquculture*, Songkhla, Deptt. Fish. MOA (Thailand)., Tech. Pap.,(**5**):26PP.

Macintosh, D.J. and M.J. Philips. 1992. Environmental considerations in shrimp farming. *INFOFISH* Int. **6** (92) : 38-92.

Ruying , S. and W. Qingying. 1992. *Laminaria* culture in china. *INFOFISH* Int., 1/92 : 40-43.

Vedavyasa Rao, P. 1995. Research and development for commercial production of marine shell fish through aquaculture in India. *Souv. INDAQUA 95: 49-57. Marine Products Export Development Authority.* Kochi (India).

Wang, J.K.V. 1990 Managing shrimp pond water to reduce discharge problem.*Aquacult Eng.*, **9:** 61-73.

Studies on some biological aspects of *Crassostrea gryphoides* (Schlotheim) at Bhatya creek in Ratnagiri, Maharashtra

M. S. Sawant and
A. M. Ranade
College of Fisheries, Konkan Krishi Vidyapeeth,
Ratnagiri - 415 629

ABSTRACT

The Bhatya creek harbours the oysters Crassostrea gryphoides, C. madrasensis *and* C. rivularis *all of which are abundant over an area of approximately 20 ha, intermittently. Attempt was done to collect the information on* Crassostrea gryphoides *(Schlotheim 1813) the maximum available species there at Bhatya creek. Studies were carried out on determination of sex ratio, seasonal gonadal changes and spawning behaviour, condition factor and percentage edibility. It was found that, among the oysters sex of which could be identified, males formed 36.13 %, females 56.75 % and indeterminants 7.12% on annual average basis. This gave the male: female ratio as 1 : 1.56.Studies on the seasonal gonadal changes and spawning, indicated that during summer almost 80 to 90 % of the oysters were with well developed gonads, in monsoon majority were in spent / resting stage and in winter the gonads were in active and ripe conditions. The observations on the percentage edibility and index of condition revealed that the values were high from January to May 1996 and June 1995, with a considerable fall from September 1995 to November 1995 which correlate with the spawning cycle of the oysters.*

Introduction

The edible oyster *Crassostrea gryphoides*, is distributed in the Bhatya creek over an approximate area of 20 ha. Several tons of oysters are exploited from this area after the post monsoon periods. These oysters are collected and marketed daily in the local retail market of Ratnagiri in live condition. The major species exploited are - *Crassostrea gryphoides, C. madrasensis, and C. rivularis* which are locally called as ' *saad kalaav* '. Studies on the biology of the oyster are a prerequsite to propogate oyster culture and the studies include those of Rao and Nayar (1956) from Madras on *C . madrasensis*, Durve (1965) from Bombay on *C. gryphoides* , Joseph and Joseph (1983) from Dakshina Kannada on *C . madrasensis*, Narasimham (1987) from Kakinada on *C . madrasensis*. From Ratnagiri, Nagabhushanam and Mane (1976) studied the reproductive biology of the Indian oyster *Crassostrea gryphoides* whereas Nagabhushanam and Bidarkar (1980) and Chavan (1982) worked on the oyster, *Saccostrea cucullata* . The present investigation covers some aspects of biology such as sex ratio, maturation and spawning and percentage edibility and condition index of edible oyster in the Bhatya creek.

Materials and methods

Samples of *C. gryphoides* for biological studies were collected once in every fortnight during June 1995 to May 1996 (except July 1995) from Bhatya creek. About 14 to 28 oysters were collected from a depth of up to seven meters at random from the oyster beds with the help of local divers. The oysters were washed and cleaned off epifauna and epiflora prior to examination. Sex and maturity stages were determined by microscopic examination of gonad smears of all individuals in each collection. Four categories of maturity stages, namely stage I (maturing), stage II (ripe), stage III (partially spawned), and stage IV (spent) were recognised following Narasimham (1987). The monthly sex - ratio data, subjected to Chi - square to see whether male female ratio was different from 1 : 1. The percentage edibility was calculated for all the collected specimens and the condition index studied in six oysters of varied size ranges from each collection. For condition factor the commonly followed method is to calculate the ratio of the dry meat weight (oven dried at 90 - 100 ^{0}C to a constant weight) to the volume of the shell

cavity and is expressed as

$$\text{Condition factor} = \frac{\text{Weight of dry meat}}{\text{Volume of shell cavity}} \times 1000$$

The condition is taken as high if the value is above 140 . Values below 70 indicate the poor conditions of oysters (Rajapandian and Rajan , 1987 ; James and Narasimham , 1994) .

Observations and results

Sex ratio

The present studies on *C. gryphoides* showed that the females invariably outnumbered the males in almost all the months , except in March , April and May 1996. The females formed 63 to 75 percent of the stock during the monsoon and winter months (June to February 1995). During summer (March to May 1996) this percentage was between 27 to 38 (Table 1).

Table 1. Percentage of male – female specimens and the sex ratio in monthly samples of *Crassostrea gryphoides* during 1995 – 96.

Month	Total number of specimens observed	Males	Percentage of males	Females	Percentage of females	M : F. Ratio
Jun. 1995	26	8	31	18	69	1 : 2.25
Jul.	-	-	-	-	-	-
Aug.	24	7	29	17	71	1 : 2.42
Sep.	33	9	27	24	73	1 : 2.66
Oct.	27	6	22	18	67	1 : 3.00
Nov.	32	8	25	24	75	1 : 3.00
Dec.	41	11	27	26	63	1 : 2.36
Jan. 1996	43	13	30	27	63	1 : 2.07
Feb.	41	10	24	27	66	1 : 2.70
Mar.	40	20	50	14	35	1 : 0.70
Apr.	44	26	59	12	27	1 : 0.46
May.	42	24	57	16	38	1 : 0.66

The males formed 22 to 31 percent of the stock during monsoon and winter months . During summer (March to May 1996) this percentage increased and was between 50 to 59 in different months. The indeterminate oysters occurred in October 1995 , December 1995 to May 1996 with their percentage occurrence between 5 to 15. Thus during the study period , males formed 36.13 %, females 56.75 % and the indeterminants 7.12 % on an annual average basis. This gave the male to female ratio as 1 : 1.56.

In order to ascertain whether the observed male to female ratio in each month differs significantly from the theorotical 1 to 1 ratio , the chi-square values for each month were calculated (Table 2) . There was a significant deviation from the 1 : 1 ratio in all the months , except March and May 1996 due to preponderence of males.

Table 2. Results of Chi - square test in the sex ratios of *Crassostrea gryphoides* from June 1995 to May 1996.

Months	**Males**	**Females**	**DF**	X^2
June 1995	8	18	1	5.76
July 1995	-	-	-	-
August 1995	7	17	1	4.16
September 1995	9	24	1	6.80
October 1995	6	18	1	6.00
November 1995	8	24	1	8.00
December 1995	11	26	1	6.08
January 1996	13	27	1	4.90
February 1996	10	27	1	7.80
March 1996	20	14	1	1.05*
April 1996	26	12	1	5.14
May 1996	24	16	1	1.60*

* Not significant at 5% level.

Maturation and spawning

The course of reproductive activity of the oyster in the Bhatya bed was monitored to determine the right time when spat collectors are to be set up . The results reported here are based on the observations made over a continuous period of 12 months from June 1995 to May 1996 . It was found that during summer (March to May 1996 and June 1995) almost 80 to 90% of the oysters were with well developed gonads. In males, the testicular laminae were densely packed with the spermatozoa . In females the follicles were packed with the ripe ova , polygonal or suboval in shape . In June 1995 most of the gametes were found to be released indicating this to be a major spawning period . In monsoon (from July to October 1995) majority of the oysters (90 to 95 %) were in spent / resting stage. This may be due to a reduction in salinity in the Bhatya creek. Decrease in salinity influences the reproductive cycle in oysters (Thangavelu and Sanjeevaraj, 1988) and thereby progressively increases the number of spent / resting oysters in these four months indicating the completion of the reproductive cycle .

In winter (i.e from November 1995 to January 1996), the gamatogenesis was initiated again with the increase in salinity and the gonads were found to be in active and ripe conditions. Finally from January to March 1996 about 50% of the oysters were in partially spawned stage and the spawning continued till May 1996 and June 1995 .

All the four maturity stages (Active , Ripe , Partially spawned and Spent / Resting) were observed in the months from January to May 1996 and June 1995 . Their distribution suggests that the oysters undergo maturation and spawning more than once during this period.

Percentage edibility and condition index

The observations on the percentage edibility were carried out by examining 393 oysters and for index of condition , by examining 88 oysters, collected during the period June 1995 to May 1996.

Percentage edibility: The monthly variations in the average

percentage edibility or meat yield of these oysters is given in Table 3. The percentage edibility remained high (between 5.0 to 9.0) from January to May 1996, the highest value (8.5) being in June 1995. Values were low (between 3.0 to 5.0) in July to December 1995 the lowest value (3.35) being in November 1995 . It was found that these variations in the percentage edibility were closely correlated with the spawning cycle of the oysters under investigations . The spawning in *C . gryphoides* commenced in April 1996 and ended by June 1996 . The percentage edibility values were high during the spawning period, whereas there was a fall in the percentage edibility during August to December 1995. Finally from January 1996 , as the oysters started recovering to normal again and started entering the active phases , the values of the percentage edibility also rose and remained high up to June end.

Table 3. Monthly variations in average percentage edibility and condition index in *Crassostrea gryphoides*.

Months	Percentage edibility	Condition index
Jun. 1995	8.50	148
Jul.	-	-
Aug.	5.20	98
Sept.	5.40	79
Oct.	4.50	52
Nov.	3.35	58
Dec.	4.10	102
Jan. 1996	5.90	123
Feb.	5.90	140
Mar.	7.50	128
Apr.	6.79	146
May.	7.96	143

Condition index: The monthly variations in the average index of condition in the oysters taken as a whole are given in Table 3. In the present case the index of condition showed the same pattern of

changes as that in the percentage edibility. The condition index remained high from December 1995 to May 1996 (and June 1995), the highest value (148) being in the month of June 1995. The values were low between the period July to November 1995, the lowest value (52) being in the month of October 1995. Thus these variations in the condition index were similar to that in the percentage edibility.

The quality of meat of the oyster, *C. gryphoides* shows definite seasonal changes and the percentage edibility and the index of condition values remain high during the period January to June, qualifying it fit for human consumption.

Discussion

Earlier studies on *C. gryphoides* indicated the predominance of females from May to August while the males predominated over the females in September and October. From November to April Durve, (1965) observed the alternate predominance of either of the sex. Narasimham' s (1987) studies on *C. madrasensis* showed the proportions of females to be significantly higher than that of the males, throughout the year. In the present studies the proportion of females was significantly higher than that of the males in all the months, except the summer months.

Studies on sexuality, seasonal gonadal changes and spawning in oysters have earlier been done by Durve (1965) on *C. gryphoides* from Bombay waters, Nagabhushanam and Bidarkar (1980) on *Saccostrea cucullata* from Ratnagiri waters, Rajapandian and Rajan (1983) on *C. madrasensis* from Tuticorin, Joseph and Joseph (1983) on *C. madrasensis* from Mulki estuary (Dakshina Kannada), Narasimham (1987) on *C. madrasensis* from Kakinada and Sarvesan *et al.* (1990) on *C. madrasensis* from Muttukadu backwater, Madras and the present work on *C. gryphoides* from Ratnagiri (Bhatya) waters. Thangavelu (1988) has observed the effect of salinity on the spawning, development and setting of spats of the oyster *Ostrea madrasensis* (Preston) in Pulicate lake. Sarvesan *et al.* (1990), have

also observed that the maturation, spawning and the spatfall of the oysters are influenced by salinity and temperature . Earlier, Durve and Bal (1962) in their preliminary observations had also found that the spawning of the oyster , *C . gryphoides* begins in July and lasts till early October . Narasimham 's (1987) studies on *C. madrasensis* of Kakinada bay showed that spawning takes place during January to June. Mane and Nagabhushanam (1988) reported that, *C. gryphoides* from Ratnagiri had shown that spawning commences in September and continues till the onset of the monsoon.

The oysters are known to show variations in the quality of their meat depending upon their physiological conditions , environmental factors and the seasons. Assessment of quality is done by estimating the percentage edibility and index of condition and has been done for oysters by Durve (1964), Joseph and Joseph (1983) , Nair and Nair (1987) , Rajapandian and Rajan (1983) , Rainer (1989) , James and Narasimham (1994) , Rosique and (1995).

Durve (1964) had observed a close correlation between the gonadal cycle and the condition index in the oyster , *C. gryphoides.* He stated, that the oysters were fatty and cream coloured from November to June when they were not spawning. Joseph and Joseph (1983) have shown that in the Mulki estuary the oysters (*C. madrasensis*) were in their best condition from late May to September . In *C. madrasensis* from the Cochin backwaters, Nair and Nair (1987) observed higher values of condition index during April to October and thus , concluded that the oysters were in the best condition during this period.

Acknowledgements

We express our thanks to the authorities of Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri and Associate Dean, College of Fisheries, Ratnagiri for providing necessary laboratory and library facilities.

References

Chavan, J . B . 1982 . *Studies on rock oyster Saccostrea cucullata (Born) species resources. M.Sc* . Thesis, Konkan Krishi Vidyapeeth, Dapoli, India .

Durve, V. S., 1964. On the percentage edibility and index of condition of the oyster *Crassostrea grypkoides. J. Mar. biol. Ass. India.* **6** (1): 128 – 135.

Durve, V . S ., 1965 . On the seasonal gonadal changes and spawning in adult oyster *Crassostrea gryphoides* (Schlotheim) *J. Mar. biol. Ass. India.* **7** (2) pp : 328 - 344 .

Durve, V . S . and D . V . Bal, 1962 . Preliminary observations on the growth of spat of the oyster *Crassostrea gryphoides* (Schlotheim) *J . Mar . biol. Ass . India* . **4** (2) pp : 206 – 213

Joseph, Mohan M ., and Shantha Joseph ., 1983 . Some aspects of experimental culture of the oyster *Crassostrea madrasensis* (Preston) *Proc . Symp . Coastal . Aquaculture,* 2 : 451 - 455 .

James , P . S . B . R . and K . A . Narasimham., 1994 . Molluscs , *Handbook on Aquafarming.* MPEDA publication. 1 - 87 .

Mane, U . H . and R. Nagabhushanam, 1988 . Reproduction in edible bivalve shellfishes of Ratnagiri coasts. *CMFRI bulletin . 42* Part-1 : 167-176

Nagabhushanam, R and U. H. Mane., 1976. A study on the reproductive biology of the Indian oyster *C. gryphoides Nat. Sci. J. Marath. Univ.* 15: 245 – 258.

Nagabhushanam, R. and D.S. Bidarkar, 1980. Reproductive biology of Indian Rock oyster *Crassostrea cucullata. Indian J. Fish.* **24** (1& 2) pp : 135 – 142

Narasimham, K . A ., 1987 . Biology of *Crassostrea madrasensis* of Kakinada. *CMFRI bulletin. 38* . Oyster culture status and prospects . 40 - 47 .

Rao , V . K . and K. Nagappan Nayar 1956 . Rate of growth in spat and yearlings of the Indian back water oyster. *C. madrasensis* Preston *Indian . J. Fish.* **3** (2) pp : 231 - 260.

Rajapandian , M . E . and C.T. Rajan, 1983 . Studies on maturity stages and spawning periodicity of *Crassostrea madrasensis* (Preston) at Tuticorin bay . *Proc . Symp . Coastal Aquaculture* , 2 : 475 - 478.

Rainer , J . S ., 1989 . Calculation of condition index in oysters . *J . Shellfish . Res.* **8** (2) pp : 487 .

Rosique , M . J , J. Cano, J. Rocamora, 1995 . Seasonal variation in condition indexes and chemical composition of the body in the European flat oyster (*Ostrea edulis* L) in the *Mar Menor* (Murica, South eastern Spain) *Iberus* **13** (2) : 9-22.

Sarvesan, R, V. Sreenivasan, K. Satyanarayana Rao, R. Thangavelu and P. Poovannan., 1990 . Reproductive biology and setting of spat of *Crassostrea madrasensis* (Preston) in Muttukadu backwater . *J . Mar . biol . Ass . India* . **32** (1 & 2) : 119 - 128 .

Experimental fattening of the green mud crab Scylla oceanica (Dana)

M.K. Anil and C. Suseelan

Central Marine Fisheries Research Institute, Kochi-682 014

ABSTRACT

Results of the experimental fattening of postmoult soft crabs ('Water crabs') of Scylla oceanica *(Dana) in the size 550 g and above in a brackishwater pond of 0.05 ha area at Cochin during August 1992 - April '93 are presented. Four fattening trials were attempted under varying salinity conditions (5-29 ppt) and stocking densities (2.5-5 crabs/ 10 m^2) for 45-60 days, feeding with trash fish, slaughter house waste and/or clam meat at the rate of 7% of body weight. Experiments have shown that 45 days fattening with a stocking density of 1 crab /3m^2 is ideal for better survival and economic returns in tide-fed conditions. Although no significant change was observed in the total weight of crab during fattening, the average protein content of the meat increased from 8.33% to 14.93% with a decrease in moisture content from 87.15% to 80.93%. Economic analysis of crab fattening trials indicated gross profit ranging from Rs. 13300 to 50400 in 5-6 crops a year.*

Introduction

For centuries, mud crabs of genus *Scylla*, also known as green crabs or mangrove crabs constituted an important secondary crop in the traditional prawn or fish culture systems of Asian countries. In India the mud crabs have come into prominence since early eighties with the commencement of live crab export to the South East Asian countries like Singapore, Malaysia and Hong Kong. This has created a renewed interest in the exploitation as well as in the production of mud crabs through aquaculture.

Technology of mud crab farming can be broadly categorised into 'grow-out culture' and 'fattening'. Grow-out culture refers to farming of undersized crabs for longer period, usually 3 to 6 months, to produce marketable sizes, whereas fattening is holding of market-size crabs for 2 to 4 weeks time to acquire certain desired biological characteristics (Chong, 1993). During grow-out culture the stocked animals moult several times before they are harvested, while during fattening, the animals are usually harvested before they moult . In the latter-case, therefore, there is hardly any change in the size of the animal at the time of harvest. The aim of fattening is either to produce female crabs with ripe gonads or to convert post-moult soft crabs to hard shelled crabs. Crabs with well developed ovary or roe filling the body cavity is considered a delicacy in many countries. Such animals fetch very high prices in restaurants and markets. Therefore female crabs with immature ovary are fattened by giving proper feed until their ovary develops and fills up the body cavity. Experienced farmers can identify the crabs with fully developed ovary by looking through the carapace, holding the crab against sun light or a bright light source.

Newly moulted crabs have soft shell and watery meat and hence are known as 'water crabs' which fetch very low price compared to hard shelled crabs. The soft shelled crabs are fattened by proper feeding for two weeks to one month, and by this time their flesh becomes firm and shell hardened. Hardness of the shell is judged by pressing the sternites of the cephalothorax.

In spite of the decline in catches due to overfishing and destruction of mangrove areas which form their natural habitat, the fishing pressure on the resource is ever on the increase all over the world because of very high price of the commodity. One of the ways of protection of natural stock is the utilisation of the under sized and post moult soft crabs by grow-out and fattening type of aquaculture. Keeping the above points in mind fattening trials were conducted and the results are presented in this paper. In order to assess the change in biochemical composition during fattening, the proximate composition of meat was analysed before and after fattening and the results presented and discussed.

Material and methods

The crab fattening experiments were carried out in a brackishwater pond of 0.05 ha (Fig. 1) situated in Vypeen island. The pond was connected to Cochin backwater by a 5m wide canal of about 200 m length. The pond had a strong bund of 3 m width at the top. A sluice gate of 1 m width connected the pond to the tidal canal. Average depth of the pond was 1.5 m.

In order to prevent escape of crabs over the bund, a perimeter fencing of 1m height was constructed using nylon netting of 20 mm mesh size. The netting was supported by split bamboo sticks fixed on the dyke at 2 m intervals. The net was fixed in such a way that the lower portion of the same was buried in soil and secured firmly with bamboo pegs. The sticks were planted in slanting manner overhanging the pond to prevent

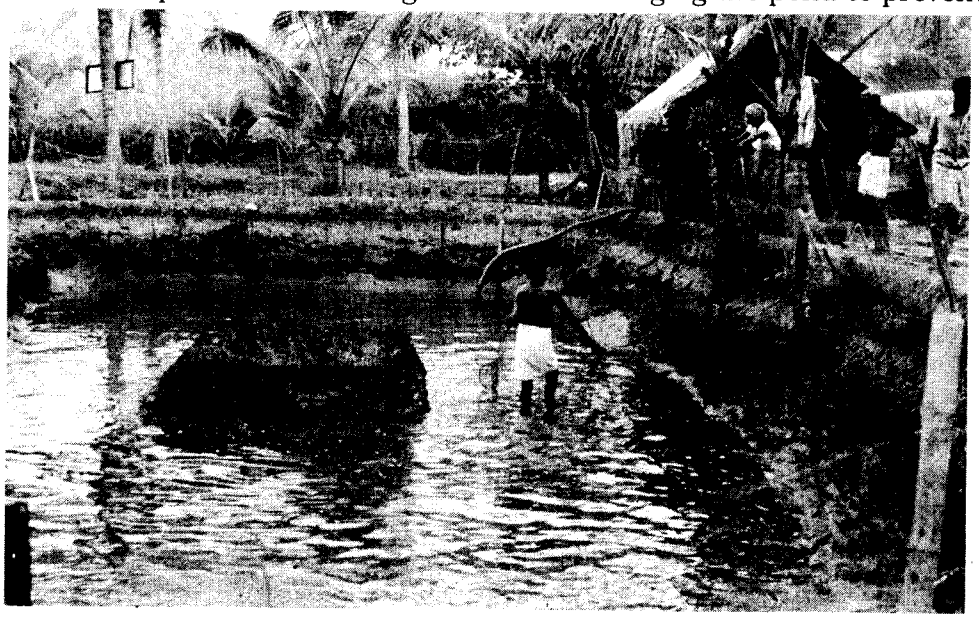


Fig. I. Final harvesting of crabs in fattening pond

crabs from climbing over the fence. Bund near the sluice gate was reinforced with bamboo matting as the crabs showed a strong tendency to burrow near the sluice gate. A watchman kept vigil to prevent poaching. Four fattening trials were conducted between August 1992 and April 1993. The fattening period varied from 45 to 60 days.

During the experiments, physico-chemical parameters like temperature, salinity, dissolved oxygen and pH were measured at an interval of 7 days. Standard methods AOAC (1965) were used for determination of moisture, ash, crude protein, total lipid, crude fibre and nitrogen free extract (NFE).

Results

The pond was first conditioned by adding lime at the rate of 600 kg/ha after reducing the water level to the minimum. Next day water was let in during high tide. The incoming water was screened using a nylon net screen to avoid entry of competitors. Soft crabs weighing above 550 g in body weight were purchased from local fishermen and active crabs with all the appendages intact were used for stocking. The carapace length of the crabs ranged from 15 to 20 cm. The crabs were fed with either trash fish, slaughter house waste or clam meat at the rate of 7 % of body weight. Boiling the slaughter house waste before feeding was found to reduce water pollution. When trash fish was available in bulk, it was salted and stored and used for feeding when trash was scarce. Water exchange was done by tidal flushing.

Four fattening trials were conducted and the experimental details are summarised in Table 1.

Table 1. Details of crab fattening experiments

Particulars	Trial-1	Trial-2	Trial-3	Trial -4
Area of pond(ha)	0.05	0.05	0.05	0.05
Duration (days)	45	45	45	60
Stocking density (No/m^2)	0.25	0.35	0.35	0.5
Number of water crabs stocked	125	175	175	250
Total weight of water crabs stocked(kg)	95.75	140.8	145.7	192
No. of hard shelled crabs harvested	98	142	154	183
Total weight of hard shelled crabs harvested (kg)	75	114	128	143

No. of soft shelled crabs at harvest	18	22	17	36
Total weight of soft shelled crabs at harvest	13	17.5	13	28
No. of dead or missing crabs	6	6	2	22
No. of damaged crabs	3	5	2	9
Survival rate (%) including Damaged crabs	95.2	96.6	98.9	91.2
Crabs used for sale* (%)	92.8	93.7	97.7	87.6

* *Both soft and hard shelled*

In the *first trial*, 125 soft crabs ('water crabs') weighing 95.75 kg were stocked and the stocking density was 0.25/m^2. Among the stocked crabs, 30 crabs were individually tagged using numbered plastic tags. These tags were tied to the base of the last leg of the animal after noting the weight. During the first trial, salinity was low, ranging between 5.0 ppt and 7.5 ppt. The water temperature, pH and dissolved oxygen values were 25-26.7° C, 7.2-7.5 and 2.1-6.7 ml/l respectively.

Partial harvesting started from 20th day onwards using baited ring nets. All the crabs thus caught were individually tested for their shell condition by pressing the sternites of cephalothorax. Those with hardened shells were segregated and marketed. The crabs whose shells were not hardened properly were returned to the pond for further fattening. Total harvesting was done on the 45th day after completely draining the pond, by hand picking or using scoop nets locally called 'bols'. All the tagged crabs were carefully cleaned of mud and algae attached to the tags and weighed. Though a maximum individual weight increase up to 30 g/ crab was noticed, majority of the crabs showed little change in weight even after a period of 45 days of experiment. Out of the 125 crabs stocked, 98 crabs weighing 75 kg were sold as hard shelled meat crabs and 18 as water crabs. Apart from this, 3 crabs were caught severely damaged and 6 were either dead or missing. Survival rate was 95.2 % which included hard shelled, soft and damaged crabs at the time of harvesting. The percentage of crabs suitable for sale (undamaged hard or soft shelled ones) was 92.8.

During the *second trial*, stocking density was increased to 0.35/ m^2 and altogether 175 soft crabs were stocked within a week. Salinity recorded at the beginning of the experiment was 6.5 ppt which gradually increased to 24.2 ppt by the end of the trial period. The pH and temperature values ranged from 6.8 to 7.8 and 25.2 to 28.3°C respectively. Dissolved oxygen varied between 2.1 and 7.0 ml/l. Out of the 140.8 kg of soft crabs stocked, 114 kg numbering 142 were harvested in hard shell condition. The survival rate of marketable individuals was 93.7 %. Twenty two crabs remained in soft condition, 5 were caught in damaged condition. Actual survival rate was 96. 57%.

In the *third fattening trial*, stocking density was the same as that of the second trial but the feeding schedule was changed from once a day to twice a day. Only 30 % of the daily ration was given in the morning, the remaining 70% in the evening. During the experimental period the salinity varied narrowly, ranging from 24.2 to 27.5 ppt. Dissolved oxygen varied between 2.9 and 7 ml/l. Water temperature and pH were in the range of 27.3-29°C and 7.3-7.8 respectively.

During the third trial the total weight of soft crabs stocked was 145.75 kg. Out of this 128 kg numbering 154 crabs was harvested in

Fig. 2. Tying harvested crabs for sale.

hard shelled state and 17 crabs weighing 13 kg remained in soft condition. Total mortality was 2 and another 2 crabs were caught in damaged condition. Actual survival rate worked out to 98.85% of which percentage of crabs suitable for sale formed 97.7 %.

During the *fourth trial*, the stocking density was increased to 0.5/ m^2 and 250 crabs weighing 192 kg were stocked. It took two weeks to stock the crabs. During this cycle salinity ranged 27-29 ppt, temperature 28-29.7°C and pH 7.2 to 8.1. Harvesting began from the 20th day onwards. A total of 183 crabs weighing 142 .75 kg was harvested during the fattening period of 60 days and 36 crabs weighing 28 kg remained in soft condition. Dead and damaged ones were maximum in this trial which numbered 22 and 9 respectively. Actual survival rate worked out to 91.2%. The rate of recovery of animals suitable for marketing (Fig.2) was the lowest, being 87.6% among all the four fattening trials.

Biochemical changes during fattening

In order to assess the change in biochemical composition during fattening, the proximate composition of crab meat was studied before and after fattening and the results are presented in Table 2.

Table 2. Proximate composition of meat of soft shelled and hard shelled (fattened) crabs (Standard deviation of the mean is given in parenthesis)

Constituents	Percentage of wet meat weight	
	Soft crab	Fattened crab
Moisture	87.15	80.925
	(1.371)	(1.911)
Crude protein	8.328	14.93
	(0.663)	(0.716)
Ash	3.559	3.315
	(0.241)	(0.343)
Fat	0.293	0.2873
	(0.19)	(0.021)
Crude fibre	Negligible	Negligible

The values of the different biochemical constituents in the two different conditions of crab body would reveal that considerable change occurs in the contents of moisture and crude protein during the transformation period. The protein content was 7.282-9.143% in the soft shelled condition and 13.93-16.03% in fattened condition with average values of 8.33% and 14.93% respectively. As the protein content increased, the water content showed a reduction from the average value of 87.15% in 'water crab' to 80.93% in fattened crab. The differences in protein and water content were found statistically significant. Other parameters analysed, such as, ash content, fat and NFE did not show any statistically significant difference between soft and fattened crab. The crude fibre was negligible in the meat of both the types of crabs indicating very low content of non digestible carbohydrate in their meat.

Economics of crab fattening

Based on the results obtained from the fattening experiments, the economics of crab fattening has been worked out for a 0.05 ha farm for one year separately for each type of experimental trials with varying stocking densities and feeding schedule. The comparative cost and earnings arrived at are shown in the Table 3.

Table 3. Economics of mud crab fattening in 0.05 ha farm at Vypeen Island (Values in Rs.)

Particulars	Trial-1	Trial-2	Trial-3	Trial -4
I. Initial investment				
1.Cost of land	25000	25000	25000	25000
2.Watchman's shed	5000	5000	5000	5000
3.Pond construction	4000	4000	4000	4000
4.Sluice gate	3000	3000	3000	3000
5.Fencing	750	750	750	750
Total	37750	37750	37750	37750
II. Annual fixed cost				
1.Opportunity cost of land (@10%)	2500	2500	2500	2500

2.Depreciation (20% of initial investment excluding land cost	2550	2550	2550	2550
3.Interest (20% of initial investment)	7550	7550	7550	7550
Total	12600	12600	12600	12600
III. Operating cost				
1.Pond preparation	300	300	300	300
2.Cost of soft crab(@80/kg)	7660	11280	11660	15360
3.Feed	777	1134	1182	1555
4.Labour charges	3000	3000	3000	3000
Total (per crop)	11737	15714	16142	21215
5.Annual working cost (6/5 crops/year)	70422	94284	96852	106075
IV. Annual expenditure (II+III)	83022	106884	109452	118675
V. Annual income				
1.Income from the sale of hard shelled crabs @ 200/kg	15000	22800	25600	28550
2.Income from the sale of soft shelled crabs @ 80/kg	1040	1400	1040	2240
Total	16040	24200	26640	30790
3.Annual income from 6/5 crops	96240	145200	159840	153950
VI. Gross profit (V-IV)	13218	38316	50388	35275

As the fattening period was 45 days each for the first 3 trials, the annual expenditure and income were calculated assuming that at least 6 such fattening operations could be carried out in a year. However in the case of the 4th trial, computations were made on the assumption that 5 trials could be taken in a year since the duration lasted for 60 days.

The initial investment of fattening operation included cost of land, cost of construction of watchman's shed, cost of construction of pond, cost of sluice gate and expenditure for perimeter fencing. Total initial investment was Rs. 37,750, which remained the same for all trials since all the trials were carried out in the same pond.

Annual fixed cost was calculated taking 10% of the cost of land as its opportunity cost, 20% as depreciation on the initial investment and

interest at the rate of 20 % of initial investment. The calculated value of initial investment for all the 4 trials was Rs. 12,600.

Operating cost included cost of pond preparation, cost of stocking material (soft crabs), cost of feed and labour charges. Charges towards pond preparation included cost and transportation charges of lime, labour charges for strengthening bund etc. Feed materials such as trash fish, slaughter house waste etc. were purchased at Rs. 3-4 /kg. Cost of salt used for storage of trash fish also included under the cost of feed. Cost of stocking material was the most dominant variable among the operating cost. Consequently the operating cost/crop was lowest in the first trial (Rs. 11,737) and highest in the fourth trial (Rs. 21,215) corresponding with the lowest and highest stocking densities. The cost/crop of the third and fourth trials were Rs. 15,714 and Rs. 16,142 respectively.

Annual working cost was calculated for 6 crops in the case of first three trials and five crops in the fourth trial and the values worked out to Rs. 70,442, Rs. 94,284, Rs. 96,852 and Rs. 106,075 respectively.

Annual expenditure was calculated by adding the annual fixed cost and annual working cost. Income/crop included the revenue obtained from the sale of the hard shelled crabs or meat crabs harvested during the trial and the income from the sale of soft crabs at the time of the final harvest of each trial. The meat crabs were sold at Rs. 200/kg and soft crabs at Rs. 80/kg. Income per crop was the highest in the fourth trial (Rs. 30,790) and the lowest in the first trial (Rs. 16,040). There was no significant difference in the income/crop for the third and fourth trials (Rs. 24,200 and Rs. 26,640). Though the stocking densities were different for the last two trials, the annual income realised from these trials did not show significant variation (Rs. 159,840 and Rs. 153,950) because of the lesser number of cycles in the last trial. In the second trial the annual income was Rs. 1,45,200 which was lower than that of the third trial with an annual turnover of Rs. 1,59,840. The gross profit was calculated by subtracting the annual expenditure from the annual income. It is seen that the profit was the highest in the third trial (Rs. 50,388/annum) as against the decreasing profits realised for the 2nd (Rs. 38,316), 4th (Rs. 35,275) and 1st (Rs. 13,218) trials.

Discussion

An important problem associated with mud crab farming is the inherent tendency of crabs to escape from the farm during the period of culture. In order to prevent the escape of crabs, different management measures such as fencing the pond, reinforcing the dyke with concrete/ bricks, proper feeding schedule, adequate water exchange etc. are resorted to. Of these fencing is done invariably and for this various kinds of materials are used. In Taiwan, the fattening ponds are constructed with vertical brick walls (Chen, 1990) whereas in Indonesia the farmers use bamboo stakes for fencing the pond (Cholic & Hanafi, 1992).

During the present study the culture pond was provided with nylon netting which proved to be very effective to prevent the escape of crabs and is cheaper than other materials like wire mesh, asbestos sheet or bamboo fencing. Further, nylon net fencing was much easier to construct and cheap. As in the case of the export of processed fishery products wherein quality assurance is an essential aspect, the live crab export also demands quality assurance like healthy and disease free condition for trade. In the event of crabs attempt to escape from the pond by climbing over the dykes and fencing, injuries are bound to occur on the ventral side of the crab leading to infections. As the presence of scratches on the body or shell breakage in the crabs would disqualify their acceptability to exports, care has to be taken to prevent occurrence of scratches or shell breakage during culture operation. It is observed that the chances of such damages on the body of crabs are extremely low when the fencing is done with nylon netting.

Assorted sizes of crabs falling between 550-2,000g were used in all the four trials since the preference for live crab export was within the above range and also due to non consistency in the availability of any particular size range in the backwater capture fishery which formed the source of material for the experiment.

The present study on the fattening of large size crabs was a maiden attempt to find out the ideal stocking density yielding the maximum economic returns. The stocking density was 0.25/m^2 in the first trial, 0.35/m^2 in the second and third and 0.5/m^2 in the fourth trial. The duration of fattening was uniformly 45 days for the first 3 trials and 60

days for the fourth trial. The percentage survival at the time of final harvest varied significantly between different trials. In the first experiment the survival was 95.2%, which increased to 96.6% in the second trial and 98.9% in the third trial. The final survival rate in respect of 4th trial went down drastically to 91.2%. The rate of survival of crabs was good in spite of the low salinity of the pond ranging 5.0-7.5 ppt in the first trial. The most favourable salinity for mud crab farming is reported to be 15-35 ppt (Liong, 1994). Although the fattening commenced with low salinity conditions (6.5 ppt) in the second trial the salinity values increased gradually to as high as 24.2 ppt, and it remained more or less constant level of 27-29 ppt in the third trial. It is possible that the higher survival rate recorded for these two trials could have been favourably influenced by the higher salinity regime prevailing in the pond as most of the other environmental factors and pond management procedures remained more or less same. The high survival rate of 98.7% was recorded in the third trial in which the only variable factor that could be attributed to this is the difference in the feeding schedule during that trial. The feeding schedule during the third trial was changed from once a day to twice a day, about 30% of the daily ration was given in the morning and the remaining 70% in the evening.

In the 4th fattening trial though higher salinity condition (27-29 ppt) prevailed in the pond, mortality was maximum. The percentage of marketable crabs was also lower than all other experimental trials (87.6%). This poor performance coincided with deteriorating water conditions on several occasions as evident from the smell of water caused by the excess metabolic wastes released by the denser population and the lack of adequate water exchange. The temperature of the pond water was also higher than in all the previous trials because of the summer season (April), which would have adversely affected the crabs as evident from the sluggish nature of the animals and fouling of epiphytic algae on the crabs body. Taiwanese crab farmers, who stock crabs of 8-12 cm CW corresponding to about 200-250 g in weight use one crab/m^2 as optimum stocking density in summer (Sivasubramoniam and Angell, 1992). In the present study, 0.5/m^2 stocking density corresponds to about 400g/ m^2 assuming that the average weight of crabs stocked was about 800g. The Taiwanese use pump sets for water replenishment as and when

required whereas in the present study the water exchange was by tidal action only which would not have ensured adequate water replenishment commensurate with the crab biomass. From the above observation it can be presumed that the optimum stocking density of crab of about 800g average weight during summer period could be around 0.35 crabs/m^2 under tide fed conditions. This works out to 3500 crabs/ha water area for fattening in tide fed brackishwater condition.

The food given to the crabs consisted of fresh trash fish, salted fish and slaughter house waste. A feeding rate of 7% of body weight was adopted for trash fish and slaughter house waste while a reduced rate of 5% of body weight was used when salted fish was offered. The crab showed varying preferences for different food items when three types were offered together. The first preference was shown to trash fish, then to salted fish and finally to slaughter house waste. Chen (1990) reported a feeding rate of about 1% of body weight with trash fish and sometimes with freshwater hornshell snail during fattening of female crabs of size 220-250g size. Snail meat is added in the diet to facilitate development of gonads, as the fattening is aimed at producing crabs with well developed roe rather than converting 'water crabs' to meat crabs in Taiwan.

According to Rattanchote and Dangwatankul (1992) a higher rate of feeding is practiced in Thailand where trash fish and horse mussel are given at the rate of 7-10% of body weight once or twice in a day. De Silva (1992) used offal, clam meat and fish for feeding mud crabs and found that first preference was for clam meat and then for offal. Cholic and Hanafi (1992) reported the use of dried trash fish at higher feeding rates of 10-15% of body weight. The feeding rate of 5-7% of body weight followed during the present experiment appears to be reasonable considering the prevailing environmental conditions of the brackishwater farms of Kerala coast which are tide fed. The slaughter house waste when offered in fresh condition was found to pollute the water as evident from the colour change and foul odour. Though this problem could be partly overcome by half cooking of meat before use, regular use of slaughter house waste in crab fattening pond is considered undesirable from the view point of environmental safety.

Fattening in cages or pens is considered more advantageous as

the stocking rates in such systems could be considerably high (Natarajan and Thankaraj, 1983; Bensam, 1986; Marichamy *et al.*, 1986). In Philippines as many as 18 crabs are stocked in a cage of 140x70x25 cm size with 18 compartments, keeping one crab in each compartment (Larda, 1992). In Indonesia crabs are stocked in individual compartments at the rate of $40/m^2$ in which condition the mortality rate is less than 5% (Cholic and Hanafi, 1992). This is in contrast to a stocking rate of $2/m^2$ for crabs of 150-200 g size in pond systems. Observation on crab fattening in wooden cages (2.5x2.5x2 m) which was commenced in small scale in Cochin backwaters in recent years have shown that as many as 25-30 'water crabs' of size 550g and above are successfully raised at a time without mortality. This high stocking density is possible since the cages facilitate constant water replenishment in the open backwater providing very ideal condition for the crab. From this it would appear that the optimum stocking density of 0.35 crabs/m^2 worked out for fattening ponds could be further increased by resorting to frequent water exchange and aeration coupled with appropriate feeding as practiced for shrimps in the semi-intensive systems. Further under such improved environmental conditions a denser population can be raised safely since no moulting takes place during fattening period which would make the animals vulnerable for cannibalism and consequent mortality.

A clear change was noticed in the biochemical composition of meat during fattening. The average moisture content decreased from 87.15 % to 80.93% whereas the crude protein content increased from 8.33 to 14.93 % during the transformation. This would indicate that the water profusely absorbed during moulting gets replaced with protein during fattening apparently with little change in body weight.

Economic analysis of mud crab farming practiced in most of the East Asian countries (Anon, 1992; Kathirvel, 1983; Viswakumar,1993) have established that the culture is highly profitable when compared with other forms of aquaculture due to the increasing price live mud crabs command in the international markets.

In the crab fattening experiments the initial investment and fixed cost were the same for all the four trials as they were conducted in the same pond. Among the operating costs, cost of soft crabs was the most

important variable since the stocking size was big. The operating cost per crop was lowest in the first trial corresponding to the lowest stocking density and highest in the fourth trial corresponding to the highest stocking density tested. The operating cost of the second and third trial did not differ much because of the uniformity of stocking density and the meagre variation in the weight of animals. Feed, which was given according to the biomass of the stocked animals also showed a similar pattern with respect to its cost.

The lowest income per crop was obtained in the first trial and the highest in the fourth trial corresponding to the lowest and highest stocking densities used in these trials, but the annual income was higher in the third (Rs. 1,59,840) and second (Rs. 1,45,200) trials. Even though the income per crop was the highest in the fourth trial, the annual income worked out for this trial was lower than that of the third trial because of the lesser number of trials in the former case. The gross profit from the fourth trial amounted to Rs. 35,275 which was less than the gross profit from the third (Rs. 50,388) and second (Rs. 38,316) trials as the operating cost was the highest in the fourth trial. This would probably indicate that stocking density of $0.5/m^2$ or above cannot be recommended under the present level of management. The lowest profit obtained at the stocking density of $0.25/m^2$ shows the under-utilization of the pond. The higher gross profit obtained in the third trial as compared to the second trial shows that even at the same stocking density profit can be increased significantly by adopting better management techniques.

References

Anon. 1992. Filipino crab. *Aqua farm news*, **10**(2): 1-20.

AOAC 1965. *Official methods of analysis of the Association of Official Analytical Chemists*, 10th Edition, Publ. by the Association of Official Analytical Chemists, Washington D.C.

Bensam, P. 1986. A culture experiment on the crab *Scylla serrata* (Forskal) at Tuticorin during 1975-77 to assess growth and reproduction. *Proc. Symp. Coastal Aquaculture*, **4**: 1186-1189.

Chen, I.O. 1990. *Aquaculture in Taiwan*. Fishing News Books, U.K. 173 p.

Cholik, F. and A. Hanafi 1992. A review of the status of the mud crab (*Scylla*

sp.) fishery and culture in Indonesia. In: *The mud crab*, Report of the seminar on mud crab culture and trade, Surat Thani, Thailand, Nov. 5-8, 1991, P.13-28.

Chong, L.P. 1993. The culture and fattening of mud crabs. *Infofish Intl.*, **3**: 46-49.

Desilva, L.B.D. 1992. Results of mud crab fattening. . In: *The mud crab*, Report of the seminar on mud crab culture and trade, Surat Thani, Thailand, Nov. 5-8, 1991, P. 155-159.

Kathirvel, M. 1983. Crab resources and prospects of crab culture. Mariculture potential of Andaman and Nicobar islands - An indicative survey. *Bull. Cent. Mar. Fish. Res. Inst.*, **34**: 66-68.

Larda, D.I. 1992. Mud crab fattening practices in the Philippines. In: *The mud crab*, Report of the seminar on mud crab culture and trade, Surat Thani, Thailand, Nov. 5-8, 1991, P. 151-154.

Liong, P.C. 1994. Crab culture present status, future prospects. *Aqua International*, **2**(4&5): 4-11.

Marichamy, R., M. Manickaraja and S. Rajapackiam 1986. Culture of mud crab *Scylla serrata* (Forskal) in Tuticorin Bay. *Proc. Symp. Coastal Aquaculture.* **5**: 1176-1182.

Natarajan, P. and G. Thankaraj 1983. Studies on the growth of edible crab *Scylla serrata* in a brackishwater cage. In *Proc. Natl. Sem. Cage and Pen Culture*, Fisheries College, TNAU, Tuticorin, 107-110.

Rattanachote, A. and R. Dangwatangul. 1992. Mud crab (*Scylla serrata*) fattening in Surat Thani province. . In: *The mud crab*, Report of the seminar on mud crab culture and trade, Surat Thani, Thailand, Nov. 5-8, 1991, P.171-177.

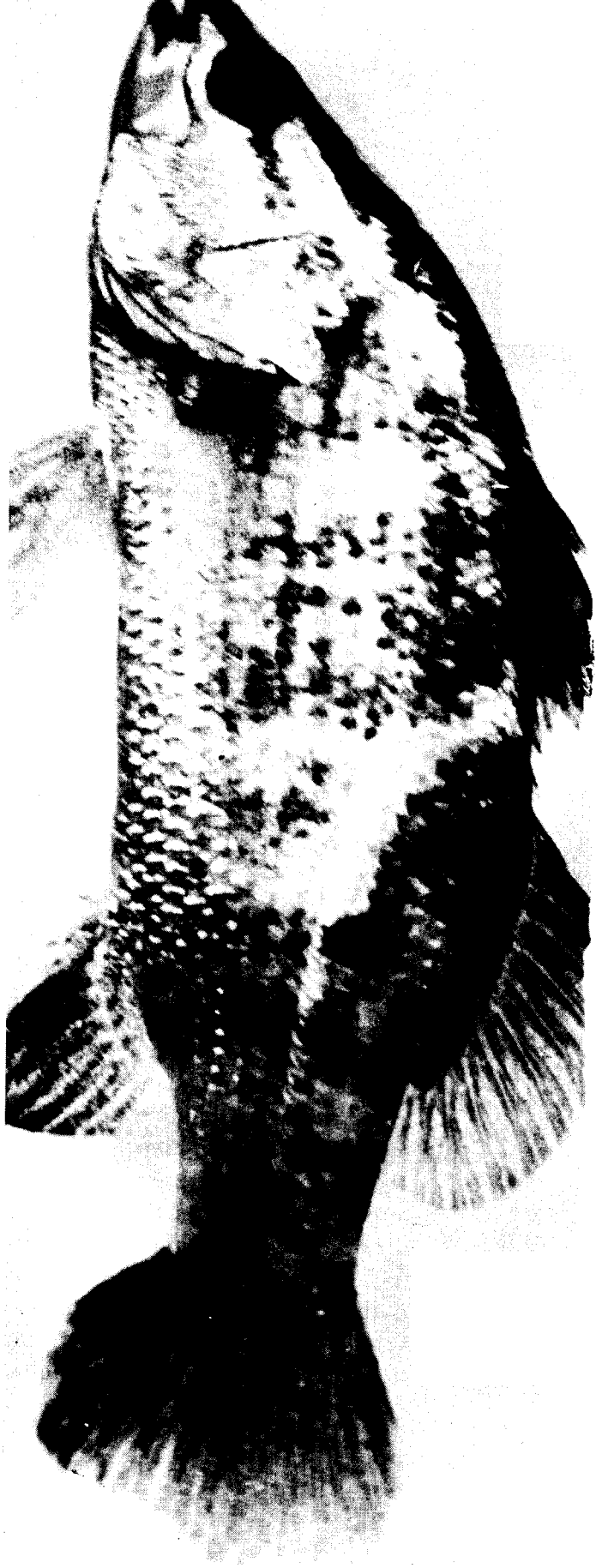
Sivasubramaniam, K. and C. Angell 1992. A review of the culture, marketing and resources of the mud crab (*Scylla serrata*) in the Bay of Bengal region. In: *The mud crab*, Report of the seminar on mud crab culture and trade, Surat Thani, Thailand, Nov. 5-8, 1991, P. 5-12.

Viswakumar, M. 1993. Mud crab farming techniques. In: *Shrimp lobsters mud crabs*, (Publ.) MPEDA, Cochin. P. 65-72.

Captive broodstock development and breeding of seabass *Lates calcarifer* (Bloch) in India

A.R. Thirunavukkarasu, M. Kailasam, P. Kishore Chandra, P. Shiranee, Mathew Abraham A.V.K. Charles and R. Subburaj

Central Institute of Brackishwater Aquaculture, Chennai - 600 034



ABSTRACT

*Seabass (*Lates calcarifer*) is an ideal candidate for an eco-friendly sustainable aquaculture in India. Large scale farming is hampered due to non-availability of adequate quantity of seed. Hatchery technology for commercial production of seabass seed has been developed for the first time in India at the Central Institute of Brackishwater Aquaculture, Chennai. Protocols for captive broodstock development, induced maturation, breeding and larval*

rearing have been standardized. Adult fishes in the weight range of 2.5 to 10.0 kg, collected from the artisanal fishery were stocked in 100 tonne capacity RCC broodstock holding tanks and maintained from December '95 @ 1 kg/m³ density. Fishes were fed @ 5% of the body daily with frozen trash fishes and the 80% water exchange was done daily with proper health, water and feed management. Fishes attained gonadal maturity in the fish holding tanks during May - June. Males were in oozing condition. Maturity in females was advanced through LHRH-a hormone pellet implantation @ 100µg/kg. Gravid fish was induced to spawn spontaneously by the administration of single dose of LHRH-a hormone @ 60-70µg/kg body weight and for males 30-35 µg/kg. Second spawning of the same female on subsequent day was also observed. Fertilization rate varied from 70 to 80% with hatching rate of 60-90%. Details are discussed in the paper.

Introduction

Asian seabas *Lates calcarifer* is one of the potential candidate species for farming in coastal and inland water both in pond and cage culture systems. It is a highly relished fish, with a capacity to grow fast and ability to withstand wide fluctuation in environmental conditions. Seabass is extensively cultured in South East Asian countries like Thailand (Sakras 1982, Wangsomnuk *et al.*,1979),Indonesia (Kungvankiji, 1986), Phillippines (Forotes, 1985, 1986), Malaysia (Ali, 1986) and Australia (Mac Kinnon, 1987). In India too, in the coastal traditional farms seabass is cultured to a limited extent. However, seabass farming on large scale adopting better management practices is hampered due to the non-availability of adequate quantity of quality seed. Central Institute of Brackishwater Aquaculture, Chennai took up seabass seed production technology development on priority basis and made a breakthrough in 1997 (Thirunavukkarasu, 1997; Kailasam *et al.*, 1998). One of the important prerequisites for the successful seed production under captive condition is the availability of gravid fishes. Collection of gravid fishes from wild is unpredictable and risk is involved in transporting them to hatchery site and maintaining them till the fish spawns. Hence, captive broodstock development and inducing them to spawn in captivity is important to enhance the possibilities of mass production of seabass seed.

Materials and methods

Adult seabass were procured from the wild catch and farm reared stock and transported to fish hatchery site at CIBA experimental field station, Muttukkadu. The fishes were initially examined for parasitic infection, if any, and after prophylactic treatment with antibiotics, they were maintained in acclimatization tanks for ten days. There after fishes were transferred to the broodstock fish holding tanks for captive maturation.

The fish broodstock holding tanks were 100 ton net water capacity in dimension of 12m x 6m x 2m. Feeding was done with trash fishes like tilapia (*Oreochromis mossambicus*), sardines (*Sardinella sp*) and horse mackerel (*Decapterus sp*) @ 5% of the body weight once a day. The broodstock holding tanks were cleaned regularly. Water exchange was done to the extent of 80% daily with water drawn from bore wells in the intertidal area. Important water quality parameters like temperature, salinity, pH, dissolved oxygen, nitrite and ammonia were regularly monitored.

The health of broodstock fishes was periodically monitored, when parasite infection was noticed fishes were treated. The maturity stages of the captive broodstock fishes were examined regularly. A simple biopsy method was adopted to assess ovarian maturation of females from April onwards. A polyethylene cannula of 1.2 mm dia. was inserted through the genital pore to a distance of 10 cm. The other end of cannula is gently aspirated by mouth withdrawing the cannula shortly. The content of the cannula was emptied into a watchglass and examined microscopically, the diameter of ova was measured using ocular micrometer. Females with ova diameter of more than 450 μ was taken for induced spawning trials. Oozing males were taken for induction of spawning. In case of necessity the females maturity was accelerated by implanting LHRH-a hormone pellet intramuscularly. For induction of spawning, females were administered with LHRH-a (Lutenizing Releasing Hormone analogue) intramuscularly. The dosage rate was 60-70 μg/kg of body weight for females and 30-35 μg/kg for males. After hormonal injection, fishes were released at the rate of 2 males for one female in 20-ton capacity rectangular RCC tanks.

After spawning, fertilized eggs collected through scooping/siphoning were washed and transported into 500 liter capacity rectangular FRP tanks for hatching. The eggs spawned were counted, collected randomly by one liter of water from the spawning tank and counting the number of eggs in sub sampling 100 ml. This is repeated 2-3 times and the fertilized and unfertilized eggs were separated for commuting the fertilization rate. The percentage of hatching was also estimated.

Results and discussion

Broodstock procurement, transport, acclimatization, maturation

Adult fishes with the size of 2.5 and 8.0 kgs were procured from commercial catches of Muttukkadu coastal waters. The fishes were mainly caught by hook and line. Since the fishes were caught from nearby area of hatchery site, open containers like troughs, buckets were used with aeration. With an objective of studying the feasibility of long distance transportation of brooder, adult fishes were transported from Papakoil Village (Nagapattinam) to Muttukkadu hatchery site and covering a distance of 354 kms. The transportation duration was 12 hours and 100% survival was obtained. The stocking density was maintained @ 4 Kg, 5 Kg and 6 Kg/m^3 in the transportation tanker. Water quality parameter over transportation duration is presented in Table 1.

Table 1. Water quality parameters in relation to biomass during transportation

	Tank-1	Tank-2	Tank-3
Tank capacity	4000 Liters	4000 Liters	4000 Liters
Biomass of Fish (Kg/Ton)	4	5	6
Transport Duration (Hrs)	11	11	11
Transport Distance (Km)	354	354	354
Sampling Interval(Hrs)	0 6 12	0 6 12	0 6 12
Water temp.(°C)	23.0±29.0±24±1	23±29±24±	23±29±24±
	0.8 1	0.8 1 1	0.8 1 1

Salinity(ppt)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
$_pH$	8.08 ± 0.42	8.09 ± 0.39	8.10 ± 0.52	8.08 ± 0.42	7.82 ± 0.36	7.86 ± 0.42	8.08 ± 0.42	7.8 ± 0.49	7.84 ± 0.52
D.O.(ppm)	5.6 ± 0.7	5.4 ± 0.8	5.1 ± 0.6	5.6 ± 0.4	5.2 ± 0.4	4.9 ± 0.3	5.5 ± 0.5	4.8 ± 0.6	4.2 ± 0.4
Nitrite(ppm)	0.017 ± 0.003	0.021 ± 0.007	0.026 ± 0.006	0.017 ± 0.003	0.02 ± 0.008	0.021 ± 0.007	0.017 ± 0.003	0.036 ± 0.009	0.042 ± 0.010
Ammonia(ppm)	0.025 ± 0.003	0.036 ± 0.007	0.040 ± 0.009	0.025 ± 0.003	0.04 ± 0.009	0.048 ± 0.008	0.025 ± 0.003	0.052 ± 0.011	0.06 ± 0.012

During transportation, the salinity was maintained at 4 ppt. In all the densities water temperature varied from 23°C to 29.0 ± 1°C. No significant variation was observed between densities. pH values were observed to be lowered from the initial value of 8.08 ± 0.42 to 7.80 ± 0.52 in the container with 6kg/m^3 fish with marginal decline. Dissolved oxygen value gradually reduced from initial level of 5.6 ± 0.6 ppm to 5.1 ± 0.6 ppm and 4.9 ± 0.3 ppm respectively in the densities of 3 kg/m^3 and 5 kg/m^3. The initial nitrite content was 0.017 ± 0.003 ppm. As the duration of time increased the nitrite level also increased. The Nitrite level was 0.026 ± 0.006 ppm in lower density (3kg/m^3), 0.031 ± 0.007 ppm in the density of 4kg/m^3 and 0.042 ± 0.010 ppm in higher density of 6kg/m^2 after 12 hours transportation showing gradual increase.

Ammonia concentration was initially 0.025 ± 0.003 ppm, the level increased with duration to 0.040 ± 0.009, 0.048 ± 0.008 and 0.06 ± 0.012 after 12 hours in densities of 4kg/m^3, 5kg/m^3 and 6kg/m^3 respectively showing increasing ammonia concentration with transport duration. The higher concentration of ammonia in higher density when compared to that of lower density is indicating the water quality deterioration. This increase might have due to the high metabolic activities of the fish biomass in high density medium.

Fishes after brought to the hatchery were examined for pathogenic infections. As a prophylactic measurement fishes were treated in the potassium permanganate 1.0 ppm solution for 5 minutes or acrifalvine

1-ppm for 10 minutes or Furazolidone @ 10 ppm for one hour. After conditioning in the acclimatization tanks for ten days, healthy females were transported to fish broodstock holding tanks.

Fishes were fed with trash fish @ 5% of the body weight daily. Broodstock holding tanks were cleaned and water was changed daily. Water quality was monitored regularly. Values of some of the important water quality parameter in the fish broodstock holding tanks (Monthly mean values) are presented in Table 2. The water temperature ranged from 28.77 ± 1.17°C (October) to 32.92 ± 1.3°C(June) and salinity values ranged between 25.54 ± 1.41 ppt in December and 31.91 ± 1.22 ppt in June. pH ranged from 7.28 ± 0.28 during September, to 8.3 ± 0.11 in July. Dissolved oxygen content observed were minimum during June (5.4 ± 0.6 ppm) and maximum during April (7.84 ± 0.95). Maximum concentration of ammonia 0.0854 ± 0.068 ppm was observed during June and minimum value of 0.006 ± 0.03 ppm was recorded during March. Nitrite level in the broodstock holding tanks were between 0.0008 ± 0.04 ppm (December) and 0.0854 ± 0.018 ppm (June). Feed management and water quality maintenance for the broodstock holding tanks are major requirements for the maturation of the captive broodstock. Feeding with trash fish is commonly done in many of the hatcheries. Banchang *et al.* (1989) have suggested the following suitable range of water quality in the broodstock tanks. Temperature 29-32°C, salinity 29-32 ppt, pH 6.8-8-0, dissolved oxygen more than 6 ppm, ammonia less than 0.5 ppm and nitrite less than 1 ppm. In the present study also broodstock tank water quality parameters were maintained within the required range except that of salinity fluctuating from 25.54 ± 1.41 ppt to 31.91 ± 1.22 ppt. The reduction in salinity was due to monsoon rain during these months. The salinity reduction leads to the resorbtion of gonads in the subsequent months and further maturation was observed after May showing the importance of salinity in the gonadal maturity. Providing quality feed is an important factor in the captive maturation, spawning and quality of the eggs of seabass. Banchang *et al.* (1986) observed poor hatching rate of eggs and low survival rate of larvae and fry when the broodstock fishes

were fed with poor nutritional diet. They also further noticed improvement in the condition when the fish were fed with good quality marine fish supplemented with Vitamin E once a week @ 30mg/kg of fish.

Table 2. Water quality parameters of brood stock fish holding tank.

Months 1997	Water temp (°C)	Salinity (ppt)	pH	D.O. (ppm)	Ammonia (ppm)	Nitrite (ppm)
Jan	30.5±1.3	29±0.85	7.67±0.14	6.8±0.88	0.0013±0.04	0.023±0.02
Feb	30.37±0.98	30±1.41	7.89±0.17	7.71±0.33	0.081±0.02	0.071±0.011
Mar	32.1±1.2	30.4±0.92	7.96±0.14	7.3±1.58	0.006±0.03	0.014±0.031
Apr	32.46±1.22	31.3±1.13	7.94±0.03	7.8±0.95	0.0175±0.08	0.018±0.001
May	32.86±1.27	31.15±0.77	8.03±0.11	8.02±0.75	0.013±0.004	0.016±0.002
Jun	32.92±1.3	31.91±1.22	7.31±0.13	5.4±0.6	0.0389±0.027	0.0854±0.068
Jul	31.15±1.15	29.5±1.11	8.3±0.11	6.83±0.56	0.0125±0.006	0.016±0.01
Aug	31.26±0.95	29.95±0.97	7.95±0.15	7.89±0.47	0.077±0.07	0.0585±0.014
Sep	29.79±1.51	30.6±1.09	7.28±0.28	7.53±0.78	0.0155±0.02	0.041±0.034
Oct	28.77±1.17	29.8±0.9	7.36±0.14	7.18±0.19	0.095±0.08	0.004±0.1
Nov	28.96±0.69	27.06±1.2	7.65±0.18	7.77±0.1	0.0415±0.011	0.006±0.003
Dec	28.91±1.14	25.54±1.41	7.75±0.17	7.07±0.11	0.0931±0.04	0.0008±0.04

The health of the captive broodstock has to be monitored carefully since the broodstock is kept at higher density than in their natural habitat and the probable contamination of the broodstock holding system due to accumulation of nitrogenous loading to deterioration of water quality. In the broodstock maintained in captivity the common parasites encountered were the protozoan *Trichodina* sp, monogeneans *Diplectinum latesi* and *Dactylogyrs* sp, crustacean like *Caligus* sp, *Lernanthropus* sp, *Heridinnea* like leach and Bacteria *Vibrio* sp. The intensity of the infection of parasites

was found to be high during November-January where salinity and temperature were recorded low during these months. Mortality of captive seabass due to monogenic parasite *Diplectinum latesi*, has been observed by Rajendran *et al.* (1998a, 1998b). However these problems could be overcome through periodic treatment with 100-ppm formalin for 1 hour for crustacean parasites and with 1-ppm organophosphorus pesticide dichlorvos for 1 hour for monogenic parasite infections.

Maturation and induced breeding

The maturity stages of the fish was assessed regularly. The egg diameter of the captive broodstock fishes during the months from May to October is presented in Table 3. Fishes were found to have varying sized ova in the ovary indicating protracted spawning of individual fish in a season. During the season May-October, the egg size varied from 360μ to 462μ. Fishes with average ova diameter more than 450μ was selected for induction of spawning. Matured males with oozing milt by gently pressing the abdomen were selected for spawning. To accelerate the maturity of females LHRH-a hormone pellet was implanted intramuscularly in three fishes. Results on the influence of LHRH-a pellet implantation on ovarian development is presented in Table 4. The dose of the hormone was @ 100μg/kg body weight of the fish. The hormone pellet was prepared following procedure of Parazo *et al.* (1990) with some modifications. The implanted pellet facilitates the slow release of the hormone accelerating the maturation. Within a period of 20-49 days after LHRH a implantation the ova diameter was found to increase to the required size of more than 450μ. Female fishes with initial average ova diameter 0.4 mm was found to have increased ova dia. of 0.54 mm after 25 days of LHRH a pellet implantation @ 125μg/kg body weight during May 1992 (Mathew Abraham *et al.*, 1998). In the present study also acceleration of maturation was observed when hormone pellet 100 μg/ kg body was implanted in maturing fish.

Table : 3. Acceleration of ovarian maturation by LHRH - a - pellet implantation.

Fish No.	Length (mm)	Weight (kg)	Initial mean ova diameter (μ)	Hormone	Dosage (μg/kg)	Response
1.	860	10	391	LHRHa Pellet	100	During last week of June '97, after 20 days of LHRHa pellet implantation, mean ova diameter increased to 473 μ.
2.	820	9	363	LHRHa	100	After 38 days, during first week of July '97, ova mean diameter increased to 458μ
3.	650	6	346	LHRHa pellet	100	After 49 days, during 3rd week of July '97, the oocyte mean diameter measured was 454μ.
4.	790	8	354	No hormone pellet implantation (Control)		After 45 days (July '97), the mean ova diameter was increased to 398μ. However, the ova attain the mean size of 451μ during the first week of September '97 only after 80 days.
5.	760	7	355	No hormone pellet implantation (Control)		Mean oocyte diameter was found to be 402μ during July '97 and increased to 464μ during first week of October '97 only after 109 days.

Crim (1985) and Crim *et al.* (1987) have stated that the LHRH-a pellet would be gradually released into the circulatory system chronically stimulating the pituitary gonadal axis for an extended time period. They have also demonstrated the release of gonadotropin hormone (GTH) for several days following the *in vivo* single implantation of LHRH-a pellet. The acceleration of gonadal maturation through pellet implantation was demonstrated by other workers also (Naa cario, 1987; Garcia, 1990).

The results of the induced breeding experiment in *Lates calcarifer* during 1997 and 1998 breeding seasons are presented in Table 4. In the first experiment, fishes were treated with LHRH-a hormone initially @ 100μg/kg body weight for females and @ 35μg/kg body weight for males. However, spawning was not successful and plugging of genital pore was observed. In the subsequent trials, the hormone dosage was administered

to the fishes @ 60-70 μg/kg body weight for females and 30-35 μg/kg body weight for males. In these cases spawning was successful after 32-36 hours of hormone injection. In many cases, second spawning was also observed on subsequent days. The total number of eggs per spawning released varied from 0.70 million to 1.46 million. Rate of fertilization was 70 to 80% in the first spawning and in the second spawning it was between 40 and 60%. The fertilized eggs were transparent, floating on the surface with diameter 0.78 mm to 0.80 mm. The unfertilized eggs were opaque and settled at the bottom. Fertilized eggs were collected from spawning tank by scooping/siphoning and transferred to incubation tanks. Eggs were maintained at the density of 80-100 nos/1 for hatching. Hatching took place after 17-18 hours with the water temperature of 27-28°C. The hatchlings were then distributed into larval rearing tanks for rearing.

Induced breeding of seabass was successful with a single dose of LHRH-a @ 60-70 μg/kg body weight for females and 30-35 μg/kg body weight for males but at higher dose of 100 μg/kg body weight it was found to give negative result of plugging. Similar observation of decline in spawning was observed by Garcia (1990) when the fish was administered with higher dose of 150-300 μg/kg body weight of the fish. Possible selfsuppression action by LHRH-a with GTH releasing mechanism would occur when the hormone is administered at higher dose. Peter (1989) has observed suppression of serum GTH level in circulatory system after three daily injection of high dose of super active LHRH-a in mature gold fish. Based on several trials Garcia (1989 a) suggested a dose of 38.75 μg/kg body weight for females for inducing sequential spawning. The present study also showed a single dose of LHRH-a @ 60-70 μg/kg for females and 30-35 μg/kg body weight for males are optimum for successful spawning of seabass. Garcia (1989 b) observed spawning of seabass without hormone injection when the ova diameter was more than 0.5 mm however opined that LHRH-a will be highly effective for obtaining eggs on demand from matured females.

Second spawning was observed on subsequent days in most of the cases. In general the number of eggs obtained in the first spawning was

Table : 4. Details of induced breeding experiments in *Lates calcarifer* (Bloch)

Expt. No.	Date	Length (mm)	Weight (Kg)	Mean ova diameter (μ)	LHR Ha dose		No. of eggs spawned (million)		Spawning time after hormone injection (hr)	Rate of fertilization (%)		Rate of hatching (%)		Remarks
					Female	Male	First spawing	second spawning		First spawing	second spawning	First spawing	second spawning	
1.	24.06 '97	860	10.0	473	100									Plugged. No spawning, even after 48 hrs of injection, stripping attempted. But, eggs not fertilised.
2.	12.07 '97	820	9.0	458	70	35	1.46	1.30	35	70		80		Spawning spontaneous and natural. Second spawning observed 24 hrs after first spawning. First spawned eggs successfully fertilized. But, no fertilization in second spawned eggs.
3.	23.07 '97	650	6.0	454	70	35	4.05	1.00	34	80		90		Spawning spontaneous and natural. First spawning eggs fertilized, second spawning observed 24 hrs after first spawning. But, not fertilized.
4.	06.09 97	790	8.0	451	70	35 30*	1.20	0.90	32	70	40	60	25	Spontaneous and natural spawning. Eggs fertilized in both the spawnings and hatching was successful for second spawning, fresh males were introduced with hormone treatment.
5.	04.10 '97	760	7.0	464	70	35	1.10	1.00	34	80	60	70	53	Spawning spontaneous and natural. Both first and second spawned eggs fertilized and hatched.
6.	12.07 '98	640	6.0	452	70	35	0.78	-	35	60	-	60	-	Spawning successful
7.	28.07 '98	750	7.5	461	70	35	0.68	1.0	35	65	52	68	70	Spawning successful
8.	13.08	600	4.5	453	70	35	0.80	-	34	62	-	71	-	Spawning successful
9.	30.08 '98	760	7.0	454	70	35	1.21	1.0	35	71	62	69	48	Spawning successful
10.	16.09 '98	800	8.5	462	70	35	1.0	1.9	35	60	48	70	51	Spawning successful

more than that of second spawning. Similar observation was made by Almendras *et al.* (1988) in seabass when the fish was induced for multiple spawning by LHRH-a pellet implantation. However, Suzuki (1983) has stated no correlation between the incidence of multiple spawning and eggs fertilizability or hatchability. In the present study also, more number of eggs and higher fertilization rate were observed in two case. However, in some cases fertilization was not effected in the second spawning possibly because the males being small could not cop up with the milt requirement for the subsequent spawning. In one case successful fertilization in the second spawning were effected by replacing the male. In most of the trials, fertilization and hatching were similar to that of first spawning implying that the spawning response depends on the overall fertilization rate roughly around 70-80% in the first spawning and 40-60% in the second spawning. The hatching rate ranged from 60-90% in the first spawning and 25-61% in the second spawning. Lim *et al.* (1986) also observed fertilization rate ranging from 72.8 to 94.9% in the induced breeding trials. However, the rate of fertilization and hatching depends upon the condition of the brood fishes and other factors like water quality etc.

The technology developed at the Central Institute of Brackishwater Aquaculture on captive broodstock maintenance, induced breeding and larval rearing of *Lates calcarifer* is standardized and upgraded for better management. This would help in the large-scale production of seabass in India, the candidate species for diversification of sustainable aquaculture.

Acknowledgement

The authors are indebted to Dr. G.R.M. Rao, Director, Central Institute of Brackishwater Aquaculture for the constant encouragement and guidance for this work.

References

Ali, H.M. 1987. Seabass *(Lates calcarifer)* spawning in tanks in Malaysia. In : J.W. Copland and D.L. Grey (Editors), *Management of wild and cultured Seabass/Barramundi (Lates calcarifer).* Proceeding of an International workshop held at Darwin N.T., Australia, 24-30 October, 1986. ACIAR Proceedings No.20 129-131.

Almendras, J.M., C. Duenas, J. Nacario., N.M. Sherwood., L.W. Crim 1988. Sustained hormone release III. Use of gondadotropin releasing hormone analogues to multiple spawning in seabass, *Lates calcarifer. Aquaculture*, **74** : 97-111.

Banchang T., Sangklod, C., Siit, B., Samodo, K.Y. and Hanungisn 1989. Propagation of seabass *Lates calcarifer* in captivity. *Sea Farming Development Project* INS7811008 - Manual UNDP, 55 pp.

Barlow, C., K. Williams and R. Rimmer, 1996. Seabass culture in Australia. *Info Fish International* 2/96. 26-33.

Crim, L.W., 1985 Methods of acute and chronic hormone administration in fish In : C.S. Lee and L.C. Lias (ED). *Reproduction and culture of Milkfish*, Oceanic Institute And Tungkang Marine Laboratory. 22-24 April, 1985. Taiwan, pp 1-13.

Fortes D. Romeo 1985. Induced spawning of seabass *Lates calcarifer* (Bloch) in the Philippines. In. J.W. Copland and D.L. Grag (Editors). *Management of wild and cultured seabass/Barramundi (Lates calcarifer).* Proc. Inst. Workshop held at Darwin, N.T. Australia. 24-30. Sept. 1986, 123-125.

Garcia, L.M.B., 1989 a. Dose dependent spawning response of mature female seabass *Lates calcarifer* (Bloch) to pelleted Lutenizing Hormone Releasing Hormone analogue (LHRH-a). *Aquaculture* **77** : 85-96.

Garcia, L.M.B. 1989 b. Spawning response of female seabass *Lates calcarifer* (Bloch) to a single injection of Leutinizing Hormone Releasing Hormone analogue: Effect dose and initial oozyte size. *J. Apply. Ichthol.* **6** : 167-172.

Garcia, L.M.B 1990. Advancement of maturation and spawning of *Lates calcarifer* (Bloch) using pelleted Lutenizing Hormone analogue and 17 Methyltestosterone. *Aquaculture* **86** : 333-345.

Kailasam, M., A.R. Thirunavukkarasu, P. Kishore Chandra, P. Shranee and K.V. Rajendran, 1998. Induction of maturity and spontaneous spawning of captive broodstock of Bhetki *Lates calcarifer* (Bloch) through hormonal manipulation. Paper presented in *"National workshop on Recent advances in Hormonal Physiology of fish and shellfish Reproduction"*. CIFA, Bhubaneshwar, 16-18 April 1998, PP-30 (Abstract)

Kungwankiji, P., 1986. Induction of spawning of seabass *Lates calcarifer* by hormone injection and environment manipulation. In. J.W. Copeland and D.L. Grey (Ed). *Management of wild and cultured Seabass/Barramundi (Lates calcarifer).* Proc. Inst. Workshop held at Darwin, N.T., Australia 24-30, Sept. 1986. 120-122.

Lim, L.C., H.H. Heng and H.H. Lee 1986. The induced breeding of seabass *Lates calcarifer* (Bloch) in Singapore. *Singapore J. Primary Ind.* **14** : 81-95.

Mathew Abraham, L.Krishnan, A.R. Thirunavukkarasu, and K. Gopinathan, 1997. First report on development of captive broodstock and induced maturation of the seabass *Lates calcarifer* (Bloch) from India. *J. Mar. Biol. Assoc., India* **39** (122) : 178-182.

MacKinnon, M.R. 1987. Pelleted barramundi show potential for commercial farming. *Australian Fish.*, **46** (7): 42-44.

Nacario, J.F., 1987. Releasing hormone as an effective agent in the induction of spawnning in captivity of seabass *Lates calcarifer*. In J.W. Copland and D.L. Grey (Ed.) *Management of wild and cultured Seabass/Barramundi Lates calcarifer*. Proc. Inst. Workshop held at Darwin N.T., Australia, 23-30, Sept. 1986. 126-128.

Parazo, M.M., L.M.B. Garcia, F.G. Ayson, A.C. Fermin, J.M.E. Almendros, D.M. Reyers and E.M. Avila Jr. 1990. Seabass hatchery operation. *Aquaculture Extension Manual* Np. **18**. SEAFDEC. 36 pp.

Peter, R.E., 1980, Serum gonadotropin levels in mature male gold fish in response to Lutenizing hormone releasing hormone (LHRH) and Des. Glu. 10 (D.Ala). LHRH ethylamide. *Can. J. Zool.* **58** : 110-104.

Rajendran, K.V., A.R. Thirunavukkarasu, Mathew Abraham and M. Kailasam 1998. Disease and Pathogens of seabass *Lates calcarifer* (Bloch), *Fishing chimes* (1998). Vol.No.8. 16-18.

Rajendran, K.V., Ar.R. Thirunavukkarasu and T.C. Santiego, 1998. Mortality of captive seabass *Lates calcarifer* (Bloch) due to monogenic parasite, *Diplectanum latesi*, Tripathi 1957. *J. Aquacul. Tropics* (In press).

Sakarsin, 1982. The experiment an cage culture of seabass *Lates calcarifer* (Bloch) in different growing densities. Working paper. *Second symposium on Brackishwater fish culture*, Brackishwater Research Division, Thailand.

Suzuki, R. 1983. Multiple spawning of the cyprinid toad *Misgurnis anguillicauddes*, *Aquaculture* **31** : 2333-243.

Thrunavukkarasu, A.R. (1997) Marine fin fish seed production. Paper presented in "Indo-Egyptian Workshop", CMFRI, Kochi, Dec. 8-9, 1997.

Wangsommunk. S. and S. Manevonk, 1973. Results of experiments and artificial breeding and larval rearing of seabass *Lates calcarifer* (Bloch) *Songkhla Marine Fisheries station*, Thailand. Contribution No.5, 20 pp.

Description of a simple prototype pelletizer for preparation of hormone pellets

L. Krishnan

Central Marine Fisheries Research Institute, Cochin - 682014

ABSTRACT

The design and working of a simple prototype pelletizer for preparation of hormone pellets, to be used in cultivable fishes, for inducing maturation as well as spawning is described.

Introduction

Administration of reproductive hormones in the form of pellets has come to be accepted in recent years as the method of inducement for maturation and spawning in fishes. It has already been established that implantation of hormone in a pelleted-powder matrix assures long term hormone delivery in the treated animal. The fact that the high potency analogues of Luteinizing hormone releasing hormone (LHRHa) in a cholesterol matrix pellet is able to provide prolonged releasing hormone activity has been demonstared in mammals (Kent *et al.*, 1980), and fish (Crim *et al.*, 1983). Two distinct advantages are offered by the pelleted hormone technique. Pellets are easy and cheap to fabricate and pellets maximise the effect of hormonal application in species where prolonged and elevated hormone levels are a pre-requisite for spawning (Garcia, 1989). Crim (1985) describes methods for acute and chronic hormone administration in fishes.

Lee *et al.* (1986) described a cheap and simple device for pellet making using 2 plastic sheets with holes drilled in one of them to form the pellet die. Many workers like Sherwood *et al.*,(1988) used a pellet press - parr instrument Co., Moline IL in making the pellets.

This paper describes the design and working of a low cost, portable and simple pelletizer.

Description of pelletizer

The instrument (Fig. 1) is made of two 'S' shaped cast iron pieces secured in the anterior 2/3 rd with a thread nut and bolt forming the fulcrum, but still allowing free movements of both the limbs like a 'Plier'. The 'handle' portion of the instrument is 14 cms long, rectangular in cross section and has a thickness of 1 cm The anterior short portion in front of the fulcrum is 6 cms. long. The anterior upper and lower arms are provided with a cylindrical piston (4.5 cms long and 3mm dia) and the socket (length 3.4 cms and dia. 3.3 mm) respectively which help in the making of the pellet.

A rectangular piece of cast iron (2.5 cms long/5mm thick) is fixed vertically at the centro-dorsal portion of the anterior dorsal arm. The top portion of this piece is cut into a 'U' shaped slot. The central portion of a 'M' shaped iron lever (2mm thick and 5mm wide) is loosely hinged on to the 'U' shaped slot at the top of the vertical piece of cast iron with the help of a pin allowing free movement of the lever backwards and forwards. The posterior arm of this 'M' shaped lever is attached to a handle lever in a loose manner with the help of a hinge pin. The handle lever is also supported in the middle with the help of a thread bolt 4 cms long. The

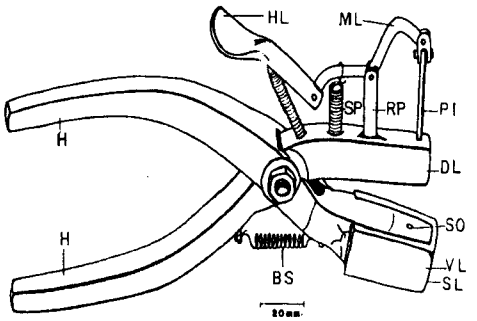


Fig. 1. Line drawing of the pelletizer showing the different parts.

LEGENDS

H	-	Handle
HL	-	Handle lever
SP	-	Spring
ML	-	M Shaped Lever
RP	-	Rectangular Piece of Iron
PI	-	Piston
DL	-	Dorsal Lip
SO	-	Socket
VL	-	Ventral Lip
SL	-	Sleeve
BS	-	Basal Spring

upper end of this bolt is shaped like a bulb and fits itself loosely into the groove like lower portion of the handle lever in one way retaining it and in another way acting as fulcrum on which the handle lever moves. The thread bolt itself is screwed into a slot drilled on the dorsal lip of the instrument.

When the handle lever is pressed, the posterior tip of the 'M' shaped lever is raised and indirectely lowering the anterior tip since the lever is loosely hinged in the centre. A 2 cms long spring attached a little ahead of the posterior edge of the 'M' shaped lever (and further fixed on the dorsal side of the instrument) allows the 'M' lever to return to its original position and in turn allows the handle lever also to come to the resting position, when the pressure on the handle lever is lessened.

The anterior tip of the 'M' lever is attached to a 4.5 cms long cylindrical piston having a dia. of 3 mm. The 'M' lever and the piston are connected through a movable link piece allowing free movement. The piston also passes through a neatly drilled cylindrical socket cutting through the dorsal lip of the instrument vertically. A portion of the piston always remains inside the socket even when the anterior upper and lower arms of the pelletizer are apposed to each other. The internal dia. of the cylindrical socket is 3.3 mm and measures 1.7 cms in length.

The anterior portion of the ventral lip of the pelletizer is also rectangular and has a well polished upper surface with a width of 1.7 cms. In the centre of this polished surface is also a vertically drilled cylindrical socket of an internal dia.of 3.3 mm and going down to a length of 1.7 cms and opening on the ventral side of the ventral lip. This cylindrical socket is positioned such that once the upper and lower lips of the pelletizer are apposed to each other the sockets of the respective lips become contiguous with one another forming a single socket.

When the handle lever is pressed, the cylindrical piston, already having passed through the cylindrical socket on the dorsal lip also moves freely through the socket on the ventral lip and juts a little outside the ventral side of the ventral lip of the pelletizer.

The rectangular dorsal surface of the lower lip of the pelletizer has two longitudinal grooves on either side. These grooves facilitate sliding

in of a metallic, box like rectangular sleeve (Length 3.8 cms, 2.1 cms wide) which has its upper portion exposed. Once this sleeve is fitted to the dorsal grooves and pressed, it slides inside fitting tightly like a jacket on the lower lip of the pelletizer and at the same time closing the opening of the cylindrical socket on the ventral side of the ventral lip. Once the sleeve is removed, the ventral opening is exposed.

The upper and lower lips of the pelletizer are kept opened with the help of the basal spring attached horizontally on both the arms of the pelletizer.

Working of the pelletizer

The pelletizer is useful in the preparation of pellets containing LHRHa hormone in a cholesterol matrix, the methodology of which is described by Lee *et al.* (1985). After a thorough cleaning of the instrument, the metal sleeve is first slided on to the anterior lip of the ventral arm of the instrument. The dried hormone mixture kept ready is then filled little by little into the socket on the ventral lip. Intermittently the mixture is compressed by depressing the handle lever. Once the socket is $3/4^{th}$ full, the remnants of the hormone mixture sticking on the sides of the sockets are all carefully shoved into the socket. The hormone mixture is then compressed progresively harder using the handle lever controlling the piston. The metal sleeve is then slided out exposing the ventral opening on the ventral lip. The pellet is then ejected out by pressing the handle lever carefully and the pellet is collected in a container. The pelletizer is then cleaned using a cloth dipped in alcohol helping the removal of the sticky cholesterol remnants. At a time a single pellet of 10 mm length with 3 mm dia. can be extruded by operating the pelletizer. This pellet can be cut into two using a sharp blade. The pellet is then dried for two hours inside an incubator (set at 37° C). The pellet can further be kept closed inside a glass vial padded with cotton and stored in a refrigerator for further use.

Discussion

Procedural guides to the production and implantation of LHRHa cholesterol pellet into milk fish is given by Lee *et al.* (1985,1986). Crim (1985) described methods for acute and chronic hormone administration in milk fish. But it was Lee *et al.* (1986) who devised a simple method for

making of pellets using two plastic plates. The pellet mould consists of one plastic plate with 15 holes drilled on to it. An undrilled plastic sheet of similar diamension was used as a base for the drilled sheet. The hormone mixture is filled into the holes of the top sheet using a nail and compressed well with a hammer. The pellets 2.4 mm in dia. by 5.0 mm are then ejected out pushing them out with a hard substance. The design and model of the hormone pelletizer given in this work is the first of its kind in India. The pelletizer is portable, easy to make and handle, and very useful in the making of pellets even in field conditions. The pellets made using this device were tried in *Lates calcarifer* with good success (Anon, 1992).

Acknowledgements

The inspiration, support and funding for designing this instrument given by Dr.K.Alagarswamy, Director, C.I.B.A., Chennai is gratefully acknowledged. Thanks are also due to Dr.K.V.Ramakrishna and Sri.K.N.Krishnamurthy of C.I.B.A., Chennai for their consistent encouragement.

References

Anon, 1992. Towards the development of captive broodstock of Bhekti. Progress at C.I.B.A., *Fishing Chimes*, **12(** 6): p.32.

Crim, L.W., 1985. Methods for acute and chronic hormone administration in fish. In : C.S. Lee and I.C Liao (Editors) *Reproduction and culture of milk fish.* Oceanic Institute and Tungkang Marine Laboratory,Taiwan: 1-13.

Crim, L.W., D.M. Evans, and B.H. Vickery, 1983. Manipulation of the seasonal reproductive cycle of the land locked salmon (*Salmo salar*) by LHRH analogues administred in various stages of gonadal development. *Can. J. Fish Aquat. Sci.* **40** : 61 - 67.

Crim, L.W., A.M. Sutterlin, D.M. Evans, and C. Weil, 1983. Accelerated ovulation by pelleted LHRH analogue treatment of spring spawning rainbow trout (*Salmo gairdneri*) held at low temperature. *Aquaculture*, **35** : 299 - 307.

Garcia, L. Ma.B., 1989. Dose depended spawning response of mature female sea bass *Lates calcarifer* (Bloch), to pelleted luteinizing hormone-releasing hormone analogue (LHRHa). *Aquaculture*, **77** :. 85 - 96.

Kent, J.S., B.H. Vickery, and G.I. Mc Rae, 1980. The use of cholesterol matrix pellet implant for early studies on the prolonged release in animals of agonist analogues of luteinizing hormone releasing hormone. *The int. symp. Controlled release of bioactive materials.* Fort Lauderdale. Florida, U.S.A.

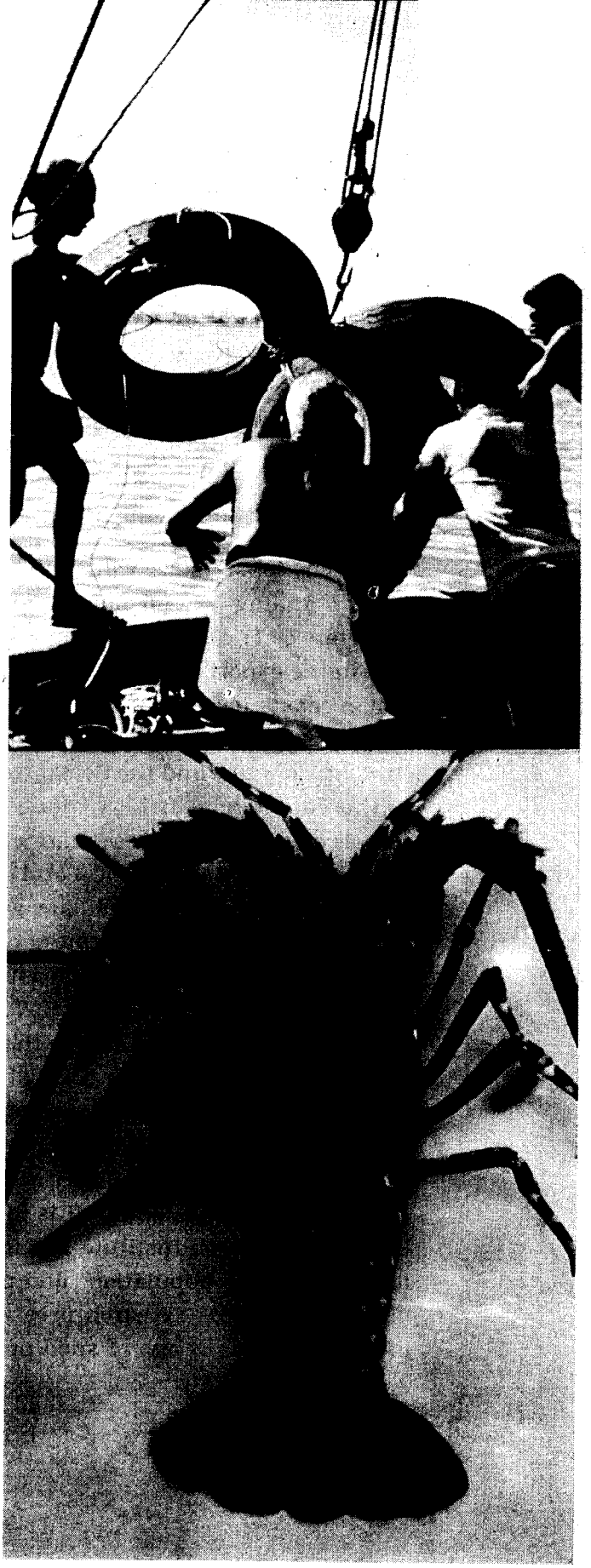
Lee, C.S., C.S. Tamaru, and L.W. Crim, 1985. Technique for hormone implantation for the milk fish *Chanos chanos* (Forskal) In : C.S. Lee and I.C. Liao (Editors). *Reproduction and culture of milk fish.* Oceanic institute and Tungkang Marine Laboratory : 215 - 226.

Lee, C.S., C.S. Tamaru, and C.D. Kelly, 1986. Technique for making chronic release LHRHa and 17 α methyltestosterone pellets for intra muscular implantation in fishes. *Aquaculture*, **59** : 161 - 168.

Sherwood. N.M., L.W. Crim, J. Carolsfed, and S.M. Walters, 1988. Sustained hormone release. 1. Characteristics of an *invitro* release of gonadotropin-releasing hormone analogue (Gn RH-A) from pellets. *Aquaculture*, **74** : 75 - 86.

Creation of artificial habitat for spiny lobsters in the sea off Vellapatti, Gulf of Mannar

M. Rajamani
Tuticorin Research Centre of CMFR Institute, Tuticorin-628 001



ABSTRACT

An experimental artificial habitat for spiny lobsters was created in the sea off Vellapatti, a fishing village near Tuticorin in the Gulf of Mannar during June 1997. A total of 49 modules fabricated out of 147 stoneware pipes were used to create the artificial habitat which covered a floor area of approximately 1000 sq. m. Inhabitation of lobsters in the artificial habitat was recorded for the first time three months after the installation of the modules. Both Panulirus ornatus *and* P.homarus *were encountered in bottom set gill net catches operated in the vicinity of the artificial habitat.*

P.ornatus *was the dominant species constituting on an average 76.8 % of the total lobster catches. The size (total length) of the lobsters captured from the artificial habitat ranged from 115 to 255 mm and from 135 to 165 mm in* P.ornatus *and* P.homarus *respectively. The importance of artificial habitat in the production, conservation and optimum exploitation of the spiny lobster resources from the sea is discussed in the paper.*

Introduction

Spiny lobsters are valued as one of the prime sea foods all over the world standing second only to shrimps in terms of their commercial importance. The increased demand for live lobsters and frozen lobster tails in the export market has given a new thrust to lobster fishing along Indian coasts recently. There are six species of spiny lobsters in Indian waters, out of which only two species namely the Ornate spiny lobster *Panulirus ornatus* and the Scalloped spiny lobster *P.homarus* contribute to the commercial fishery along Tuticorin coast. Their life cycle is complex requiring diverse marine habitats ranging from open ocean to inshore seagrass beds and coral reefs (Cobb and Phillips, 1980). The juveniles of spiny lobsters are gregarious in nature and concentrate around rocky outcrops, groups of urchins and sponges and feed on echinoids and small molluscs mostly during night time (Khandker, 1964; Davis, 1971 and 1978; Berrill, 1975). The availability of suitable shelters adjacent to the foraging area is the major limiting factor for the survival of juvenile lobsters. Such natural ecosystem has been damaged along Indian coast to a very great extent both by adverse environmental conditions and human interference resulting in a gradual decline in the population of spiny lobsters in Indian waters over the years (Kagwade *et al.*, 1991; CMFRI, 1995). It is in this context that the Central Marine Fisheries Research Institute has undertaken various research activities to replenish the population and increase the production in the sea as part of its various research programmes in mariculture. Development of artificial reefs is one of such programmes.

The technology of artificial reef is primarily based on the simple principle that the crevices present in the artificial structures provide shelter and thus protect the organisms from predators. Incidentally, such objects also serve as base for the development of algae and for the attachment of various sedentary organisms which ultimately results in the formation of a fertile feeding ground for the organisms while they themselves taking shelter in the crevices. However, later it was found out by various investigators that the instinctive behaviour of the organism is one of the major factors that constitute the fundamental linkage between the object and the organism. The present paper gives an account on the creation of artificial habitats (artificial reefs) for spiny lobsters in the sea off Vellapatti, a coastal village near Tuticorin in the Gulf of Mannar, involving artisanal fishermen.

Vellapatti is a small fishing village about 10 km north of Tuticorin on the south-east coast of Tamil Nadu. Fishing is the main avocation of the villagers with nearly 95% of the population engaging in activities connected with fishing. They operate mostly bottom-set gill nets for fishing crabs and lobsters at a depth of about 5 to 10 m. The craft used for the fishery is vallam (plank-built boat). Although fishing activities are going on round the year, their income from the fishing is meagre most of the months due to poor catch and most of the villagers live below poverty line. In order to create an awareness among the traditional fishermen on the importance of artificial habitat as a tool to increase spiny lobster production in the sea, Vellapatti was selected as the sea off Vellapatti was found to be suitable for creation of artificial habitat for spiny lobsters and also as the fishermen were cooperative. Detailed discussions were held with the fishermen and members of the fishermen society of Vellapatti and the role of artificial habitats in increasing the lobster resources in the sea was explained to them before actually commencing the work.

Materials and methods

A total of 147 stone- ware pipes, each pipe with a length of 60 cm and a diametre of 20 cm were used, out of which 49 modules were

fabricated on the shore at Vellapatti during the last week of May, 1997. Each module consisted of three pipes i.e two pipes were placed at the bottom horizontally over which the third pipe was placed. All the three pipes were tied firmly with a nylon rope. They were loaded into five vallams (country boats) belonging to the fishermen and were transported to the sea off Vellapatti in the morning on 1st June 1997. After locating the suitable ground the modules were carefully lowered into the sea and placed on the floor at a depth of about 6 metres between Van Island and Karsewar Island. After all the modules were released, their position was observed by a SCUBA diver. The area covered by the modules is estimated to be approximately 1000 sq. m.

After the installation of the modules on the floor of the sea fishing was carried out by the commercial fishermen as and when the conditions were favourable and the catches were brought to the landing centre. The landing centre was visited weekly once and the catches of lobsters and other species landed from the artificial habitats were recorded. Only lobsters were measured for their total length and carapace length. The weight of the individual lobsters also was taken.

Results and discussion

Fishing was carried out in the artificial reef area by the fishermen of the Vellapatti village using bottom-set gill nets as and when the conditions were favourable for fishing. Regular fishing from the artificial reef area commenced during the month of September '97. Thereafter, fishing was carried out in the artificial reef area in October '97 and then in January and February '98. The catch of lobsters recorded during October, January and February was in the order of 5.4, 16.5 and 17.7 kg constituting 9.1 %, 5.0 % and 2.0 % of the total catches landed by the bottom-set gill nets during the three months respectively (Table 1). Both the species of spiny lobsters (*P.ornatus and P. homarus*) were encountered in the catches. *P.ornatus* was the dominant species with an estimated catch of 30.4 kg forming 76.8 % , the rest being formed by *P.homarus* (Table 2). From 1.19 kg the catch of *P.ornatus* increased to 16.2 kg in

January and then showed a marginal decline in February with an estimated catch of 13.0 kg. In the case of *P.homarus* the catch was more or less at the same level in October and February (4.2 and 4.7 kg) with an insignificant landing of 0.3 kg in January. It may be mentioned here that the composition of *P.homarus* was high initially i.e in October, forming 78.0 % of the total lobster catches. Although the catch was maintained at the same level in February also, its composition was only 26.6 % as *P.ornatus* became the dominant one.

Table 1. Catch and effort of spiny lobsters from the artificial reef area

Months	Fishing days	No. of Units	Estimated total catch (kg)	Lobsters kg	Lobsters %	C/E
October '97	27	9	59.4	5.4	9.1	0.6
November	No fishing					
December	No fishing					
January '98	16	24	326.5	16.5	5.0	0.7
February	24	48	869.7	17.7	2.0	0.4
March	No fishing					
Total	67	81	1255.6	39.6	3.2	0.49

Table 2. Species composition of lobsters in the artificial reef area (%)

Months	Estimated total catch of lobsters (kg)	*P.ornatus* Kg	*P.ornatus* %	*P.homarus* Kg	*P.homarus* %
October '97	5.42	1.19	22.0	4.23	78.0
November	No fishing				
December	Nofishing				
January '98	16.5	16.2	98.4	0.27	1.6
February	17.7	13.0	73.4	4.7	26.6
March	No fishing				
Total	39.6	30.4	76.8	9.18	23.2

The size (total length) of *P.ornatus* collected from the artificial reef area ranged from 115 to 255 mm in male and from 155 to 235 mm in

female. Large-sized lobsters were recorded in the catches landed during January in both sexes.

Although detailed information is available on the creation of artificial habitats for fin fishes in various parts of the world, information on the creation of artificial habitat for spiny lobsters are rather limited. Chittleborough (1970) has reported that in Western Australia, juvenile populations of *P.cygnus* are limited by the availability of diurnal shelter near the centre of their range. According to Davis (1985) the structures provide a temporary shelter for the lobsters and a refuge from noise, turbidity and physical disruption during the marina construction. According to him shelter may become a limiting factor for lobster production only when there is high recruitment in the fishery. Otherwise such structures may simply invite population from other areas without any significant increase in the production. According to Herrnkind *et al.* (1975) the availability of shelters adjacent to the foraging area is the major limiting factor for the survival of the Florida spiny lobster, *P.argus*.

The resource potential of spiny lobsters in the fishing grounds along Tuticorin coast in the Gulf of Mannar has been studied in detail by Kagwade *et al.* (1991) and Rajamani and Manickaraja (1991; 1995; 1997a; 1997b). Kagwade *et al.* (1991) have warned that as the population of spiny lobsters in Indian waters is not dense enough to support a sustainable fishery, effort should not be increased to capture more of *P.ornatus* and *P.homarus* from the present fishing grounds. They have also suggested that trawling should be intensified in deeper waters as the concentration of lobsters in the shallow waters is very much limited. It is suggested, based on the results obtained from the present investigation, that artificial habitats may be created in shallow waters in certain selected places along Indian coast both for increasing the production and for conservation of the spiny lobster resources from over exploitation.

The investigation carried out in the sea off Vellapatti has clearly shown that after the installation of artificial shelters there is a remarkable increase in the landing of lobsters as admitted by the local fishermen. On seeing the effect of artificial shelters on lobster landing the

fishermen of the village are more enthusiastic and they want to take up the programme on a large scale. If vast areas are covered with several modules it becomes a commercial fishing ground. What has been created in the sea off Vellapatti is only one small unit. Many such small units form one group and several such groups form one zone. Such zones become the fishing ground for commercial fishing activities. The present investigation carried out by the Central Marine Fisheries Research Institute involving artisanal fishermen has clearly demonstrated that such artificial habitat attract spiny lobsters and become permanent habitats for them. As the installation of artificial habitats involves huge investment, both Government and non- Government Organizations should come forward to help the fishermen societies so that the programme can be taken up on a large scale covering many coastal villages so as to increase the production in the sea which will benefit the fisherfolk to a very great extent.

Acknowledgements

The author is grateful to Dr.M. Devaraj, Former Director, Central Marine Fisheries Research Institute, Cochin, for encouragement and providing necessary facilities to carry out the investigation. He wishes to express his thanks to Shri O.M.M.J.Habib Mohamed, Technical Assistant, for his help in the collection of data.

References

Berril,M. 1975. Gregarious behaviour of the spiny lobster *Panulirus argus* (Crustacea: Decapoda). *Bull. Mar. sci.* ***25:*** 515-522.

Chittleborough,R.G. 1970. Studies on recruitment in the Western Australian Rock Lobster, *Panulirus longipes cygnus*. Density and natural mortality of juveniles. *Aust. J. Mar. Freshw. Res.* ***21:*** 131-148.

CMFRI, 1995. Marine fish landings in India during 1985 - 95. *Mar. Fish. Infor. Serv.* T & E *Ser.* ***136:*** 1-28.

Cobb, J.S. and B.F.Phillips, eds. 1980. The biology and management of lobsters, Vol. II. *Ecology and Management. Academic Press,* San Francisco. 330 pp.

Davis, G.E. 1971. Aggregations of spiny sea urchins, *Diadema antillarum* as shelter for young spiny lobsters, *Panulirus argus. Trans. Amar. Fish. Soc.* ***100:*** 586-587.

Davis, G.E. 1978. Field evaluation of a tag for juvenile spiny lobsters, *Panulirus argus. Trans. Amar. Fish. Soc.* ***107:*** 100-103.

Davis, G.E. 1985. Artificial structures to mitigate marina construction impacts on spiny lobster *Panulirus orgus. Bull. Mar. Sci.* **37** (1): 1-2.

Herrnkind, W.F., J.A.Vanderwalker and L.Barr. 1975. Population dynamics, ecology and behaviour of spiny lobsters, *Panulirus argus,* of St. John, U.S.V.1. : (IV) habitation, patterns of movement and general behaviour. Pages 31 - 45 *In :* S.A.Earle and R.J. Lavenberg, (eds.) *Results of the TEKTITE program: coral reef invertebrates and plants. Nat. Hist. Mus. Los Angeles County Sci. Bull. 20.*

Kagwade, P.V., M. Manickaraja, V.D. Deshmukh, M. Rajamani, E.V.Radhakrishnan, V.Suresh, M.Kathirvel and G.S.Rao. 1991. Magnitude of lobster resources of India. *J. mar. biol.Ass. India.* **33** (1 & 2): 150-158.

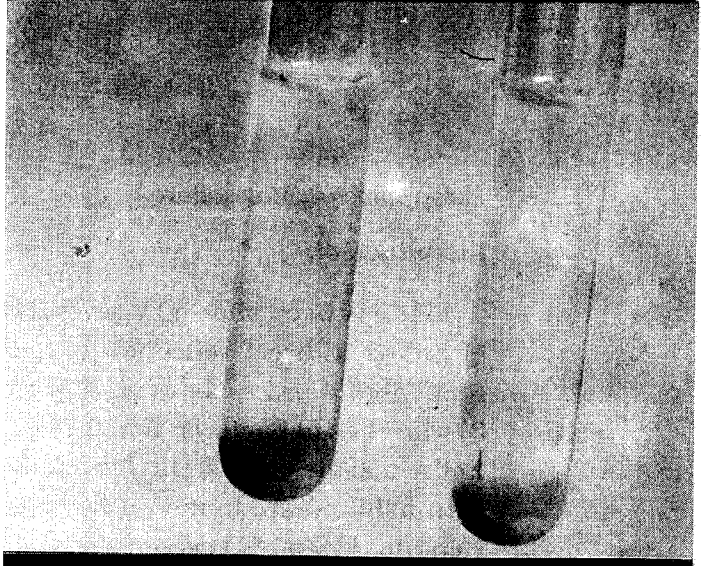
Khandker, N.A. 1964. Sponge as a shelter for young spiny lobster. *Trans. Amer. Fish. Soc.* ***93:*** 204.

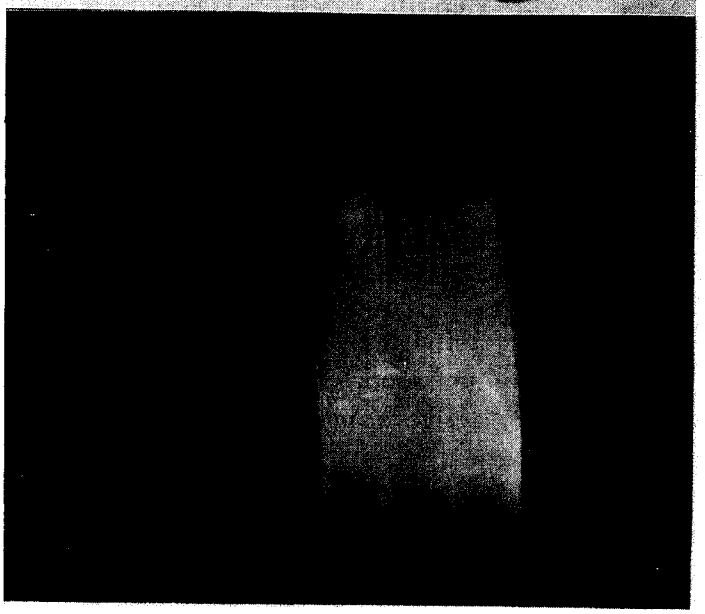
Rajamani,M. and. M.Manickaraja 1991. On the collection of spiny lobsters by skin divers in the Gulf of Mannar off Tuticorin. *Mar. Fish. Infor. Serv.* T & E. *Ser.* No. ***113.*** 17-18.

Rajamani, M. and M. Manickaraja. 1995. Fishery of the Painted Crayfish *Panulirus versicolor* in the Gulf of Mannar. *Mar. Fish. Infor. Serv.* T & E. *Ser.* No. ***140:*** 6-7.

Rajamani, M and M. Manickaraja. 1997 a. On the fishery of the spiny lobster off Tharuvaikulam, Gulf of Mannar. *Mar. Fish. Infor. Serv.,* T & E *Ser.* No. ***146:*** 7-8.

Rajamani, M. and M. Manickaraja 1997 b. The spiny lobster resources in the trawling grounds off Tuticorin. *Mar. Fish. Infor. Serv. T&E. Ser.* No. ***148:*** 7-9.





Studies on the oxidative positive gram negative rods in the perennial and pokkali fields around Cochin

S. Pramila and V. Chandrika
Central Marine Fisheres Research Institute, Cochin-682014

ABSTRACT

Out of the three observations made during the period May and June 1997, totally 13 isolates of oxidase positive gram negative rods were encountered from water and sediment samples of perennial and pokkali fields. Three strains were producing green fluorescent pigment. Fluorescent pigment production was enhanced by dipotassium hydrogen phosphate when compared to glycerol and magnesium chloride. Five cultures showed antagonistic activity against all the test pathogens, namely, Vibrio anguilarum, Mycobacterium *and* Cytophaga *and were found highly resistant to penicillin.*

Introduction

It has been shown by several workers that the normal bacterial flora of fish is a direct reflection of bacterial population of the environment they inhabit (Bauman *et al.*, 1971).It has been observed that as many as 37 varieties of bacterial flora are encountered in fresh water and 40 types in the marine ecosystem. *Pseudomonas* species are frequently associated with fish and are found on fish eggs, skin, gills and intestine (Inglis and Hendrie, 1993). As *Pseudomonas* species are so widespread and numerous, they may at times become involved in disease processes and act as secondary invaders of fish due to stress and other factors. Some species are reported as primary pathogens, principally, *Pseudomonas anguillisceptica*, *Pseudomonas fluorescens* and *Pseudomonas putida* are also found to cause septicaemia in finfish, brownspot disease in shrimps and bacterial necrosis in molluscs.

The *Pseudomonas* species are reported to be having antimicrobial activity against other microorganisms (Laine *et al.*, 1996). Padilla (1990) has reported that the bacteriocin of *Pseudomonas* spp. strain R10 was active *in vitro* against several enteropathogenic bacteria.

In the present study an attempt was made to obtain the oxidase positive nonfermentative, gram negative rods of perennial and seasonal ponds around Cochin and identify and isolate the pathogenic *Pseudomonas* species. Also, the characterization of the bacteria, including morphology, growth characters, biochemical activities, utilization of organic compounds for growth, sensitivity to antimicrobial agents and antagonistic activities were studied based on standard procedures (Shewan *et al.*, 1960).

Materials and methods

Samples from pokkali and perennial ponds located near Narakkal in Vypeen island were collected during the period from May and June and the hydrological parameters were determined. Samples were subjected to quantitative and qualitative analysis as per the scheme given (Table 1)

Table : 1. Pattern of analysis of samples for isolation of *Pseudomonas* spp.

water and
sediment samples
↓
enrichment in
malachite green broth
↓
isolation by pour plate method
Seawater +TTC
Pseudomonas agar + TTC
Cetrimide agar + TTC
↓
selection of *Pseudomonas*
by cytochrome oxidase test positive
pattern of glucose metabolism
in H&L test
↓

Oxidative		Alkaline	No reaction
↓	↓	↓	↓
green pigment	no pigment	no pigment	no pigment
↓	↓	↓	↓
Pseudomonas (Group I)	*Pseudomonas* (Group II)	*Pseudomonas* (Group II)	*Pseudomonas* (Group IV)

Total plate count and identification of the colonies are carried out using three media namely, seawater agar, *Pseudomonas* agar, for fluorescein (Hi Media) and cetrimide agar (Hi Media). Addition of Triphenyl Tetrazolium Chloride (TTC) in the selective media reduces the tetrazolium salt by bacterial oxidative enzymes and leads to the formation of a water insoluble red coloured compound, Formazan, which helps in identifying the colonies.

Identification of colonies was done based on two basic tests namely,

the oxidase test and Hugh and Leifson's oxidation fermentation test. Oxidase test is carried out using the cyto-chrome oxidase reagent-N',N',N', tetramethyl paraphenylene diamine dihydrochloride in which a filter paper is dipped and the culture is streaked using a loop. Immediate appearance of a deep purple colour indicated the positive reaction. Hugh and Leifson's medium is prepared and distributed into tubes, which is sterilized and stab inoculated to find out the glucose metabolism.

Composition of Hugh and Leifson's medium

Seawater agar: Peptone-1%, Agar-agar-2%, Ferric phosphate- 0.01%, Aged seawater-100ml, pH-7.2, 15 lbs., 30mins.

***Pseudomonas* agar:** Tryptone-1%, peptone-1%, agar agar-1.5%, Dipotassium hydrogen phosphate-0.15%, magnesium sulphate-0.15%, distilled water-100 ml; pH-7.2+/- 0.2%. 15lbs, 15mins.

Cetrimide agar: Beef extract-1%, peptone-1%, sodium chloride-0.5%, cetrimide-0.03%, agar-agar-1.2%, distilled water-100ml, and pH at 25°C 7.3+/-0.1.

Hugh and Leifson's glucose: Peptone-1%, sodium chloride-0.5%, glucose-1%, agar-agar-0.3%, distilled water-100ml, phenol red-1cc/100cc of 0.1% solution , dipotassium phosphate-0.3%.

The following tests were conducted to arrive at the species level identification:

1. Growth at 5°C and 37°C
2. Growth at different salt concentrations.
3. Hydrolysis of organic compounds like starch, arginine, casein, gelatin etc.
4. Utilization of citrate as sole carbon source.
5. Penicillin sensitivity.

Because of importance of *Psendomonas* spp in bacterial denitrification processes, utilization of nitrogenous compounds like urea, asparagine, cysteine, glutamic acid, aniline ammonium chloride, ammonium oxalate etc were tested in peptone water containing glucose and the respective nitrogen compound.

Antibiotic sensitivity studies: The sensitivity tests were carried out as a means to arrive at the species level identification. Penicillin (15 mcg per disc), Kanamycin (30mcg per disc), Ampicillin (30mcg per disc) etc. were used to study the sensitivity effects of these antibiotics.

Antagonistic activity studies: Cross streaking method and agar diffusion method were used toughened out the inhibitory effects of the pathogens.

Well plate method: The culture centrifugate was swabbed on the SWA agar medium and wells were cut in the medium to which pathogen broth cultures were added. Presence or absence of growth indicates the inhibitory action of *Pseudomonas* on the test pathogens. In the cross streak method, the test pathogens were streaked across the *Pseudomonas* culture in the petri plate, containing medium.

Results and discussion

It was found that the optimum count of forming units were obtained in dilutions of 10^3 for water in both perennial and seasonal ponds and at 10^4 for sediment., cetrimide agar media did not support the growth of environmental bacteria. It was found that surface water gave more count compared to sediment. In the present study conducted in early monsoon and monsoon period, high numbers of *Pseudomonas* occurred in the selective medium, *Pseudomonas* agar, F. Maximum bacterial populations were observed during monsoon months and the primary environmental factors supporting the pathogenic *Pseudomonas* included moisture, temperature, acidity, organic and inorganic matter supplied.

The fertility of the pokkali field was indicated by the colour of fermentation and hydrogen sulfide production in TAB BART Bio indicator medium, as the colour was more in pokkali water sample. 31 motile gram negative rods were found to be oxidase positive, out of which 13 were identified to be non fermentative based on oxidation fermentation characters in Hugh and Leifson's glucose medium.

In the H&L medium, seven isolates (22.58%) were found to show oxidative type of metabolism and four (12.9%) showed alkaline. Two (6.45%) of the isolates did not show any change. These isolates were

identified to be belonging to different groups (*Pseudomonas* Group I, Group II, Group III and Group IV) based on further evidences like presence of fluorescent pigment.

Three of the isolates were showing green fluorescent pigment. It is well known that the fluorescent pigment production property is unstable and is dependent on the nature of the medium for its manifestation, which was found to be enhanced by the addition of dipotassium hydrogen phosphate to the medium.

Six of the cultures (46.15%) were found to produce hydrogen sulphide in cysteine medium. Out of the 13 cultures 38.46% exhibited moderate growth as well as poor growth. 15.38% exhibited no growth at all. Poor growth in 5°C indicated *P. fluorescens, P. putida and P. anguillisceptica.* Growth at 37°C was there for P.*fluorescens* and *P.putida.* But *P.anguillisceptica* was unable to grow at 37°C. Inspite of these results it is seen that temperature alone cannot be used for the polarly flagellated gram negative rods because of their normally wide temperature range.

Most of the isolates were found to be growing well in peptone glucose medium without sodium chloride and in media containing 5%NaCl. Higher concentrations of sodium chloride (7% and 10%) did not support the growth of bacteria. Another feature was the absence of fluorescence in the medium. At 7% NaCl concentration only one isolate was showing good growth, which was identified as *Alteromonas piscicida.* Isolates incapable of growing at higher concentrations of NaCl were designated as *P. anguillisceptica.*

Proteolytic and amylolytic activity of the isolates were tested and the results showed that 53.84% of the isolates liquefied gelatin and 76.92% fermented arginine. Amylolytic activity of the isolates was poor since only one isolate hydrolysed starch.

The utilization of citrate as sole carbon source also formed important criterion in identifying the species. 69.73% were capable of using citrate. Species level identification is given in the Table 2.

Table 2 - Species identification tests for identification of *Pseudomonas* spp.

Culture No.	Growth at different concentrations of Sodium Chloride				Gelatin liquefaction	Fluorescence	Casein	Starch hydrolysis	Arginine	Citrate as carbon source	Identified genera
	at 0% NaCl	at 5% NaCl	at 7% NaCl	at 10% NaCl							
1	+	+	-	-	-	-	-	-	+	+	*Pseudomonas putida*
3	+	+	-	-	-	-	-	-	+	+	*Pseudomonas putida*
4	-	-	-	-	-	-	-	-	+	-	*Pseudomonas putida*
5	++	+	-	-	-	+	+	+	+	+	*Pseudomonas fluorescens*
6	-	-	-	-	-	-	-	-	-	-	*Alcaligenes faecalis*
7	++	+	-	-	-	-	-	-	+	+	*Pseudomonas putida*
8	+	++	-	-	-	-	+	-	+	-	*Pseudomonas fluorescens*
9	++	+	-	-	-	-	+	-	-	+	*Pseudomonas anguilliseptica*
18	+	+	-	-	-	-	+	+	-	-	*Alteromonas piscida*
25	++	+	-	-	-	+	+	-	+	+	*Pseudomonas fluorescens*
26	+	-	-	-	-	-	-	-	+	+	*Pseudomonas putida*
28	++	+	-	-	-	-	+	-	+	+	*Pseudomonas fluorescens*
29	+	+	-	-	-	-	-	-	+	+	*Pseudomonas fluorescens*

Utilization of nitrogenous compounds: After the incubation period, nitrate reduction as tested and ammonia production was the dominating reaction, which is at its maximum in inorganic substances like ammonium chloride and ammonium oxalate (100% in both). The nitrate reduction capability was recorded high when ammonium chloride was used as a substrate. These results indicated that the involvement and importance of *Pseudomonas* spp. in the nitrogen cycle. Although denitrifiers are usually distributed in the aquatic environment as pure cultures, only 5% of the bacterial species are endowed with the ability to

liberate free nitrogen from nitrate or nitrite in the presence of abundance of organic matter (Zobell, 1946). Most of the *Bacillus*, *Alealigenes*, *Pseudomonas* , *Serratia* and *Vibrio* were found to be extremely active nitrate reducers. Ammonification and peptonisation are mainly done by Pseudomonadales and Eubacteriales. Chandrika (1984), reported that *Pseudomonas*, the veteran degrader of organic matter in the marine ecosystem dominated the pre monsoon during 1974-75.

Antibiotic sensitivity tests: Sensitivity studies with three antibiotics showed that all isolates were resistant to penicillin and ampicillin while 46.15% were sensitive to kanamycin. It can be inferred that the predominant flora of bacteria comprising *Psendomonos spp.* were quite resistant to antibiotics.

Antagonistic activity studies: By the well plate studies it was found that 38.73% of the isolates were actively inhibiting *Vibrio anguilarum* and 53.84% were inhibiting *Cytophaga*, the test pathogens. 96.15% of the isolates were found to be inhibiting both *Edwardsiella* and *Mycobacterium* (Table 3). Also well plate method was found to be giving good results compared to cross streak method. It appears that *Pseudomonas* is a natural competitor, which can be use to control other pathogens. Padilla (1990) has reported that the bacteriocin of *Pseudomonas* spp. strain R10 was active *in vitro* against several enteropathogenic bacteria.

Table 3 Antagonistic activity of selected *Pseudomonas* spp on some test pathogens (well plate method)

Organism	Test pathogens			
	Vibrio anguilarum	*Cytophaga*	*Edwardsiella*	*Mycobacterium*
Pseudomonas putida	+	+	+	+
Pseudomonas putida	+	+	+	+
Pseudomonas putida	-	+	-	
Pseudomonas fluorescens	-	+	-	+
Alcaligenes faecalis	-	-	-	-
Pseudomonas putida	-	-	-	-

Pseudomonas anguillisceptica	-	-	-	-
Alteromonas piscicida	-(+ in one well)	-	-	-
Pseudomonas fluorescens	-	-	+	-
Pseudomonas putida	+	+	+	+
Pseudomonas fluorescens	Slight growth in one well	-	-	-
Pseudomonas fluorescens	Slight growth in wells	+	+	+
Pseudomonas fluorescens	+	+	+	+

Most number of isolates occurred in surface water compared to sediment and it was found that the selective medium gave more number of *Pseudomonas* isolates. The presence of fluorescence under U-V light was anguillisceptica important character in identifying isolates, in the present study. Another important observation was that bacterial denitrification is one of the important functions of pseudomonadales and inorganic nitrogenous compounds were found to be degraded more quickly when compared to organic compounds. Also, the pathogenic *Pseudomonas* were found to be mesophilic in nature and proteolytic activity was more compared to amylolytic activity. In the present study, it was seen that, most isolates were quite resistant to antibiotics like penicillin and ampicillin. Inhibitory activity was well demonstrated against some important test pathogens like *Vibrio, Cytophaga, Edwardsiella,* and *Mycobacterium.*

Acknowledgements

The authors thank the Director, CMFRI, Kochi, for facilities. The first author is thankful to ICAR for Junior Research Fellowship.

References

Bauman, L., P. Bauman, and M. Mandel, 1971. Taxonomy of aerobic marine bacteria. *Journal of Bacteriology,* 110, 402-429.

Buchanan, R., E., And N.E. Gibbons, (Ed.), 1974. *Bergy's manual of determinative bacteriology*. Eighth edition. The Williams and Wilkins company, Baltimore, 1268pp.

Chandrika, V., 1984. *Studies on the ecophysiology of some heterotropic and indicator bacteria in the marine environment of Kerala.* Ph.D. Thesis, Cochin University of Science and Technology, Cochin, India, 304 pp.

Horsely, R.W., 1977. A review of bacterial flora of teleosts and elasmobranchs including methods for its analysis. *Journal of Fish Biology*, 10, 529-553.

Inglis, V., M.S. Hendrie, 1993. *Pseudomonas* and *Alteromonas* infections. *Bacterial diseases of fish*, Edited by Inglis, V. and Roberts. R.J., pp 167-174.

Laine, M.T., M.T. Karwoski, L.B. Raaska, and T.M. Mattila, 1996. Antimicrobial. activity of *Pseudomonas* sp. against food poisoning bacteria and moulds. *Letters in Applied microbiology*, 22, 214-218.

Padilla, C., P. Brevis, O. Lobos, and E. Hubert 1990. Bacteriocin activity of *Pseudomonas* sp. on enteropathogenic bacteria in an artificial aquatic system. *Letters in Applied Microbiology*, 23, 371-374.

Shewan, J.M., G. Hobbs, and W. Hodgekiss, 1960. A determinative scheme for the identification of certain genera of gram negative bacteria with special reference to the Pseudomonadaceae. *Journal of applied Bacteriology*, 23, 379-390.

Zobell, C.E., 1946. *Marinue Microbiology*, Waltham, Mass, U.S.A, 240pp.

Sample preparation methods for isolation of *Mycobacterium* spp. from cultured fish and environmental samples

M.M. Latha and V.Chandrika
Central Marine Fisheries Research Institute, Cochin - 682 014

ABSTRACT

Fish mycobacteriosis is a problem to which more than 150 species of fish are susceptible. In order to isolate non-pathogenic and fish pathogenic mycobacterial species from environmental samples, three different methods were evaluated. Shaking and membrane filtration methods were adopted to retrieve maximum acid-fast strains from fish and environmental samples. Decontamination with 4% sodium hydroxide facilitated the isolation of acid-fast bacteria in the selective media, killing all the contaminants like heterotrophs and saprophytes. Centrifugation procedure at 4000 r.p.m for 20 minutes eliminated the contaminants from the sample and allowed only acid-fast bacilli to grow in the selective media. Centrifugation was carried out twice with distilled water. Peizer TB and LJ slopes worked well for selective isolation of fish pathogenic Mycobacteria *from the centrifugated samples. It is believed that all the three sample preparation methods will be useful tool to study the fastidious fish pathogenic* Mycobacteria *from environmental samples.*

Introduction

Studies on opportunistic pathogenic bacteria were carried out extensively in Cochin backwaters and aquaculture ponds. But studies on acid-fast *Mycobacteria* from environmental samples are very few. *Mycobacteria* are ubiquitous in nature and are isolated with relative ease from fishes (Kubota *et. al.*, 1970; Land and Abernathy, 1978; Kusuda *et. al.*, 1987) from soil, stream beds, and cattle drinking troughs (Donoghue *et. al.*, 1997) and from water (Ivanainen *et. al.*, 1997; Neumann *et. al.*, 1997; Dailloux *et. al.*, 1992) has not been reported from water and sediments of aquaculture ponds.

The objective of the present study was to find out a standardised and short-cut procedure for the maximum retrieval of environmental *Mycobacteria* species from water, sediment and fish samples collected from perennial aquaculture ponds.

The normal cell morphology and bio-chemical potential of *Mycobacteria* spp. was also examined to distinguish non-pathogenic and pathogenic species especially based on lipid hydrolysis as all lipolytic forms are considered virulent. Virulence in turn is influenced by season and geographic 'niche' in which these pathogens are found. Therefore, a seasonal examination is needed on the three criteria to find out non-pathogenic and pathogenic strains of *Mycobacteria* studied, (1) pleomorphism exhibited by the stains (2) the presence of mycolic acid (3) the hydrolysis of Tween '80 ie. lipolytic strains, The above morphological and bio-chemical potential factors vary in pathogenic and non-pathogenic stains of environmental *Mycobacteria* which is suggested.

Materials and methods

In the present study, the fish, water and sediment samples were collected from perennial aquaculture ponds which were above 6 kms apart and along 9°55' - 10°10'N and 76°20'E. The perennial pond was located at Krishi Vigyan Kendra, Narakkal and other is a polyculture pond of Valappu.

Monthly sampling was carried out from the two stations for the period from October to December 1999. The sample was brought to the CMFRI bacteriology laboratory in asceptic condition within 2 hrs for the bacteriological investigation. Fish (Tilapia - *Oreochromis niloticus*) sediment and water samples were brought to the laboratory in an ice-box (+4°C). Sediment, water as well as skin, gill, stomach, intestinal samples of fish were taken for isolation of *Mycobacteria* to know the occurrence, distribution and percentage composition of *Mycobacteria* in these samples.

In this study, the retrieval of *Mycobacterium spp.* from various samples has been standardised by incorporating methods like membrance filtration, mechanical shaking, decontamination and centrifugation procedures. The above procedures were carried out as the *Mycobacteria* are highly fastidious and won't occur easily in synthetic media. The two synthetic media recommended for isolation of *Mycobacteria* are Loewenstein - Jensen's and Peizer TB media. In the present study total heterotrophs (TPC) was also monitored using Nutrient agar to know the percentage of *Mycobacteria* in these sample. Flowchart -I shows the general procedure followed for isolation.

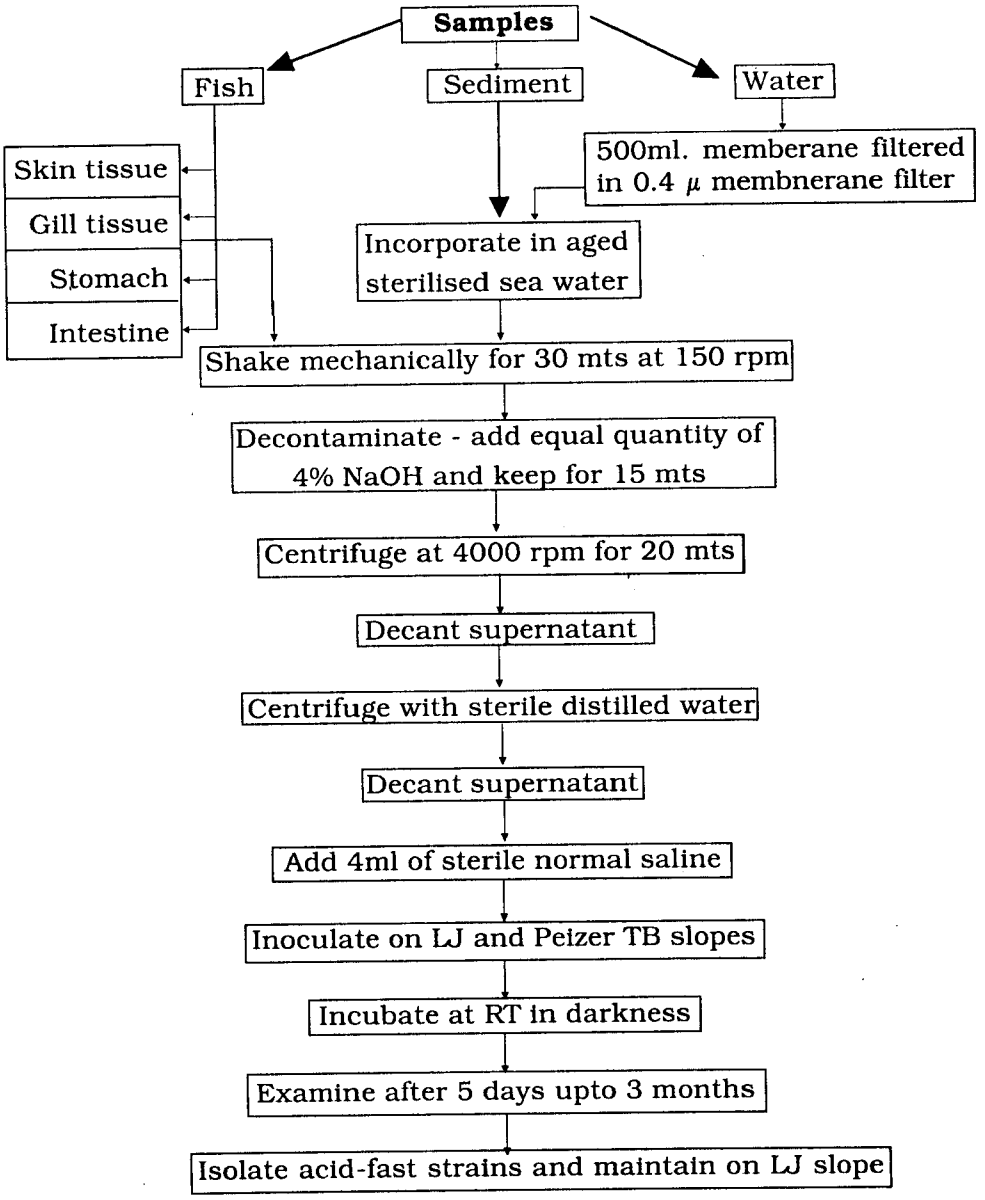
I. *Membrane filtration:* About 500 ml of water sample was membrane filtered (Whatman) using 0.4μm filters in sterile condition and TPC of heterotrophs was taken before and after the membrane filtration.

The membrane filtration technique was followed by mechanical shaking in water samples. Mechanical shaking will dismantle the adhered bacteria from the organic particles into the suspension thereby increasing the incidence of *Mycobacteria.* The decontamination procedure may vary according to the microbiologist and one of the simplest method is adopted in the present study due to its ease in its application.

II. *Mechanical shaking:* Fish and sediment samples were smashed well (1 gm each) placed in 10ml and 99 ml aged, presterilised sea water and was kept for shaking in a mechanical shaker at 150 rpm for 30 mts. The membrane filter used for the filtration of water sample was also subjected

Flow chart - I

Procedure Followed to Isolate Mycobacteria



to shaking. The data on TPC was also collected to compare the difference in total plate count before and after mechanical shaking.

III. *Decontamination procedure:* The decontamination method adopted by Marks and Thomas (1958) was followed and according to the method, 4% NaOH was poured in equal quantity of the shaken sample, mixed well and kept for 15 mts. Only the suspension was used for further procedure. The total plate count of the acid-fast bacteria are also taken before and after the decontamination procedure to understand the effect of the decontamination procedure.

IV. *Centrifugation*: The speed of cetrifugation was 3000 rpm for 20 mts. The supernatant was discarded and the centrifugation was repeated in the same speed with distilled water.

V. *Inoculation:* The supernatant of the centrifugate samples are decanted and added with 4ml of normal saline into each tube and shaken well. Duplicates of LJ and Piszer TB slopes were inoculated with 0.1 ml (for fish and sediment samples) and 0.2 ml (for water sample) of the samples.

VI. *Incubation:* Incubation was done at RT (28±2°C) and in complete darkness because some species of *Mycobacteria* were showing photoactivation capacity which was helpful for the classification by considering, the colour of the pigment produced after one hours' exposure to light. The slopes were kept in slanting portion for the first 24 hours so that the bacterial propagules may get absorbed on the slope and then kept straight, for the rest of the incubation period. In 3-5 days, colonies started to appear on slopes. The general TPC with or without NaOH procedure is also done in the same way with nutrient agar. The mycobacterial colonies on the slopes and plates are stained by Ziehl - Nelson's staining technique. Acid-fast ones were isolated for further bio-chemical and physiological studies.

Results

Membrance filtration

Mycobacteria spp. are sparsely distributed in surface water, hence sample preparation methods are suggested to retrieve maximum of them

on selective media. The membrane filtration method was adopted as an additional sample preparation method for surface pond water. The total plate count before and after membrane filtration, the TPC of aerobic hetrotrophic bacteria in two stations from October to December 99 is given in Table 1 and 2. In KVK (Table 1) the highest count obtained was during November 99 the count being 200 x 10^{-3}, and the lowest TPC encountered during October and December 1999 the count being 10 x 10^{-5}. Compared to this the TPC obtained without membrane filtration was much lower, the count ranging from 2 x 10^{-5} at the month of October to 45 x 10^{-3} at the month of November.

Total Heterotrophs (TPC) encountered in Valappu is given in Table 2. In this station, after membrane-filtration the highest and lowest values obtained were during November, the count being 36 x 10^{-3} and 10 x 10^{-4} respectively. The lowest count encountered without membrane filtration was 2 x 10^{-5}in the post-monsoon month of October 1999.

There is every possibility of obtaining more *Mycobacterium* by sample preparation methods and the incidence of *Mycobacteria* will be more in fish and environmental sample after membrance - filtration. This study will highlight the importance of these sample preparation methods in obtaining the highly fastidious *Mycobacteria* in synthetic organic media.

Table 1. Effect of membrane filtration on general TPC from water samples of KVK aquaculture pond

Months / Procedure	With filtration	Without filtration
October	10 x 10^{-5}	15 x 10^{-3}
	83 x 10^{-3}	2 x 10^{-5}
November	200 x 10^{-3}	45 x 10^{-3}
	34 x 10^{-5}	3 x 10^{-5}
December	28 x 10^{-3}	8 x 10^{-3}
	10 x 10^{-5}	4 x 10^{-5}

Table 2. Effect of membrance filtration on general TPC from water samples of Valappu aquaculture pond.

Months / Procedure	With filtration	Without filtration
October	23×10^{-3}	9×10^{-3}
	18×10^{-5}	2×10^{-5}
November	36×10^{-3}	11×10^{-3}
	10×10^{-4}	3×10^{-4}
December	21×10^{-3}	13×10^{-3}
	28×10^{-5}	6×10^{-5}

Mechanical shaking

Table 3 and Table 4 shows the count obtained before and after the mechanical shaking procedure at 150 rpm for 30 mts. The procedure was found to be effective in increasing the TPC of heterotrophic bacteria. During the sample preparation method, all the samples are subjected to this procedure and considerable difference in bacterial count was obtained in water samples.

The total heterotrophs (TPC) encountered was very high in Valappu and recorded upto 176×10^{-3} during the month of November. The TPC in the same month was only 11×10^{-3}/ml without the shaking procedure. In all the three months, the TPC was recorded high in which the lowest count recorded as 24×10^{-4}/ml in the month of November. The lowest TPC count was recorded 1×10^{-3}/ml without mechanical shaking procedure during the month of December.

Table 3. Effect of mechanical shaking on general TPC from water sample of Valappu aquaculture pond.

Months / Procedure	After shaking water sample	Before shaking water sample
October	96×10^{-3}	16×10^{-3}
	27×10^{-5}	2×10^{-5}
November	176×10^{-3}	111×10^{-3}
	24×10^{-4}	7×10^{-4}
December	39×10^{-3}	1×10^{-3}
	43×10^{-5}	12×10^{-2}

Table 4. Effect of mechanical shaking on general TPC from water samples of KVK aquaculture pond.

Months / Procedure	After shaking water sample	Before shaking water sample
October	122×10^{-3}	31×10^{-3}
	22×10^{-5}	2×10^{-5}
November	210×10^{-3}	42×10^{-3}
	40×10^{-5}	8×10^{-5}
Dcember	27×10^{-3}	3×10^{-3}
	6×10^{-5}	11×10^{-5}

Table 4, illustrates TPC of KVK, the highest count encountered was during November (210×10^{-3}/ml) and lowest during December (6×10^{-5}). In October, highest count obtained was 122×10^{-3}/ml, but the highest count before shaking was 42×10^{-3}/ml and the lowest being 2×10^{-5}/ml in October.

From this account, it is clear that there is defenite enhanced occurrence of growth in TPC and *Mycobacteria* after the shaking procedure. So it is concluded that the sample preparation methods are essential for the maximum number of *Mycobacterial* spp. retrieval from environmental samples.

Decontamination procedure

This is considered to be the most important sample preparation method as it is making the isolation of *Mycobacteria* more easy by selectively enhancing only the occurrence of *Mycobacteria* as 4% NaOH will permit only the bacteria having mycollic acid in their cell wall to grow.

Table 5 and 6 shows the TPC of *Mycobacteria* at the station of KVK before and after the decontamination procedure. All the six samples like skin, gill, stomach, intestine of tilapia and sediment and water samples were subjected to decontamination procedure.

Table 5. Retrieval of *Mycobacteria* from samples of KVK aquaculture pond before decontamination.

Months/ Samples	Skin	Gill	Stomach	Intestine	Sediment	Water
October	-	-	5×10^{-4}	-	12.14×10^{-2} 3.7×10^{-4}	-
November	37×10^{-6} 17.2×10^{-4}	-	5×10^{-4} 2.88×10^{-6}	7.69×10^{-4} -	- -	-
December	0.85×10^{-6}	1.85×10^{-2} 1.85×10^{-4}	12.5×10^{-4}	2.6×10^{-4}	-	-

Table 6. Retrieval of *Mycobacteria* from samples of KVK aquaculture pond after decontamination.

Month/ Samples	Skin	Gill	Stomach	Intestine	Sediment	Water
October	0.76×10^{-3}	3×10^{-4}	1.66×10^{-3} 106×10^{-5}	2×10^{-3} 1.55×10^{-5}	4×10^{-4}	-
November	-	0.86×10^{-2} 2.58×10^{-4}	14.6×10^{-3} 1.12×10^{-5}	0.90×10^{-5} 1.8×10^{-3}	4×10^{-4} 18×10^{-2}	-
December	41.4×10^{-3} 57.57×10^{-4}	0.94×10^{-4}	5.35×10^{-3}	21.6×10^{-5}	8.57×10^{-2}	-

Table 3 shows the count of TPC and *Mycobacteria* obtained in gill, stomach, intestine, sediment and water samples mycobacterial TPC in all the three months. Skin sample was showing the absence of *Mycobacteria* during November '99. The lowest *Mycobacterial* count was encountered in skin sample the count being 0.76×10^{-3}/gm during October. The skin sample harboured the highest count 37×10^{-6} and 17.2×10^{-4} during November '99. Water samples in all the three months before and after decontamination procedure is devoid of *Mycobacteria*. During December, the lowest *Mycobacterial* count observed was 0.85×10^{-6} from the skin sample.

Table 7 and 8 shows *Mycobacteria* TPC encounted at Valappu before and after decontamination procedure respectively. After decontamination, all the six samples showed mycobacterial occurrence except stomach during December. In the month of October, skin sample harboured highest TPC before decontamination procedure the count being 140.4 x

10^{-4}/gm and 130.28 x 10^{-6}. But the corresponding value after decontamination was 3.3 x 10^{-3}/gm and 9.16 x 10^{-5}/gm. In Table 7 the lowest TPC was recorded from stomach the count being 0.775 x 10^{-4}/gm. In water samples, *Mycobacteria* occurred only in the month of December '99, the count encountered as 2 x 10^{-2}/ml. The highest TPC encountered was 53.68 x 10^{-5} in skin during November and lowest was recorded during December, the count being 0.95 x 10^{-2}. In all the three months, water samples were showing mycobacterial representation, the highest count recorded during October and lowest count during December. The decontamination procedure was effective for the retrieval of *Mycobacteria* from sediments as the count obtained after decontamination was more when compared to the count obtained before decontamination, except during October'99.

Table 7. Retrieval of *Mycobacteria* from samples of Valappu aquaculture pond before decontamination.

Months/ Samples	Skin	Gill	Stomach	Intestine	Sediment	Water
October	140.4 x 10^{-4} - 130.28 x 10^{-6}	-	.775 x 10^{-4}	2.4 x 10^{-4}	213 x 10^{-2} - 0.9 x 10^{-4}	
November	1.08 x 10^{-6}	4.16 x 10^{-4}	-	2 x 10^{-4}		
December	0.95 x 10^{-6}	6.9 x 10^{-3}	-	-	-	2 x 10^{-2}

Table 8 - Retrieval of *Mycobacteria* from samples of Valappu aquaculture pond after decontamination

Months/ Samples	Skin	Gill	Stomach	Intestine	Sediment	Water
October	3.3 x 10^{-3} 9.16 x 10^{-5} -	10.8 x 10^{-3} 11.6 x 10-5 1 x 10^{-4}	5.5 x 10^{-3} 2 x 10^{-5} 1.7 x 10^{-5}	6.9 x 10^{-3} 3 x 10^{-5} 12.38 x 10^{-5}	2.94 x 10^{-2}	12 x 10^{-2} 16 x 10^{-4}
November	53.68 x 10^{-5}	4 x 10^{-2}	1.7 x 10^{-3}	10.6 x 10^{-3}	5 x 10^{-4}	1 x 10^{-4} 4 x 10^{-2}
December	2.06 x 10^{-2} 2.06 x 10^{-4}	0.95 x 10^{-2}	-	1.8 x 10^{-3}	32.14 x 10^{-2} 1.78 x 10^{-4}	1 x 10^{-2}

Discussion

Mycobacteriosis or fish tuberculosis is a serious infectious disease and a threat to aquaculturists. The causal organism *Mycobacterium* (the same genus of bacteria that causes tuberculosis in humans) has been isolated from different sources. Bacterial tuberculosis had been studied in yellow tails by Kubota *et. al.* (1970) and in mountain white fish by Land and Abernathy (1978). An epizootic of mycobacteriosis had been reported in yellow tails in 1987 by Kusuda *et. al.* Some mycobacteria are highly pathogenic but when isolated from environmental samples most of them are non-pathogenic. They are simply there as normal flora. Certain generalisation can be made regarding disease-causing *Mycobacteria.*

1) Most of them are aerobic.

2) All are acid-fast rod shaped or highly pleomorphic cocco-bacilli in adverse environmental conditions.

According to Dailloux *et. al.* (1992), water is the natural habitat of *Mycobacteria*, both fresh and salt water. Neumann *et. al.* (1997) has isolated *Mycobacteria* from water and they had incubated the samples on LJ slopes after enriching by filtration. It the present study, membrane-filtration procedure was adopted for making the incidence of *Mycobacterium* maximum possible. Throughout the study period, water samples were devoid of *Mycobacteria.* This may be due to the less number of mycobacterial cells which is insufficient to grow into the medium. Surface water showed high TPC in three months the count being 16×10^{-4}/ ml in October. Mechanial shaking was not used by any of the workers during the isolation procedure, and it is suggested that as it gives enhanced mycobacterial occurrence it may also be included in the isolation procedure.

For the decontamination procedure of the various samples, each laboratory was having their own standardised method of choice. Dalsgaard *et. al.* (1992) used the decontamination procedure by Beerwerth *et. al.* (1967) given in Procedure I. With this method, it was possible to isolate the fastidious *Mycobacteria.* After adding the decontaminant, a mixture

of 5% oxalic acid and 0.1% malachite green are added to neutralise the sample. Ivanainen *et. al.* (1997) tried two decontamination methods to isolate *Mycobacteria* from brook waters. The decontaminants used were : 0.7 mol/litre NaOH followed by 50g/litre oxalic acid and 0.9 mol/litre H Sub (2) SO Sub (4) combined with 0.5g/litre cycloheximide. The NaOH-oxalic acid method generally resulted in lower contamination and higher isolation of mycobacteria. Dalsgaard *et. al.* (1992) and Ivanainen *et. al.* (1997) used oxalic acid to neutralise the mixture. But in the present study, the neutralisation procedure was not done, the decontaminant used was 4% NaOH for 15 mts. which gave maximum retrieval of *Mycobacteria spp.*

Procedure - I

Beerwerth (1967)

Isolation procedure for *Mycobacteria*

1) Tissue treated with equal volume of 4% NaOH for 15 mts.
2) Centrifuged at 3000 rpm for 15 mts.
3) Decant supernatant.
4) Neutralise with 5% oxalic acid to which is added 0.1% malachite green for 15 mts.
5) Decant supernatant.
6) Suspend sediment in 4 ml physiological saline.
7) Inoculate 0.1 ml of this suspension on to four slants of LJ medium.

Lansdell *et. al.* (1993) isolated several *Mycobacterium* species from marine fish caught in the wild and fresh water ornamental fishes. After excising the infected tissues, the tissues were homogenized in 10 ml sterile water and an equal volume of 2-3% NaOH was added as in the present study. Each sample is mixed in a vortex mixture and allowed to remain for 15 mts at RT. Samples were centrifuged at 3000 rpm to effect 95% sedimentation rate of all bacilli present. The centrifugate was neutralised with 2NHcl. In the present observation all these methods were followed except the neutralisation procedure. Eventhough neutralisation procedure was not adopted, high counts of several *Mycobacterium* species has been recorded on Nutrient agar, LJ medium and

Peizer medium slopes.

Twelve methods for the isolation of mycobacterium were compared by Neumann *et.al.* (1997) from surface and treated waters and in each method a particular combination of decontaminants, growth medium and incubation temperature was used. The efficiency of each method was determined by calculating the positivity rate, negativity rate, contamination rate, mean number of mycobacterial colonies formed etc. It was found that 0.005% CPC was found best for treated waters. The general method adopted in the present study was found to be good for the maximum retrieval of *Mycobacteria* from water.

Isolation of *Mycobacteria* was very high using 4% NaOH as decontaminant, in all the six samples of Valappu and KVK except water sample of KVK during the study period. The absence of *Mycobacterium* in water may be due to the pH variation attained during decontamination procedure. *Mycobacteria* was absent in skin sample during November but recorded high during December. Intestine and sediment samples of KVK also showed higher values than gill and stomach.

Water samples recorded highest TPC in Valappu during the three months of study, the lowest value being $1x10^{-2}$/ml. Skin sample showed lowest count during October. Before decontamination the count encountered is $140.4x10^{-4}$ and $130.28x10^{-6}$. The corresponding count after decontamination is being $3.3x10^{-3}$ and $9.16x10^{-5}$. Sediment in October also showed high count before decontamination the count being $0.9x10^{-4}$/ gm. After decontamination, the corresponding value is only $2.94x10^{-2}$/ gm. The occurrence of highest mycobacterial count in the samples before decontamination will be quiet accidental. In the present study, both the stations were showing high mycobacterial count, after decontamination procedure.

Some of the *Mycobacteria* from environmental samples require large incubation period as they are slow-growing forms in synthetic media despite of their predominance in the environment. Diagnosis is both difficult and time consuming using conventional methods. All the sample preparation methods suggested in the present study are efficient in retrieving mycobateria from water, sediment, faeces, fishes and fish blood samples and will be useful tool in the study of both pathogenic and non-pathogenic mycobacteria from environmental samples.

Acknowledgement

The first author is thankful to CSIR, New Delhi for the award of Junior Research Fellowship, underwhich this work was carried out.

References

Beerwerth, W. 1967. Die Zuchtung Von Mycobacterien aus dem kot der Hanstiere and thee Bedeutungturdic Epidemilogie and Bekampfung der Tuberkulose, Praxis Pneumol, 21 : 189-202.

Dailloux, M.; C. Henry, D. Terver, 1992. Observation and experimental study of *Mycobacteria* in fresh water and salt water aquaria. *Rev. Sci. Eau.* Vol. 5 (1), 69-82.

Dalsgaard, I.; S. Millergaard J.L. Larsen, 1992. Mycobacteriosis in Cod (*Gadus morhua L.*) in Danish coastal waters. *Aquaculture,* ***107,*** 211-219.

Donoghue, H.D.; E. Overend, J.L. Stanford, 1997. A longitudinal study of environmental mycbacteria on a farm in South-West England. *J. Appl. Microbiol.* Vol. ***82***(1), 57-67.

Ivanainen, E.; P.J. Maritikainen, M.L. Katila, 1997. Comparison of some de-contamination methods and growth media for isolation of *Mycobacteria* from Northern Brook waters. *J.Appl. Bateriol.* 82 (1), 121-127.

Kubota, S.; M. Kimura, S. Egusa, 1970. Studies on bacterial tuberculosis of yellowtail symptemology and histophathology. *Fish Pathol. 4* : 111-118.

Kusuda, R., K. Kawakami K. Kawai, 1987. A fish pathogenic *Mycobacterium* species isolated from an epizootic of cultured yellowtail. Nissuishi. *Bull Jap. Soc. Sci. Fish 53* (10), 1797 - 1804.

Land, J.E.; C.S. Abernathy, 1978. Leisons of tuberculosis in mountain white fish. *Journal of Wildlife Diseases.* ***14 :*** 222-28.

Lansdell, W.; B. Dixon, N. Smith, L. Benjamin, 1993. Isolation of several *Mycobacterium* species from fish. *J. aqua. anim. health.* Vol. 5 (1), 73-76.

Marks, J.; C.H. Thomas, 1958. Notes on the cultivation of Tubercle bacilli. *The monthly bulletin of the Ministry of Health and the Public Health Laboratory Service* 17, 194-202.

Neumann, M.; R. Schulze Roebbecke, C. Hangenau, K. Behringeu, 1997. Comparison of methods to isolation of *Mycobacteria* from water. *Appl. environ. microbiol.* Vol. ***63*** (2), 547-552.

The role of the pineal gland and photoperiodicity on growth, metabolism, reproduction in fishes and its application in aquaculture-a review

U.S. Sethi and L. Krishnan
Central Marine Fisheries Research Institute, Kochi-682 014, India.

ABSTRACT

The pineal gland, situated on the roof of the diencephalon of the brain in fishes, acts as a photoneuroendocrine gland which secretes the hormone melatonin. The secretion of this hormone is controlled mainly by photoperiodicity. This paper is a review of the numerous works showing the role of this gland and its hormone, on growth and metabolism of the body by inhibition of secretion of thyroxin, MSH and other opioid peptides; in the control of behavioural thermoregulation; and on reproduction through gonadal development and secretion of GtH-II with other steroids. The possible use of this knowledge as a modern tool in manipulating aquaculture production is also evaluated.

Introduction

Aquaculture is a viable means of diversification of fisheries to increase fish production both for domestic consumption and export, rural

upliftment, employment and income generation to a large section of people (Krishnan, 1998). To meet the growing demand for aquaculture products, modern techniques must come up, which should be cheaper and convenient. This calls for basic research in functional physiology of the organisms, the results of which may ultimately help to modernize both the hatchery as well as culture techniques. The best example is that of the basic research on pituitary gland which revolutionized the induced breeding techniques in aquaculture industry and augmented the aquaculture production.

The pineal gland in fishes arises as a mid-dorsal neuroendodermal evagination from the roof of the diencephalon (Rasquin, 1958; Kavalier, 1980). It is basically a club-shaped or slightly elongated gland. The important contributions related to its morphology and histochemistry in fishes date back to 1905. The ultrastructure studies by electron microscope are contributed by McNulty (1978a, b, c;1979), Ueck *et al.* (1978), Falcon (1979a, b), Omura (1979, 1980), Omura and Ali (1980), McNulty *et al.* (1988), Gonzalez *et al.* (1990), Vigh *et al.* (1991), Wang (1994), Deng and Daren (1996). Basically the pineal gland consists of three parts called pineal sac, pineal thalamus and pineal stalk. Some of the workers (Yadav, 1995) have described it in two parts called pineal sac or end vesicle which is broad (anterior part) and pineal stalk which is elongated and narrow (posterior).

The pineal gland is a photoneuroendocrine gland which secretes the hormone melatonin (Gern *et al.*, 1988) and conveys information to the brain via neural pathway. Melatonin is an indole amine whose chemical composition is 5 - methoxy-N-acetyltryptamine described by several workers, and its precursor is serotonin. The pathway of biosynthesis and metabolism of melatonin in mammals has been described by Wesemann (1974) and Harper *et al.* (1979). In fishes the full pathway is yet to be studied. It is reported that serotonin- N-acetyltransferase (acrylalkylamine-N-acetyltransferase, AANAT) may be the first enzyme in the conversion of serotonin to melatonin which is

found in fishes like trout and pike (Coon *et al.*, 1999), whereas, Hydroxyindole-O-methyltransferase (HIOMT) may be catalyzed during the last step in the melatonin biosynthetic pathway (Falcon *et al.*, 1994).

Melatonin is produced rhythmically and its synthesis is regulated either directly by ambient photoperiod in fishes as reported by Falcon (1984), Gern and Greenhouse (1988), Falcon *et al.* (1989). Takabatake and Iga (1991), May and Menaker (1992), Zachmann *et al.* (1992), Thibault *et al.* (1993), Yanez and Meissel (1995), Okimoto and Stetson (1995), Balliet *et al* (1996), Messel and Yanez (1996), Molina *et al.* (1996), Popek *et al.* (1997), Iigo *et al.* (1998), Simonneauk and Pevet (1998), or by an endogeneous circadian oscillator that is entrained by the photoperiod which is reported by Kavaliers (1979), McNulty (1981), Kikuchi and Aoki (1985), Mohapatra *et al.* (1988), Iigo *et al.* (1991), Radchenko (1993), Randall *et al.* (1995) and Okimoto (1998). Temperature is also involved with the photoperiod which is described by many of the above researchers. During natural conditions, melatonin is produced at the highest level during night and the lowest during day.

Portar with his associates in 1995 and 1996 reported that melatonin levels decrease in pinealectomized fish. It was demonstrated that the pineal gland was the only organ in fish, responsible for the presence of melatonin in the blood and the level oscillated regularly over 24 hours showing low values during day and high over night (Popek *et al.*, 1997).

It will be noticed at the end that in spite of the fact that, good amounts of research work has been directed to pineal gland and its secretion in other countries, very few works are observed in India and these also are restricted to a few fresh water fishes.

This review paper reveals the role of the pineal gland and its hormone melatonin on growth, metabolism and reproduction in fishes. The possible use of this knowledge as a modern tool in manipulating aquaculture production is also evaluated.

Function of pineal gland and melatonin

The pineal gland or epiphysis secretes the hormone melatonin according to photoperiodicity. The receptor cells of melatonin are found in brain in fishes (Martinoli *et al.*, 1991; Ekstroem and Vanecek, 1992; Falcon *et al.*, 1996), in heart as in salmonids (Pang *et al.*, 1994), in retina as in gold fish (Iigo *et al.*, 1997) and controls the growth, metabolic activities and reproduction. Among them, the reproduction and growth are the two main aspects in aquaculture production point of view.

Role of pineal gland and melatonin

On reproduction

A pineal control of gonadal maturation has been shown in fishes either by pinealectomy (Devlaming, 1975; Devlaming and Vodicnic, 1978; Vodicnic *et al.* 1978; Vodicnic *et al.*, 1979; Sagi *et al.*, 1983; Garg *et al.*, 1987, 1989; Kezuka *et al.*, 1989; Popek *et al.*, 1997) or by melatonin administration (Sakena and Anand, 1977; Reiter, 1977; Keshavanath, 1981; Joy and Agha, 1991; Bromage *et al.*, 1994; Senthilkumaran and Joy, 1995; Khan and Thomas, 1996) or by both (Joy and Khan, 1991). All the above authors have reported the relation between pineal gland and melatonin with reproduction. The pineal gland controls reproduction through secretion of its hormone melatonin through pineal-hypophysis-pituitary- gonadal axis. Administration of melatonin inhibits the stimulatory effect of a long photoperiod and high temperature on the ovary in early preparatory phase. Treatment with melatonin during preparatory phase resulted in decreased ovarian weight and arrested ovarian recrudescence (Sakena and Anand, 1977). When the gold fishes were pinealectomized in spring and exposed to long photoperiod conditions, the ovaries regressed and plasma gonadotropin levels were significantely depressed compared to sham operated animals. Sham operated gold fish exposed to short photoperiod conditions in spring had regressing ovaries whereas pinealectomized animals under this regime either spawned or had ovaries in the late vitellogenic phase (Vodicnic *et al.*, 1978). The pinealectomized mullet (*Liza ramada*) exposed to long photoperiod (16L/ 8D) for 14 weeks, showed undeveloped ovaries with maximum oocyte

diameter of 100μm, whereas to short photoperiod (8L/16D) for 6 weeks, the mean diameter of oocyte was 270μm (Sagi *et al.*, 1983). In catfish like *Heteropneustes fossilis*, pinealectomy had no effect on gonadal activity during the preparatory, pre-spawning and spawning periods of the reproductive cycle. However, during the post-spawning period, under long or short photoperiod at 25° C or at gradually increasing ambient temperature, pinealectomy accelerated testicular recrudescence and secretory activity of the seminal vesicle (Garg, 1987). In *Channa punctatus*, pinealectomy results in an accelerated growth on the ovary in preparatory phase, but had no significant effect in pre-spawning or post-spawning phase (Joy and Khan, 1991). In fish like *Heteropneustes fossilis*, the gonadosomatic index (GSI) had decreased significantly by melatonin during preparatory and pre-spawning seasons, and also the melatonin administrations arrested vitellogenesis during the gonadal recrudescence (Joy and Agha, 1991). Khan and Thomas (1996) reported the role of melatonin in the control of gonadotropin-II (GtH-II) secretion in Atlantic croaker (*Micropogonias undulatus*) during different phases of day night cycle and seasonal reproductive cycle. Intraperitonial injection of melatonin during the late-night phase on the day night cycle elicited a significant elevation of plasma GtH-II levels in croaker with fully developed gonads. They reported that the stimulatory effect of melatonin was dose dependent. Melatonin inhibited LHRHa-induced GtH-II released during mid-dark phase of the day night cycle in a dose dependent manner. These authors concluded that melatonin can directly influence the pituitary to stimulate GtH-II release. The pineal gland can affect one of the phase of gonad maturation cycles in carp, probably through a stimulating influence of melatonin on estradiol-level in the final phase of vitellogenesis (Popek *et al.*, 1997).

By taking all the knowledge from different research groups, it is reported that pineal gland and melatonin are having either stimulatory or inhibitory effect on the gonadal development and the secretion of GtH-II with other steroids according to photoperiodicity.

Melatonin is also having the inhibitory effect on PGE (Prostaglandin E) and PGF (Prostaglandin F) and ovulation in fish like yellow perch (Stacey and Goetz, 1982). This hormone inhibits gonadal development by reducing

the sex- steroid production in certain cases (Nayak and Singh, 1987). Again in 1988 these authors reported that this gland (through pinealectomy) inhibits these sex- steroids at certain stages in same species. This is in close conformity with the earlier findings of Fenwick (1970), DeVlaming *et al.* (1975), Sunderaraj and Keshavanath (1976), Sakena and Anand (1977), Borg and Ekstroem (1981), who reported that gonadal function (wt.and histology) are inhibited with the administration of melatonin. It appears that pineal gland and melatonin are having inhibitory effect to thyroid hormone in fishes during gonadal development and maturation (Nayak and Singh, 1987a, b), which is specially required for the sex steroidogenesis in the process of reproduction.

A review of the above will reveal the fact that reducing the natural melatonin production through using of long photoperiod will augment the gonadal development, thus helping in controlled breeding of fishes.

On metabolic activity and growth

Growth is the main criteria for aquaculture production ultimately, which mainly rely on metabolic activities. The pineal gland and its hormone play the role as an intrinsic controller according to environmental stimuli (photoperiodicity). Metabolism and growth processes are mainly controlled by the endocrine glands by their hormonal secretion and some of these hormones are controlled by the pineal gland. Melatonin has an inhibitory role to the thyroid hormone (Nayak and Singh, 1987a,b) which acts as one of the main growth inducing hormone in fishes. Nayak and Singh (1987b) reported from their experiment in fish *Clarias batrachus* that melatonin is having the inhibitory role to the sex steroid in certain doses.

The pineal gland regulates carbohydrate metabolism by altering insulin responsiveness in the animal like gold fish (Delahunty and Tomlinson, 1984a). Pinealectomy in this species causes a decrease in liver glycogen stores and disappearance in plasma glucose. These effects occur independently of photoperiod acclimation and are seasonal in nature. The hormone from this gland was observed having a hypoglycemic effect in the above species (Delahunty and Tomlinson, 1984b). According

to Soengas *et al.* (1998), melatonin plays an indirect role, possibly through alterations in insulin physiology, in the regulation of carbohydrate and ketone body metabolism in brain of fish like rainbow trout. For the first time in the teleost like rainbow trout, the above authors reported the existence of changes in brain carbohydrate and ketone body metabolism due to melatonin treatment.

Removal of pituitary and epiphysis in young sturgeon, *Acipenser baeri*, significantly affects the total lipid content of the liver (Semenkova, 1984). Injection with melatonin changes lipid content in the liver of epiphysectomised fish. The nature of this change differs depending on the time of the treatment and duration of the photoperiod. Semenkova and Sautin (1990) suggested complicated interactions in the system of epiphysis-hypothalamus-hypophysis-lipid metabolism in liver of cartilaginous ganoids. Portar *et al.* (1998) investigated the role of melatonin and pineal gland as intermediaries in the transfer of photic information on daily and calender time in the control of the timing of the par-smolt transformation in the Atlantic salmon (*Salmo salar*). This is suggested by Rourke (1994) and Randall *et al.* (1994). However, the mechanism of this process is unclear.

Withyachumnarnkul (1992) suggested that the optic lobe of certain crustacea like *Macrobrachium rosenbergii* is the source of melatonin and increased the rate of limb regeneration in both eye stalk-intact and eye stalk removed groups like in fiddler crab, *Uca pugilator* (Tilden *et al.*, 1997). This is contrary to results of regeneration studies in other phyla, in which similar melatonin concentrations inhibited regeneration. Tilden *et al.* (1998) sugested that the crustaceans, however, are an exception in that melatonin production increased during the day and long photoperiods also increased the rate of limb regeneration. Therefore, melatonin may be mediating long-day effects on regeneration and other physiological process in crustaceans.

Other roles of pineal gland and melatonin

Several authors have reported that this gland and its hormone have

the role to play in the colour change mechanism in fishes (Bhargava and Jain, 1978; Fujii and Miyashita, 1978; Nayudu and Hunter, 1979; Kavaliers 1980; Matsumoto *et al.*, 1982; Iwakiri, 1983;Ohta and Ono, 1983; Kasukawa and Fujii, 1984; Sugimoto *et al.*, 1985; Nishi *et al.*, 1991; Fujii *et al.*, 1992; Takabatake *et al.*, 1992; Nishi and Fujii, 1992; Visconti and Castrucci, 1993; Filadelfi and Castrucci, 1994; Maartensson and Andersson, 1997; Goda and Fujii,1998).There is substantial evidence that the pineal is involved in determining circadian and seasonal organisation in teleosts (Kavaliers, 1979a,b,c; 1980a,b,1981a,b; Tabata *et al.*, 1991). These effects may arise from the pineal photoreceptive functions, the role in circadian integration, and temporal organisation of hormonal physiological and behavioural events through CNS modulation. The pineal gland is involved in the control of behavioral thermoregulation in fishes (Kavaliers and Ralph, 1980; Kavalier, 1982a,b; Varghese and Pati, 1997). and Pati, 1997)

The pineal gland in anadromous fish like salmonids may play a role in the migratory behaviour (Weber and Smith, 1980), and schooling activity in fish like *Chromis viridis* (Sparwasser, 1987) .The influence of the eye and pineal gland on locomotor activity rhythm of channel cat fish, *Ictalurus punctatus, and* the extent to which varying light intensity altered these activity rhythms were evaluated by Goudie *et al.* (1983). Pinealectomized fish exhibited nocturnal activity patterns, which corresponded with the exogenous photoperiod.

Photoperiodicity, control of melatonin production and their role in aquaculture

The production of melatonin is mainly controlled by the photoperiodicity of the environment suggesting that the secretion will be only in scotophase in fishes. This secretion is having mainly inhibitory role to the other hormones in certain stages and periods, which are required for somatic as well as gametic growth of the fishes. By increasing the photoperiodicity at certain stages, the secretion of

melatonin can be reduced which leads to the secretion of other required hormones for both gametic as well as somatic growth enhancement, which is the main objective in scientific aquaculture.

Several workers reported that, in fishes, photoperiodicity along with temperature play the major role in reproduction (Razani *et al.*, 1987; Richter *et al.*, 1987; Nakari *et al.*, 1987; Walsh, 1987; Fores *et al.*, 1988; Cantin, 1988; Pavlidis *et al.*, 1989; Jafri, 1989; Adams and Thorpe, 1989; Micale and Perdichizzi, 1990; Agarwal *et al.*, 1990; Grier, 1991; Srivastava and Singh, 1991; Aida, 1992; Kumari and Dutt, 1995; Taranger, *et al.*, 1998), growth (Stefansson *et al.*, 1989; Thorensen and Clarke,1989; Wright *et al.*, 1991; Parma-De-Crouk,1996) and metabolism (Boujard and Leatherland,1993).Therefore through artificial manipulation of photoperiod, we can quicken the metabolic activity, somatic as well as gametic growth in fishes and crustaceans. By using artificial illumination, in combination with temperature regulation, it is now possible to advance or delay the internal clock of fishes and thereby manipulate the timing of smolt transfer in case of salmon. By using this technique at Stirling in conjunction with a series of commercial smolt producers, Portar *et al.* (1999) have produced smolts at regular intervals through the summer, autumn and winter (Fig-1). With such smolt transfers, sectors of the industries are now producing 3-4 Kg salmon through out the year. Fig-2 shows the growth profiles achieved from a series of such 0+smolt transfer. They indicate that the plasma melatonin must be reduced below the threshold level by artificial lighting before the modified photoperiod is capable of altering the timing of smoltification. It is important that, fish in open environments are exposed to ambient light, if the additional artificial illumination is of lower intensity than natural day-time light level. If this is not done, then plasma melatonin may remain above critical threshold levels and so the fish will not respond to the light. Rimmer in Australia achieved reliable spawning in grouper using environmental control like light with temperature.

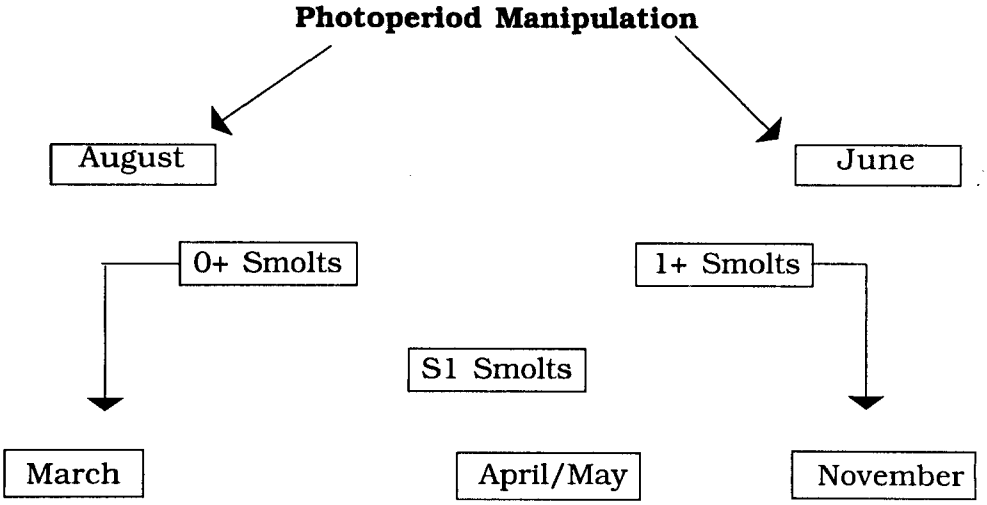


Fig. 1: All- year round smolt production using photoperiod manipulation.

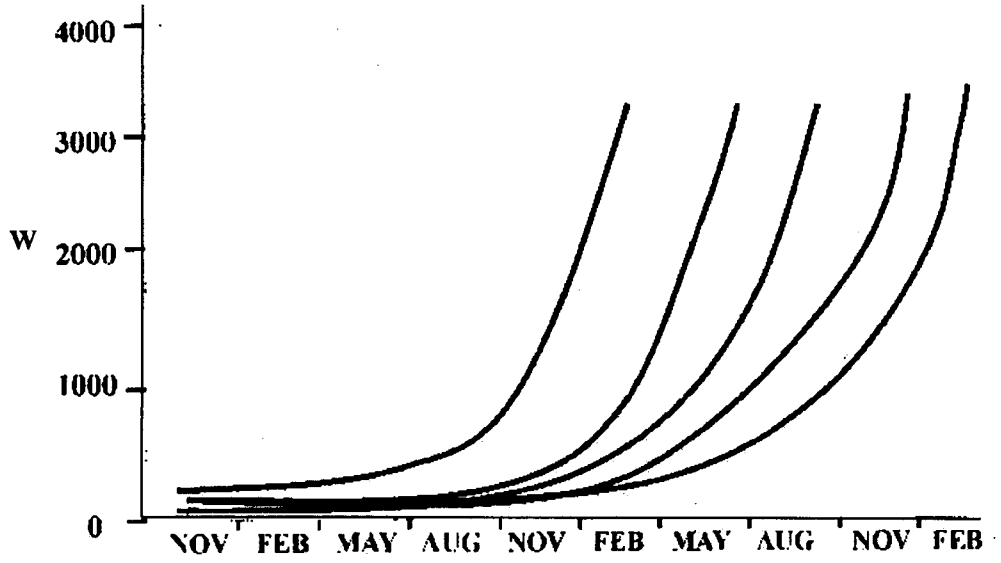


Fig. 2: Growth of 0+ and S1 smolts from seawater transfers in November, December, March, May and October upto harvest weight (3-4 Kg) Source: Figure 1 and 2, Porter *et al.*, Light manipulation, Melatonin and the All - Year - Round Supplies of 3 - 4 Kg Salmon. *Aquaculture News*, July 1999, pp.31-32.

Conclusion

A good deal of evidence has been collected suggesting that the pineal gland, upon receiving light information, directly or indirectly, produces melatonin, which controls many body functions. So, by using this technique described above i.e. by the manipulation of photoperiodicity in certain stages and seasons we can control the secretion of melatonin and ultimately increase the gametic as well as somatic growth. This is a simple technique, which may be used to induce maturation and growth thereby augmenting aquaculture production. For this, intensity of light and duration is very important. So attention must be given to standardize the light intensity along with duration. The melatonin secretion may be reduced chemically in certain stages that will increase the production of other induced hormones required for growth and reproduction. This is possible only after understanding the exact metabolic pathway of melatonin in fishes. So emphasis must be given towards research in this direction.

References

Abraham, M. and G. Sagi 1984.Photoperiod regimes and "pineal treatment" as a means of controlling gonadal recrudescence in *Liza ramada*. *Proc. Sec. Sem. German Israel Coopert. Aquac. Res.* (H. Rosenthal AND S.Saring, Eds.), Europian mariculture Soc. Bredene Belgium, **8**, pp. 105-118.

Adams, C.E. and J.E. Thorpe 1989. Photoperiod and temperature effects on early development and reproductive investment in Atlantic Salmon (*Salmo salar* L.). *Aquaculture*, **79**(1-4): 403-409.

Agarwal, P.L., M. Banerjee and K. Pandey 1990. Effect of long and short photoperiod on the initiation of ovarian recrudescence in a fresh water perch *Colisa fasciata* (Bl. & Schn.). *Bol. Fisiol.* Anim. Univ. Sao. Paulo., **14**:61-69.

Aida, K. 1992. Environmental regulation of reproductive rhythms in teleosts. *Isr. J. Aquacult. Bamidgeh.*, **44**(4):131.

Bhargava, N.H. and A.K. Jain 1978. Some aspects of colour change mechanism in the Indian freshwater siluroid, *Heteropneustis fossilis* (Bloch). *Biochem. Exp. Biol.*, **14**(4): 359-373.

Boujard, T and J.F. Leatherland 1993. Dielrhythm of food demand, liver weight and glycogen content and plasma hormonal concentrations in *Oncorhynchus mykiss* held in different photoperiod regimes. In *"Fish Nutrition in Practice"*. (S.J. Kanshik and P.Luquet, Eds.), *Inst. Nat. Reche. Agron. France*, **61**: 269-277.

Bromage,N., C.Randall, B.Davies and M.Portar 1994. Photoperiod, melatonin and fish reproduction. *Proc. Intern. Fish Physiol. Symp.* (D.D.Mackinlay.Ed.), Vancouver, BC Canada Fish Physiol. Asso. Canada, pp. 137-142.

Cantin, M.C. 1988. Oocyte developement of small mouth bass under natural and controlled photoperiod - temperature regime. In *"Aquaculture intern. Cong. Expos."*, Vancouver Trade Conven. Cent., Canada, pp.63.

Cole,W.C., and J.H. Youson 1981. The effect of pinealectomy, continuous light and continuous darkness on metamorphosis and anadromous sea lampreys, *Petromyzon marinus* (L). *J. Exp. Zool.*, **218**(3): 397-404.

Coon, S.L., V. Begay, D. Deurloo, J. Falcon and D.C. Klein 1999. Two Arylalkylamine N-Acetyltransferase Genes mediates melatonin synthesis in fish. *J. Biol. Chem.*, **274**(13): 9076-9082.

De Vlaming, V.L. 1975. Effects of pinealectomy on gonadal activity in the cyprinid teleost, *Notemigonus crysoleucas. Gen. Comp. Endocrinol.* **26**: 36-49.

De Vlaming, V.L. and M.J. Vodicnik 1978. Seasonal effects of pinealectomy on gonadal activity in the goldfish, *Carassius auratus. Biol. Reprod.* **19**: 57-63.

Delahunty, G. and M.Tomlinson 1984a. Hypoglycemic effects of melatonin in the gold fish, *Carassius auratus. Com. Biochem. Physiol.*, **78**A(4): 871-875.

Delahunty,G. and M. Tomlison 1984b. Photoperiod independent actions of pineal organ: Aspects of a pineal organ-pancreas relationship. In *"Rhythmicity in Fishes"*, **113**(4): 439-443, Annual Meet. Am. Fish. Soc., USA.

Deng H. and H.Daren 1996. The histology and ultrastructure of the pineal bodies in three species of mullets (*Mugil carinatus, M. dussumieri, M. engeli*). *J. Xiamen Univ. Nat. Sci. Xiaman Dakue Xiamen.*, **35**(2): 262-267.

Ekstroem, p. and J. Vanecek 1992. Localization of 2 - (super (125) I) iodomelatonin binding sites in the brain of the Atlantic salmon, *Salmo salar* L. *Neuroendocrinology*, **55**(5): 529-537.

Ekstroem, P., T. Van-Veen , A. Bruun and B. Ehinger 1987. GABA-immunoreactive neurons in the Photosensory Pineal organ of the rainbow trout: Two distinct neuronal populations. *Cell Tissue Res.*, **250**(1): 87-92.

Falcon, J. 1979a. The pineal organ of the pike, *Esox lucius* L. 1. A light and electron microscopic study. *Ann. Biol. Anim. Biochim. Biophys.*, **19**(2A): 445-465.

Falcon, J. 1979b. The pineal organ of the pike, *Esox lucius* L. 2. An electron microscopic study of photoreceptor cell diferentiation and regretion and variations of the photoreceptive potential in different pineal regions. *Ann. Biol. Anim. Biochim. Biophys.*, **19**(3A): 661-688.

Falcon, J. 1984. Photosesitivity and biosynthesis of indole compounds in the cells of the receptor line of the pineal organ of the pike. In *"Ocular Extraocular Process. Visual Inform.,"* (E. Zrenner, H. Meissl, Eds.), **6**(1-2): 123-128.

Falcon, J. and J.P. Collin 1989. Photoreceptors in the pineal of lower vertebrates: Functional aspects. *Experientia*, **45**(10): 909-913.

Falcon, J. and V. Begay 1998. The vertebrate photoreceptor: A cellular circadian clock. In *"Trends Comp. Endocrinol. And Neurobiol."*, (H. Vaudry, M.C. Tondon, E.W. Roubos and A.de-Loof, Eds.), *New York Acad. Sci.*, **839** : 279-283.

Falcon, J., M. Molina-Borja, J.P. Collin and S.Oaknin 1996. Age-related changes in 2-(super (125) I) iodomelatonin binding sites in the brain of seabreams (*Sparus aurata L*). *Fish physiol, Biochem.*, **15**(5): 401-411.

Filadelfi, A.M.C. and A.M.L. Castrucci 1994. Melatonin desensitizing effects on the in vitro response to MCH, alpha-MSH, isoproterenol and melatonin in pigment cells of a fish (*S. Marmoratus*), a toad (*B. ictericus*), a frog (*R. pipiens*), and a lizard (*A. carolinensis*), exposed to varing photoperiodic regimens. *Comp. Biochem. Physiol*, **109**A(4): 1027-1037.

Fores, R., J. Iglesias, M. Olmedo, F.J. Sanchez and J.B. Peleteiro 1988. Introduction of spawning in turbot *Scophthalmus maximus* L., by photoperiod control *Copenhagen. Denmark Ices* , pp:13.

Fujii, R. and Y. Miyashita 1978. Receptor mechanisms in fish Chromatophores - 4. Effect of melatonin and related substances on dermal and epidermal melanophores of the siluroid, *Parasilurus asotus. Comp. Biochem. Physiol*, **59C**(1): 59-63.

Garg, S.K. 1989.Effect of Pinealectomy, eye enucleation and melatonin treatment on ovarian activity vitellogenin levels in the catfish exposed to short photoperiod or long photoperiod. *J. Pineal Res.*, **7**(2): 91-104.

Garg,S.K. 1987.Seasonal effects of Pinealectomy on testicular recrudescence and secretory activity of seminal vesicle in the cat fish *Heteropneustes fossilis*(Bloch). *J. Fish. Biol.*, **30**(4): 377-387.

Gern, W.A. and S.S. Greenhouse 1988. Examination of in vitro melatonin secretion from superfused trout (*Salmo gairdneri*) pineal organs maintained under diel illumination or continuous darkness. *Gen. Comp. Endocrinol.*, **71**(1): 163-174.

Goda, M. and R. Fujii 1998. The blue colouration of the common sturgeon fish, *Paracanthurus hepatus. Zool. Sci.*, **15**(3): 323-333.

Goudie, C.A, K.B. Davis and B.A. Simco 1983. Influence of the eyes and pineal gland on locomotor activity patterns of channel catfish *Ictalurus punctatus. Physiol. Zool.*, **56** (1): 10-17.

Grier, H.J. 1991. Environmental control of reproduction in a live bearing fish. *Am. Zool.*, **31**(5): 36A.

Harper, H.A., V.W. Rodwell and P.A. Mayes 1979. Biosynthesis and metabolism of melatonin (Fig. 28.9). In *"Review of physiological Chemistry,"* Maruzen Asia Pte. Ltd., Singapore 9111, pp. 435.

Iigo, M., F.J. Sanchez- Vazquez, J.A. Madrid, S. Zamora and M. Tabata 1997. Universal responses to light and darkness of ocular melatonin in European sea bass. *Neuroreport*, **8**(7): 1631-1635

Iigo, M., M. Tabata and K. Aida 1997. Ocular melatonin rhythms in a cyprinid teleost, Oikawa *Zacco platypus*, are driven by light dark cycles. *Zool. Sci.*, **14**(2): 243-248.

Iigo, M., R. Ohtani-Kaneko, M. Hara, K. Hirata and K. Aida 1997. Melatonin binding sites in the gold fish retina. *Zool. Sci.*, **14**(4): 601-607.

Iwakiri, M. 1983. Action of melatonin and its derivatives on melanophores in isolated scales of a teleost fish. *J. Sci., Hiroshima Univ.* B. I. Zool., **31**(1): 85-98.

Jafri, S.I.H. 1989. The effects of photoperiod and temperature manipulation on reproduction in the roach, *Rotilus rutilus* (L.). *Pak. J. Zoll.*, **21**(3): 289-299.

Joy, K.P. and I.A. Khan 1991.Pineal-gonadal relationship in the teleost *Channa punctuates* (Bloch): Evidence for possible involvement of hypothalamic serotonergic system. *J. Pineal Res.*, **11**(1): 12-22.

Joy,K.P. and A.K.Agha 1991.Seasonal effects of administration of Melatonin and 5-methoxy tryptophanol on ovarian activity in the cat fish *Heteropneustes fossilis*(Bloch). *J. Pineal Res.*, **10**(2): 65-70.

Kasukawa, H. and R. Fujii 1984. Potassium ions act to release transmetter from *"Cholinergic"* sympathetic postganglionic fiber to the glass cat fish melanophore. *Zool. Sci.*, **1**(4): 553-559.

Kavaliers, M.1979a. The pineal organ and circadian rhythms of fishes. *"Environmental Physiology Of Fish"* (M.A.Ali, Ed.), **35**, pp. 631-645, Plenum Press, New York, USA.

Kavaliers,M. 1979b. Pineal involvement in the control of circadian rhythmicity in the lake chub, *Couesius plumbeus. J. Exp. Zool.* **209**(1): 33-40.

Kavaliers,M. 1979c. The pineal organ and circadian organization of teleost fish. *Rev. Can. Biol.*, **38**(4): 281-292.

Kavaliers,M. 1980a. Pineal control of ultradian rhythms and short-term activity in a cyprinid fish, the lake chub, *Couesius plumbeus. Behav. Neural Bio.*, **29**(2): 224-235.

Kavaliers,M. 1980b. Circadian locomotor activity rhythms of the turbot, *Lota*

lota: Seasonal differences in period length and the effect of pinealectomy. *J. Comp. Physiol.*, **136**(3): 215-218

Kavaliers,M. 1981a. Circadian rhythm of Non pineal extraretinal photosensitivity in a teleost fish, the lake club, *Couesius plumbeus*. *J. Exp. Zool.*, **216**(1): 7-11.

Kavaliers,M. 1981b. Circadian organization in white suckers *Catostomus commersoni*: The role of pineal organ. *Comp. Biochem. Physiol. A*, **68**(1): 127-129.

Kavaliers,M. 1982a. Pineal mediation of the thermoregulatory and behavioral activating effects of beta-endorphin. *Peptides.*, **3**(4): 679-686

Kavaliers,M. 1982b. Effects of pineal shielding on the thermoregulatory behaviour of the white sucker *Catostomus commersoni. Physiol. Zool.*, **35**(2): 155-161.

Kavaliers,M. and C.L.Ralph 1980. Pineal involvement in the control of behavioral thermoregulation of the white sucker, *Catostomus commersoni. J. Exp. Zool.*, **212**(2): 301-303.

Keshavanath, P. 1981. Effects of estradiol-17 beta and melatonin treatment on the hypophysis, testes and seminal vesicles of the cat fish, *Heteropneustes fossilis* (Bloch) in the pre-spawing season. *J. Agric. Sci.*, **15**(1): 136-144.

Kezuka,H., M.Kobayashi, K.Aida and I.Hanyu 1989. Effects of photoperiod and pinealectomy on the gonadotropin surge and ovulation in gold fish *Crassius auratus*. *Nippon Suisan Gakkaishi Bull. Jap. Soc. Sci. Fish.*, **55**(12): 2099-2109.

Khan, I.A. and K.P. Joy 1987. Diurnal variation in, and effects of long photoperiod-raised temperature and melatonin on hypothalamic monoamine oxidase activity in the teleost, *Channa punctatus* . *J. Interdisciplinary - cycle Res.*, **18**(4): 287-292.

Khan, I.A. and K.P. Joy 1988. Diurnal variations in hypothalmic monoamine levels in the teleost *Channa punctatus* (Bloch) in response to melatonin under two photothermal conditions. *Fish Physiol. Biochem.*, **5**(4): 187-190.

Khan,I.A., and K.P.Joy 1990. Effect of season, pinealectomy, and blinding,

alone and in combination, on hypophalamic monoaminergic activity in the teleost *Channa punctatus* (Bloch). *J. Pineal Res.*, **8** (3): 277-287.

Krishnan, L. 1998. Status of seed production and culture of brackish water fin fishes in India. In *"Technological Advancements in Fisheries"*. (MS)

Kumari, M. and N.H.G. Dutt 1995 . Photoperiodic control on the testes and pituitary gonadotropic cells in *Puntius sarana* (Hamilton). *Proc. Natl. Acad. Sci.*, India B Biol. Sci., **65**(4): 355-365.

Maartensson., L.G.E. and R.G.G. Andersson 1997. Denervation of pigment cells lead to a receptor that is ultrasensitive to melatonin and noradrenaline. *Life Sci.*, **60**(18): 1575-1582.

Martinoli, M.G., L.M. Williams, O. Kah, L.T. Titchener and G. Pelletier 1991. Distribution of Central Melatonin binding sites in the goldfish (*Carassius auratus*). *Mol. Cell. Neurosci.*, **2**(1):78-85.

Matsumoto, J., T.J. Lynch, S.M. Grabowski, J.D. Taylor and T.T. Tchen 1982. Induction of melanized cells from a gold fish erythrophoroma: Isolation of pigment translocation variants. *Science Wash*, **217**(4565): 1149-1151.

McNulty, J.A. 1978. The pineal of the troglophilic fish *Chologaster agassizzi* : an ultrastructural study. *J. Neural, Trans.*, **43**(1) : 47-71.

McNulty, J.A. 1979. A Comparative light and electronic microscopic study of the Pineal Complex in the deep sea fishes, *Cyclothone signata* and *C . Acclinidens. J. Morphol.*, **162**(1) : 1-16.

McNulty,J.A. 1986. Uptake and metabolism of indole compounds by the gold fish pineal organ. *Gen. Comp. Endocrinol.*, **61**(2): 179-186.

Mc Nulty, J.A. 1988. Ultrastructure and biochemistry of the Pineal organ in deep-sea lantern fishes (Myctophidae). *Experientia.*, **44**(9) : 740-742.

Micale V. and F. Perdichizzi 1990. Gonadal responsiveness to photoperiod extension in captivity-born *Sparus aurata* (L.) during the male phase. *Boll. Zool.*, **57**(1): 21-26.

Nakari, T., A. Sivio and S. Pensnen 1987. Effects of an advanced photoperiod cycle on the gonadal development and spawning time of 2-year-old *Salmo*

gairdneri R. reared in earth ponds under extreme annual water temperature. *Aquaculture*, **67**(3-4): 369-384.

Nayak, P.K. and T.P. Singh 1987a. Effect of melatonin and 5-methoxytryptamine on sex steroids and thyroid hormones during the prespawning phase of the annual reproductive Cycle in the fresh water teleost, *Clarias batrachus*. *J. Pineal Res.*, **4**:377-386.

Nayak, P.K. and T.P. Singh 1987b. Effect of pinealectomy on thyroid hormone (T4 and T3) level in plasma during annual reproductive cycle in the fresh water catfish, *Clarias batrachus*. *J. Pineal Res.*, **4**: 387-394.

Nayak, P.K. and T.P. Singh 1988. Effect of pinealectomy on Testosterone, Estradiol-17 beta, Estrone, and 17d-Hydroxy Progesterone Levels during the annual reproductive cycle in the fresh water *Catfish*, *Clarias batrachus*. *J. Pineal Res.*, **5**:419-426.

Nayudu, P.L. and C.R. Hunter 1979. Cytological aspects and differential response to melatonin of melanophore based colour mutants in the guppy, *Poecilia reticulata*. *Copeia*, **2**:232-242.

Nishi, H. and R. Fujii 1992. Novel receptors for melatonin that mediate pigment dispersion are present in some melanophores of the pencil fish (*Nannostomus*). *Comp. Biochem. Physiol.*, C., **1030**(2): 263-268.

Ohta, T. and N. Ono 1983. Melatonin action on fish melanophores. 3. Effects of lighting conditions during maintenance. *Int. J. Acad. Ichthyol.*, **4**(1-2): 1-5.

Omura, Y. 1979. Light and electronic microscopic studies on the pineal tract of rainbow trout, *Salmo gairdneri*. *Rev. Can. Biol.*, **38**(2): 105 - 118.

Omura, Y. 1980. Histochemical and Ultrastructural studies on the nervous organization of the Pineal organ of the ayu, *Plecoglossus altivelis*. *Bull. Jap. Soc. Sci. Fish. Nissuishi.*, **46**(12) : 1483-1488.

Pang, C.S., M.A. Ali, P.K. Reddy, J.F. Leatherland, G.M. Brown and S.F. Pang 1994. A Comparative study of picomolar affinity 2-(Super (125) I) iodomelatonin binding sites in the hearts of three salmonid species. *Fish physiol. Biochem.*, **13**(5):371-378.

Parma-De-Crouk, M.J. 1996. Growth of Pimelodus *Clarias maculatus* (Pisces, Pimelodidae) fingerlings under experimental conditions. Effect of photoperiod. *Rev. Aroc. Cienc. Nat. Litor. St. Tome.*, **27**(2): 95-102.

Pavlidis, M., V. Theochari, I. Christofidis and A. Dessypris 1989. Effects of seasonally - changing photoperiods on the 1st and 2nd reproductive cycle of the rainbow trout *(Salmo gairdneri)*: Role of Plasma thyroid hormones. In *"Aquaculture Europe - 89"*, Intern. Aquacult. Conference, Francce, **10**: 203-204.

Popek, W., P. Epler, K. Bieniarz, and M. Sokolowska - Mikolajczyk 1997. Contribution of factors regulating melatonine release from pineal gland of carp (*Cyprinus carpio* L.) in normal and in polluted environments. *Arch. Ryb. Pol. Arch. Pol. Fish*, **5**(1): 59-75.

Portar,M.J.R., C.F.Randall, N,.R.Bromage and J.E.Thorpe 1998. The role of melatonin and the pineal gland on development and smoltification of Atlantic salmon (*Salmo salar*) parr. *Aquaculture* **168**(1-4): 139-155.

Porter, M., N. Duncan and N. Bromage 1999. Light manipulation, melatonin and the All - Year - Round supplies of 3-4 Kg Salmon. *Aquaculture News*, July 1999, No: 25, pp: 31-32.

Randall,C.F., N.R.Bromage, J.E.Thorpe and M.S.Miles 1994. Photoperiod, melatonin and the timing of smoltification in salmonid fish. In *"Salmonid smoltification-4"*, 4- Intern. Salmonid Smolt Workshop, CANADA, **121**(1-3): 295.

Rasquin, P. 1958. Studies in the control of pigment cells and light responses in recent Teleost fishes. *Amer. Mus. Novitates*, **115**: 6-68.

Razani, H., I. Hanyu and K. Aida 1987. Critical daylength and temperature level for photoperiodism in gonadal maturation of gold fish. *Exp. Biol.*, **47**(2): 89-94.

Richter, C.J.J., W.J.A.R. Viveen, E.H. Eding, M. Sukkel, A.J.Rothuis, M.F.P.M. Hoof, F.G.J.V. Berg and P.G.W.J.V.Oordt 1987. The significance of photoperiodicity, water temperature and an inherent endogenous rhythm for the production of viable eggs by the African Catfish, *Clarias garlepinus*,

kept in subtropical ponds in Israel and under Israelli and Dutch hatchery conditions. *Aquaculture*, **63** (1-4): 169-185.

RourkeA.W. 1994. Melatonin and smolt status. *Proc. Intern. Fish Physiol. Sym. Univer. British Columbia, USA*,(MacKinlay, Ed.), Van. Can. Fish Physiol. Assoc., pp. 110-115.

Sagi,G., M.Abraham and V.Hilge 1983. Pinealectomy and ovarian developement in the grey mullet, *Liza ramada*. *J. Fish. Biol.*, **23**(3): 339-345.

Sakena,P.K. and K.Anand 1977.A comparison of ovarian recrudescence in the cat fish, *Mystus tengara* (Ham.), exposed to short photoperiods, to long photoperiods, and to melatonin. *Gen. Com. Endocrinol.*, **33**(4): 506-511.

Semenkova, T.B. 1984a. Hormonal effects on the liver lipids in the Siberian sturgeon *Acipenser baeri* B. from the Lena River. *Vopr. Ikhtiol.*, **24**(1): 158-164.

Semenkova,T.B. and Yu.Yu.Sautin 1990. Prolactin-regulated lipid metabolism and neuroendocrine control in the Siberian sturgeon, *Acipenser baeri*. *J. Ichthyol.*, **30**(8): 93-104.

Senthilkumaran,B. and K.P.Joy 1995. Effects of melatonin ,P-chlorophenylalanine, and alpha-methylparatyrosine on plasma gonadotropin level and ovarian activity in the cat fish, *Heteropneustes fossilis*: A study correlating changes in hypothalamic monoamines. *Fish Physio. Biochem.*, **14**(6): 471-480.

Soengas, J.L., E.F.Strong, M.D.Andres and M.Aldegunde 1998. Dose dependent effects of acute melatonin treatments in brain carbohydrate metabolism of rainbow trout. *Fish Physiol. Biochem.*, **18**(4): 311-319.

Sparwasser,K. 1987. The influence of metoclopramide and melatonin on activity and schooling behaviour in *Chromis viridis*. *P. S. Z. N.-I. Mar. Ecol.*, **8**(4):297-312.

Srivastava, S.J. and R. Singh 1991. Effects of constant photoperiod -temperature regimes on ovarion activity during the annual reproductive cycle in the murrel, *Channa punctatus* (Bloch). *Aquaculture*, **96**(3-4): 383-391.

Stacey,N.E. and F.W.Goetz 1982. Role of Prostaglandin in fish reproduction. *Can. J. Fish. Aquat. Sci.*, **39**(1): 92-98.

Steffansson, S.O., G. Naevdal and T. Hansen 1989. The influence of three unchanging photoperiods on growth and par smolt transformation of Atlantic salmon, *Salmo salar* L. *J. Fish Biol.*, **35**(2): 237-247.

Sugimoto, M., N. Oshima and R. Fujii 1985. Mechanism controlling motile responses of amelanotic melanophores in the medaka, *Oryzias latipes*. *Zool. Sci.*, **2**(3): 317-322.

Tabata,M., M.N.Maung and M.Oguri 1991. The role of eyes and the pineal organ in the circadian rhythmicity in the cat fish *Silurus aratus*. *Nippon Suisan Gakkaishi Bull. Jap. Soc. Sci. Fish.*, **57**(4): 607-612.

Taranger , G.L., C. Hauk, S.O. Stefansson, T.B. Bjorn, Walther and Hansen 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradiol-17 beta profiles in Atlantic salmon, *Salmo salar*. *Aquaculture*, **162**(1-2): 85-98.

Thorarensen, H. and W.C. Clarke 1989. Smoltification induced by a skeleton photoperiod in underling coho salmon *(Oncorhynchus kisutch). Fish Physiol. Biochem.*, **6**(1): 11-18.

Tilden, A.R., P.Rasmussen, R.M.Awantang, S. Furlang, J.Goldstein, M.Palsgrove and A.Sauer 1997. Melatonin cycle in the fiddler crab *Uca pugilator* and influence of melatonin on limb generation. *J. Pineal Res.*, **23**(3): 142-147.

Tilden, A.R., T.Mainardi, C.Holtzer and J.Alt 1998. Melatonin and circadian rhythms in the green shore crab, *Carcinus maenus*. *Bull. Mt. Desert Isl. Biol. Lab*, **37**: 103-104.

Varghese,E., and A.K.Pati 1997a. Annual cycle of thermal tolerance in sham-pinealectomized and pinealectomized air breathing catfish *Clarias batrachus*. *Biol. Rhythm Res.*, **28**(4): 453-459.

Visconti, M.A. and A.M.L. Castrucii 1993. Melanotropin receptors in the cartilaginous fish, *Potamotrygon reticulatus* and in the lungish, *Lepidosiren paradoxa*. *Comp. Biochem. Physiol*, **106**C(2): 523-528.

Vodicnic,M.J., J.Olcese, G.Delahunty and F.deVlaming 1979. The effects of blinding, pinealectomy and exposure to constant dark conditions on gonadal activity in the gold fish, *Carassius auratus. Environ. Biol. Fish.*, **4**(2):173-177.

Walsh, W.J. 1987. Patterns of recruitment and spawning in Hawaiian reef fishes. *Environ, Biol: Fish.*, **18**(4):257-276.

Wang, D. 1994. A study on microscopic structure and ultrastructure of the Pineal body in a teleost fish *(Carassius auratus). Acta Hydrobiol. Sin. Shuisheng Shengwu Xuebad*, **18**(1): 22-25.

Weber, L.J. and J.R. Smith 1980. Possible role of pineal gland in migratory behaviour of salmonids. *Proc. Sym. Salmonid Ecosystem North Pac. Ocean* (W.J. McNeil and D.C. Himsworth, Eds.). Oregen state University, USA, pp. 313-320.

Wesemann, W. 1974. Biochemistry of serotonin and synaptic membranes in Neurotransmission (Fig. 3). In *"Biochemistry of sensory functions"* (L. Joenicke, Ed .), Springer - Verlag Pub., Berlin, pp.565-590.

Withyachumnarnkul,B., K.Buppaniroj and A.Pongsa-Asawapaiboon 1992. N-aceytltransferase and melatonin levels in optic lobe of gaint fresh water prawn, *Marcobrachium rosenbergii* (DeMan). *Com. Biochem. Physiol.*, **102A**(4): 703-707.

Wright, P.J., D. Rowe and J.E. Thorpe 1991. Daily growth increments in the otoliths of Atlantic salmon parr, *Salmo salar* L., and the influence on environmental factors on their periodicity. *J. Fish Biol.*, **39**(1)., 103-113.

Yadav, B.N. 1995. The Pineal organ. In *"Fish endocrinology"*. Daya Pub., New Delhi, pp. 108-124.

Zachmann, A., J. Falcon, S.C.M. Knijff, V.Bolliet and M.A. Ali 1992. Effects of photoperiod and temperature on rhythmic melatonin secretion from the pineal organ of the white sucker (*Catostomus commersoni*) in vitro. *Gen. Comp. Endocrinol.*, **86**(1): 26-33.

Quantitative dietary requirement of threonine for *Penaeus monodon*

M. Natarajan, C. Gopal,
A. Safiullah, and D. N. Swamy.
Central Institute of Brackishwater Aquaculture, Chennai - 600 034.

ABSTRACT

Dietary requirement of essential amino acid threonine for juvenile tiger shrimp, Penaeus monodon *was determined by feeding semi-purified test diet containing graded levels of threonine (0.66, 1.20, 1.73, 2.27 and 2.81 %). Triplicate groups of shrimp (initial weight 1.44 ± 0.48 g) were fed at the rate of 15% of body weight per day for a period of 30 days. At termination, the results showed highest growth and survival and lowest FCR when fed threonine at 2.27 %. The results are discussed with reference to optimal dietary requirement of this amino acid for best results.*

Introduction

Nutritive value of dietary proteins and its utilization in shrimps depends upon the amino acid (AA) content and ratios (Wilson and Peo, 1985). The quantitative requirements of amino acids of the cultured aquatic species largely depend on the source and profile in the protein, which should meet the requirements of the animal. Studies have shown that 10 amino acids are essential for shrimps (Pascual and Kanazawa, 1986) like in all vertebrates for efficient growth and survival. Crustaceans have been reported to be poor utilisers of free amino acids (EAA) or hydrolysed protein products present in their diets (Deshimaru, 1982; Teshima *et al.*, 1992), but efficiently utilize bound amino acids. Hence,

it is necessary to study the optimal dietary requirement of EAA for shrimps before formulating practical diets for shrimps. The dietary requirement studies are done by eliminating a particular amino acid in their test diets (dose-response curve method) or by estimation of serum or muscle levels of the amino acid in free form. In shrimps such studies are scanty - arginine and methonine (Kitabayashi *et al.*, 1971; Gopal *et al.*, 1999), lysine (Akiyama, 1986; Fox *et al.*, 1992) and arginine (Chen *et al.*, 1992) to mention a few. Threonine has a major role in osmoregulation (Hartenstein, 1970) and has been studied in postlarve of *P. monodon* (Millamena *et al.*, 1997). In the present study dietary requirement of threonine for optimum growth and feed efficiency was determined for juvenile *P. monodon*.

Material and methods

The study was conducted in 15 nos. of 100 liter capacity FRP tanks with continuous flow through of seawater (salinity 25.35 ± 0.23 ppt; pH 8.32 ± 8.32 ±, temp. 27.4 ± 0.44°C). Triplicate tanks were stocked with juvenile *P. monodon* from single stock (mean weight 1.44 ± 0.48 g) @ 10 Nos./tank. The feed residue was collected and fecal matter was siphoned out daily, followed by 50% water change. The experimental duration was for 30 days.

Five purified moist diets (isonitrogenous, 40% and isolipidic, 8%) which contained casein and gelatin (3:1) as the main protein sources apart from other ingredients, were prepared according to Gopal and Paulraj, 1990. Crystalline amino acid mixture (in CMC) without threonine were added to simulate the amino acid profile of shrimp muscle (Penaflorida, 1991). Graded levels of threonine were incorporated and adjusted with glutamic acid and aspartic acid in the basal feed to give isonitrogenous diets (Table 1). The diets were neutralised and stored at - 20°C. The feeds were thawed prior to feeding. The shrimps were fed in two instalments at 15% body weight per day.

Table 1. Composition of synthetic test feeds used for determining the optimal threonine requirement in *P. monodon*.

Ingredients	Feed Nos.				
	I	II	III	IV	V
Casein	15.00	15.00	15.00	15.00	15.00
Egg albumin	1.65	1.65	1.65	1.65	1.65
Gelatin	5.00	5.00	5.00	5.00	5.00
Dextrose	7.00	7.00	7.00	7.00	7.00
Starch	25.00	25.00	25.00	25.00	25.00
Cholesterol	0.50	0.50	0.50	0.50	0.50
Lecithin	1.40	1.40	1.40	1.40	1.40
Fish oil	3.60	3.60	3.60	3.60	3.60
Sunflower oil	1.40	1.40	1.40	1.40	1.40
Agar agar	2.00	2.00	2.00	2.00	2.00
CMC	1.00	1.00	1.00	1.00	1.00
Minerals & vitamins*	6.60	6.00	6.00	6.00	6.00
Alpha-cellulose	9.68	9.68	9.68	9.68	9.68
Amino acid mix*	15.96	15.96	15.96	15.96	15.96
Threonine	0.00	0.54	1.07	1.61	2.15
Aspartic acid	2.11	1.83	1.57	1.30	1.03
Glutamic acid	2.10	1.84	1.57	1.30	1.03
Total	100.00	100.00	100.00	100.00	100.00

* As per Gopal *et al.*, 1999.

* Amino acids other than threo, asp and glu.

Freeze-dried (VIRTIS bench top freeze dryer) samples of feed and experimental animals were analysed for proximate composition (AOAC, 1990). The amino acid analysis was done using HPLC. The data were subjected to ANOVA and Aunean's multiple range tests (Snedecor and Cochran, 1967). Percentage values were arcsine % transformed before statistical analysis.

Results and discussion

Dietary threonine level significantly affects the percent increase in length, weight and food conversion ratio but not the survival rate. The control treatment gave only 20.4% increase in length and 76.2% increase in weight in 30 days. The growth in length and weight was 28.7% and 99.7%, respectively over the same period when fed 2.27% threonine, significantly higher than the control ($p>0.05$). The lowest FCR was also recorded at this level of total threonine ($p>0.05$). However, statistical analysis of data showed no difference ($p<0.05$) between 1.73%, 2.27% and 2.81% dietary threonine in terms of percent increases in length and weight as well as food conversion efficiency (Table 2). Therefore 1.73% dietary threonine could be the lower limit for this essential amino acid, below which the weight increase as well as food conversion efficiencies get reduced. There were few mortalities in all treatments as a result of molting and subsequent death/cannibalism. However, comparing the survival rates at different levels of threonine, there was a slight upward trend beyond the optimum, Shrimps were able to tolerate higher level of threonine (2.81%) even though there was decline in growth.

Variation of body amino acid level in fishes and crustaceans are quite marked, as there is differences in their physiology. Studies have shown that in crustacean muscle the levels of threonine is 30-40% less compared to fish muscle (Borgstrom, 1962). The reported levels of threonine in fishes range from 3.84 - 4.5% (Nose, 1979; Santiago and Lovell, 1988; Borlongan and Coloso, 1993) whereas in crustacean it ranges from 1.11-1.48% with an optimum of 1.40% in *P.monodon* of 0.05g size (Millamena *et al.*, 1997). In the present study it was observed that with increasing levels of threonine, the weight gain in shrimps was observed upto 2.27% and declines thereafter at higher level.

The decline in growth at 2.81% threonine suggests the possible toxicity effect of this amino acid on the shrimp. Millamena *et al.* (1997) have also reported similarly. The optimum level observed in the present study is higher than that reported by Millemena *et al.*, (1997) for shrimp weighing 0.05g. This could be attributed to the differences in the weight of animal used and the experimental conditions. Water temperatures

Table 2. Growth, food conversion ratio and survival of juvenile *P. monodon* fed diets containing graded levels of threonine for 30 days.

Threonine levels in diet (g/100g)		Initial size (Mean ± SD) $		Final size (Mean ± SD)		% size increase (Mean ± SD)		FCR	%Survival
Supp.	Total	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)		
0.00	0.66	61.67±7.52^{A}	1.85±0.73^{A}	74.22±9.02^{A}	3.25±1.20^{A}	20.39±4.53^{B}	76.21±7.99^{B}	5.51±0.40^{A}	86.68±5.77^{A}
0.54	1.20	56.17±2.01^{A}	1.28±0.72^{A}	67.86.12.92^{A}	2.27±1.16^{A}	26.38±1.47^{AB}	81.35±11.66^{B}	5.48±1.03^{A}	76.67±11.55^{A}
1.07	1.73	58.47±6.40^{A}	1.46±0.42^{A}	71.21±5.32^{A}	2.76±0.73^{A}	22.1±5.53^{AB}	88.62±8.51^{AB}	4.64±1.03^{AB}	76.67±5.77^{A}
1.61	2.27	55.90±4.25^{A}	1.38±0.20^{A}	71.85±3.86^{A}	2.76±0.46^{A}	28.70±3.95^{A}	99.68±r.49^{A}	3.68±0.08^{B}	83.33±11.55^{A}
2.15	2.81	55.13±4.67^{A}	1.24±0.37^{A}	69.71±6.25^{A}	2.47±0.77^{A}	22.43±2.25^{AB}	98.40±3.64^{A}	3.89±0.68^{B}	90.00±10.00^{A}

$ Values with same superscripts in each column are not significantly different ($p<0.05$).

could have also influenced the variation in either study. Fishes generally require higher levels of dietary proteins at warmer temperatures (Delong *et al.*, 1958). Comparison of the amino acid profile of shrimp fed increasing levels of threonine with the initial did not show any differences in case of essential amino acids. However non-EAA levels were higher in shrimp fed 2.27% threonine feed.

Conclusion

The dietary threonine requirements for juvenile *Penaeus monodon* for good growth and food conversion efficiencies are a minimum of 1.73%, with a possible upper limit of 2.27% in feed.

Acknowledgements

The authors thank the former Directors, Dr. K. Alagarswami, CIBA, Chennai and Dr. K. Gopakumar, CIFT, Kochi for coordinating the project sponsored by Department of Biotechnology, New Delhi and ICAR. Dr. G.R.M.Rao, Director, CIBA, Chennai is thanked for permitting the publication of this paper.

References

Akiyama, D., 1986. *The development of purified diet and nutritional requirement of lysine in penaeid shrimp.* Ph.D. dissertation, Texas A&M Univ., College Station.

AOAC 1990. Official methods of Analysis. Vol. 1 & 2, 15th edition, Association of Official Analytical Chemists Inc., USA.

Borgstrom, G., 1962. Shellfish proteins - Nutritive aspects. In : *Fish and Food,* (Ed.) Borgstrom, G. Publ. Academic Press London, 434pp.

Borlongan, I.G. and R.M. Coloso, 1993. Requirements of juvenile milkfish *Chanos chanos* for essential amino acids. *J. Nutr., 123*:125-132.

Chen, H.Y., Y.T. Leu, and I. Roelants, 1992. Quantification of arginine requirement of juvenile marine shrimp, *Penaeus monodon* using microencapsulated arginine. *Mar. biol., **114**:*229-233.

Delong, D.C., J.E. Halver, and E.T. Mertz, 1958. Nutrition of salmonid fishes. VI. protein requirements of Chinook salmon at two water temperatures. *J. Nutr.*, ***65*** : 589-599.

Deshimaru, O., 1982. Protein and amino acid nutrition *P. japonicus*. In: G.D.Pruder, C.J.Langdon and D.E. Conklin (Eds.) *Proc. Second Intl. Conf. on Aquaculture Nutrition : Biochemical and Physiological Approaches to Shellfish Nutrition, pp.* 106-123, Louisiana State Univ., Batton Rouge, Louisiana.

Fox, J., A.L. Lawrence, and E. Li-Chan, 1992. Apparent lysine requirement of *Penaeus vannamei* using covalent and crystalline lysine supplementation. Abstracts of Aquaculture '92, Marriott's Orlando World center, Orlando, Florida, 21-25 May, pp. 96-97.

Gopal, C. and R. Paulraj, 1990. Protein requirement of juvenile *P. indicus*. 1. Food consumption and growth. *Ind. Acad. Sci.*, ***99(5)*** : 401-409.

Gopal, C., M. Natarajan, D.N. Swamy, P. Madhavan, K.G.R. Nair, A.G.Radhakrishna, and A. Safiullah, 1999. Quantitative dietary requirement of methionine for *Penaeus monodon*. *The fourth Indian Fisheries Forum Proceedings* 24-28 Nov., 1996, Kochi : 313-315.

Hartenstein, R., 1970. Nitrogen metabolism in non-insect Arthropods (Crustacea). In: *Comparative Biochemistry of Nitrogen Metabolism* (Ed.) J.M. Campbell vol. 1. Academic Press NY 566pp.

Kitabayashi, K., K. Shudo, K. Nakamura, and S. Ishikawa, 1971. Studies on formula for Kuruma prawn-III : On the growth promoting effects of both arginine and methionine. *Bull. Tokai Reg. Fish. Res. Lab.*, ***65*** : 119-127.

Millamena, O.M., M.N. Bautista, O.S. Reyes, and A. Kanazawa, 1997. Threonine requirement of juvenile marine shrimp *Penaeus monodon*. *Aquaculture*, ***151***; 9-14.

Nose, T., 1979. Summary report on the requirement of EAA for carp. In : J.E. Halver and K. Twies (Eds.), *Finfish Nutrition and Fishfeed Technology*. Heenemann GmbH, Berlin, pp 145-156.

Pascual, F.P. and A. Kanazawa, 1986. Specific amino acid - free semi-purified diets for *P. monodon* juveniles. *Mem. Kagoshima Univ. Res. Center S., Pac.*, ***7 :*** 65-72.

Penaflorida, V., 1991. An evaluation of indigenous protein sources as potential component in the diet formulation for tiger prawn *Penaeus monodon* using essential amino acids index. *Aquaculture* ***83:*** 319-330.

Santiago, C.B. and R.T. Lovell, 1988. Amino acids requirements of Nile tilapia. *J. Nutr.*, ***118 :*** 1540-1546.

Snedecor, G.W. and W.G. Cochran, 1967. *Statistical methods.* Oxford and IBH Pub. Co., New Delhi, pp. 59-296.

Teshima, S., A. Kanazawa, and S. Koshio, 1992. Supplemental effects of methionine enriched plastein in *P. japonicus* diets. *Aquaculture*, ***101*** (1-2) : 85-93.

Wilson, R.P. and W.E. Poe, 1985. Relationship of whole body and egg essential amino acids pattern to amino acids requirement patterns in channel catfish, *Ictalurus punctatus. Comp. Biochem. Physiol.*, ***80B :*** 385-388.

Effect of manures and fertilisers singly and in combination on shrimp production and environment

M.Muralidhar, B. P. Gupta, K.O.Joseph, K.K.Krishnani and P.S.Sudheesh

Central Institute of Brackishwater Aquaculture, Chennai- 600 034

ABSTRACT

Increasing chemical fertilisers beyond certain limits is undesirable in aquaculture and their quantity should be reduced by supplementing with organic manures. Manures and fertilisers were tried singly and in combination in yard experiment in order to study the enhancement of natural productivity and impact on environment. Organic manures such as cattle manure and poultry (chicken) manure @ 1 % and 0.3 % of soil weight and fertilisers viz., urea and single superphosphate @ 50 and 30 kg/ha, respectively and combination treatment of manures and fertilisers were applied to brackishwater in cement tanks of 1 ton capacity with 10 cm layer of saline soil at the bottom. Manure plus fertiliser combination treatment registered maximum primary production and plankton density as compared to control and other treatments before stocking of shrimp larvae and during culture period. Treatment of urea + single superphosphate registered higher concentration of nutrients and lower amount of metabolites before stocking. The concentration of metabolites and nutrients after stocking of shrimp larvae and at the time of harvest did not show any negative impact on the environment. Manures plus fertilisers combination resulted in higher shrimp yield as compared to control, cattle manure, poultry manure and urea + single superphosphate in 120 days.

Introduction

Nutrients play a vital role in the biological productivity of water bodies (Das and Jana, 1996). The traditional shrimp farming highly relies on natural productivity of ponds with minimum use of inputs and consequently the production is generally low. The primary objective of fertilisation is to maintain optimum nutrient condition for sustained biological production. The significance of the use of manures and fertilisers in increasing the productivity of brackishwater ponds has been emphasised by many research workers (Bhimachar and Tripathy, 1966; Lin, 1968; Chen, 1972). Organic and chemical fertilisers have been found necessary to increase the pond productivity at a stocking density greater than 2000 fry/ha (Apud *et al.*, 1981). The food organisms growing in such enriched ponds vary with pond condition and location.

Shrimp farmers world wide use inorganic fertilisers to promote primary productivity. The addition of organic matter to natural water stimulates microbial biomass and productivity (Schroeder, 1978; Moriatry, 1986). The fertilisation of the ponds to stimulate the production of natural food is used extensively in pond based aquaculture partly to reduce the requirement for expensive supplementary diets. Even though pond fertilisation and feeding are the two important measures for increasing production, the latter due to its high cost is not popular among poor traditional shrimp farmers. Information on shrimp growth in fertiliser ponds without feed is limited.

The importance of fertilisation of ponds well in advance of stocking to allow the communities of natural food organisms has not been adequately documented. Relationships among fertilisation rates of brackishwater, primary productivity and shrimp production is not well known. Information regarding changes in physico-chemical and biological properties of soil and water after fertilisation in brackishater ponds are meager.

Materials and methods

Yard experiments were conducted at Muttukadu experimental station of Central Institute of Brackishwater Aquaculture, using one ton capacity circular cement tanks. The tanks were packed at the bottom to a height of 10 cm with dried saline clay loam soil (sand-49%, silt-15%, clay-36%, pH-8.1, electrical conductivity- 5.5 dS/m, organic carbon-0.12%, total nitrogen-0.036%), collected from the shrimp ponds near Mahabalipuram, Tamil Nadu.

In the present experiment manures and fertilisers, singly and in combination were tried without feed on enhancement of natural productivity, shrimp production and impact on environment comprising of soil and water. The treatments are 1. Cattle manure (CM) @ 1 % of soil weight 2. Poultry (chicken) manure (PM) @ 0.3 % of soil weight 3. Urea (U) @ 50 Kg/ha + single superphosphate (SSP) @ 30 Kg/ha 4. Combination of manures and fertilisers @ half of the dose when applied singly (i.e., cattle manure and poultry manure @ 0.5 and 0.15 % of soil weight and urea and SSP @ 25 and 15 Kg/ha, respectively) and 5. Control. The tanks were filled with fresh filtered seawater upto 20 cm, followed by treatment on the following day. The water level was raised slowly upto 80 cm and allowed to remain for a period of 29 days for the development of the algal bloom.

Penaeus monodon larvae (average weight 0.281g, average length 3.5 cm) obtained from shrimp hatchery of Central Institute of Brackishwater Aquaculture were stocked in cement tanks @ 8 nos./tank after 29 days of the treatment. The duration of the experiment (culture period) was 120 days. No water exchange was done and the water level was maintained constant throughout the course of the experiment by the addition of filtered seawater. Urea and superphosphate @ 10 kg/ha each were applied once in a month in all treatment tanks as a booster dose. At the end of the experiment shrimps were recovered and growth measured as weight was recorded.

Soil and water samples from experimental tanks were collected at weekly intervals throughout the experiment till harvest. Primary productivity was estimated once in every 15 days throughout the culture period using the light and dark bottle method (Strickland and Parsons, 1972). Total plankton was enumerated (APHA, 1989). Salinity, pH, turbidity, ammonia nitrogen (NH_3 –N), nitrite nitrogen (NO_2-N), Phosphate (PO_4), chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) were estimated by standard methods (APHA, 1989; Strickland and Parsons, 1972). The soil samples were measured immediately for oxidation-reduction potential (E_h) using a platinum electrode. The soil samples were dried and crushed to pass through 2 mm sieve and analysed for pH and electrical conductivity (EC) in 1:2.5 soil water ratio and organic carbon, total nitrogen and soil texture according to the methodologies given by Piper (1966) and Jackson (1967).

The experiment had a completely randomised design with three replicates. ANOVA and Duncan's multiple range tests were used to determine differences in treatment means (Gomez and Gomez, 1984).

Results and discussion

Water, soil and biological features before stocking shrimp

The weekly recorded primary production and plankton number before stocking shrimp is presented in Fig.1A. A significant difference between treatments and control was observed in enhancing primary production and plankton number. The manure plus fertiliser combination was significantly superior ($p<0.05$) over other treatments. The plankton number per liter ranged from 2000-2800 in manure and fertiliser combination followed by 1200-1900, 1600-2000, 1300-1600 and 450-550 with cattle manure, poultry manure, urea + SSP and control, respectively. High average primary production of 211.2 mg $C/m^3/h$ was registered with manure plus fertiliser treatment followed by 164 mg $C/m^3/h$ with urea + SSP. Chemical fertilisation is effective in inducing primary productivity because nutrients are rapidly incorporated by autotrophic organisms.

Manures contain undigested organic feed stuffs, liquid nitrogenous wastes and microbial cells that might have helped in increasing primary productivity. The results are in conformity with the findings of Moore (1986) with the addition of manures and Tacon (1990) with the addition of urea and superphosphate. Weekly depicted primary production values showed a decreasing trend after third week, when manures and fertilisers applied singly. Addition of chemical fertilisers might have helped in the slow mineralisation of nutrients from manures which inturn resulted in continuous increase of primary production with manure plus fertiliser treatment.

The average values of weekly-analysed turbidity and nitrate nitrogen (NO_3-N) and ammonia N and chemical oxygen demand (COD) before stocking shrimp larvae are presented in Figs. 1B and 1C. Water pH values did not vary significantly among the treatments. Initially higher turbidity values have been found with manures as compared to fertiliser and

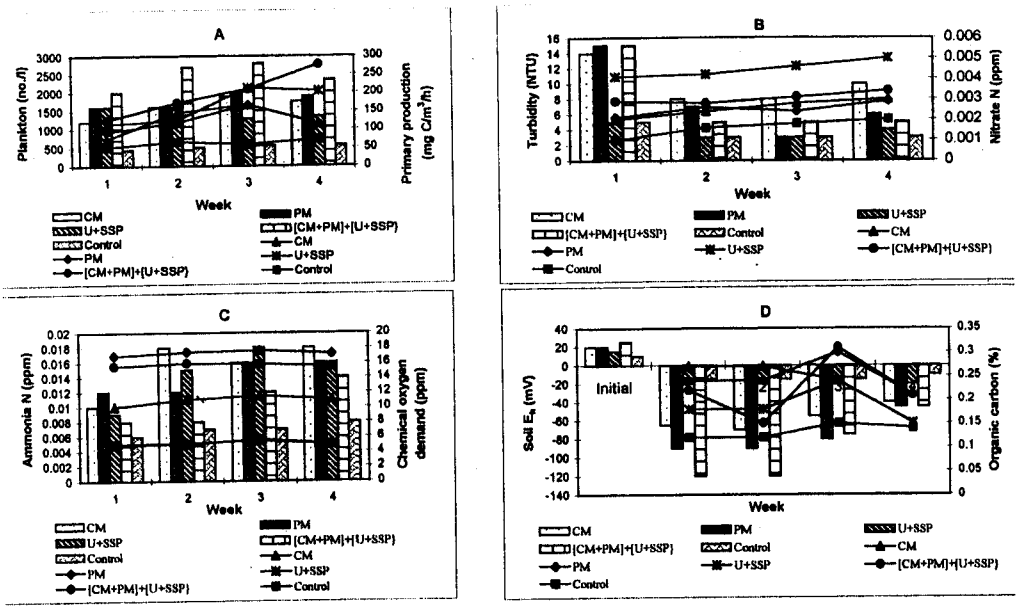


Fig. 1. Changes in biological features, water and soil quality before stocking shrimp

control treatments. Among manures high turbidity values were observed with cattle manure obviously due to high amount of cattle manure applied than poultry manure.

Nutrient concentration (NO_3-N and phosphate) has been observed more with fertiliser treatment as compared to control which, might be due to immediate release of nutrients from fertiliser. A steady increase in nutrient concentration has been recorded with progress of time. There were not many differences in metabolite (NH_3-N and NO_2-N) concentration. Higher COD and BOD_5 values have been observed with poultry manure treatment.

Weekly changes in soil redox potential (E_h) have been depicted in Fig.1D. E_h values ranged from positive (oxidised condition) before starting experiment to negative (reduced condition) immediately after imposing treatments with the progress of time. The E_h values ranged from +10 to -120 during prestocking period with all the treatments. Soil reaction (pH) and electrical conductivity (EC) values did not vary among treatments. Organic carbon content was more in manure treatments as compared to fertiliser and control treatments.

Water, soil and biological features during culture period

The range and average values of biological production, water and soil quality parameters along with standard error and significance are presented in Table 1. The weekly changes in important water and soil quality parameters are depicted in Figs. 2,3 and 4.

Primary production and plankton number were significantly more in manure plus fertiliser combination as compared to other treatments. There was no significant difference between manures and fertilisers in influencing primary production when applied singly. The primary production decreased after stocking shrimp compared to pre-stocking in all the treatments except control. A high average primary production of 99.67 mg C /m^3/h and plankton number of 2530/litre were observed with manure plus fertiliser treatment (Table 1). Higher natural productivity values can be attributed to high plankton population. Similar findings

Table 1. Effect of treatments on biological production and water and soil quality parameters.

Treatment	Cattle Manure (CM)	Poultry Manure (PM)	Urea + Single super phosphate (SSP)	(CM + PM) (Urea + SSP)	Control
		Biological Production			
Plankton (No./L)	1200 - 3000 (1600 ± 110.52)[a]	1200 - 3000 (1593 ± 103.97)[a]	800 - 2000 (1431 ± 80.04)[a]	2800 - 4200 (2530 ± 161.69)[b]	600 - 900 (783.3 ± 51.63)[c]
Primary production (mg $C/m^3/hr$)	67.6 - 93.7 (81.96 ± 15.39)[a]	62.5 - 109.3 (85.43 ± 16.42)[a]	62.5 - 93.3 (80.0 ± 13.45)[a]	83.3 - 109.3 (99.67 ± 19.21)[b]	62.5 - 88.5 (74.2 ± 6.12)[c]
		Water parameters			
NO_3 - N (ppm)	0.003 - 0.04 (0.012 ± 0.004)[a]	0.0027 - 0.038 (0.0117 ± 0.002)[a]	0.0038 - 0.036 (0.0089 ± 0.002)[b]	0.003 - 0.031 (0.0083 ± 0.002)[b]	0.002 - 0.019 (0.004 ± 0.001)[c]
Phosphate (ppm)	0.018 - 0.045 (0.037 ± 0.002)[a]	0.014 - 0.042 (0.029 ± 0.002)[a]	0.026 - 0.036 (0.03 ± 0.001)[b]	0.018 - 0.044 (0.031 ± 0.002)[b]	0.004 - 0.024 (0.015 ± 0.002)[c]
TAN (ppm)	0.018 - 0.075 (0.036 ± 0.004)[a]	0.016 - 0.08 (0.035 ± 0.005)[a]	0.016 - 0.075 (0.03 ± 0.004)[a]	0.014 - 0.088 (0.031 ± 0.006)[a]	0.01 - 0.08 (0.032 ± 0.006)[a]
Nitrite - N (ppm)	0.007 - 0.08 (0.0227 ± 0.005)[a]	0.0069 - 0.06 (0.0193 ± 0.004)[b]	0.003 - 0.05 (0.0157 ± 0.003)[c]	0.005 - 0.085 (0.0221 ± 0.004)[a]	0.006 - 0.058 (0.015 ± 0.004)[c]
COD (ppm)	18 - 46 (32.59 ± 2.15)[a]	16.2 - 45 (32.12 ± 2.34)[a]	8.5 - 45 (27.44 ± 2.74)[b]	14.8 - 38.5 (30.57 ± 2.09)[a]	4.8 - 39.5 (22.52 ± 2.77)[c]
BOD_5(ppm)	6 - 16.5 (11.55 ± 0.834)[a]	5.8 - 16.0 (11.56 ± 0.837)[a]	2.5 - 16.1 (10.51 ± 0.999)[ab]	4.9 - 14.2 (9.92 ± 0.740)[b]	2 - 14 (8.37 ± 0.878)[c]
		Soil parameters			
E_h(mV)	-100 to - 165 (-136 ± 4.987)[a]	-80 to - 180 (-134 ± 6.679)[a]	-60 to - 170 (-118 ± 6.669)[b]	-65 to - 175 (-125 ± 7.441)[b]	-60 to -150 (-91 ± 6.239)[c]
p^H (1:2.5)	7.61 - 8.69 (8.12 ± 0.069)[a]	7.74 - 8.9 (8.10 ± 0.076)[a]	7.68 - 8.53 (8.17 ± 0.062)[a]	7.7 - 8.65 (8.18 ± 0.065)[a]	8.10 - 8.56 (8.18 ± 0.064)[a]
EC (dS/m)	8.22 - 13.8 (10.64 ± 0.372)[a]	8.24 - 12.64 (10.96 ± 0.289)[a]	9.11-12.45 (11.03 ± 0.246)[a]	9.1 - 13.8 (10.51 ± 0.353)[a]	7.7 - 12.04 (10/21 ± 0.313)[a]
Organic carbon (%)	0.18 - 0.4 (0.255 ± 0.014)[ab]	0.22 - 0.35 (0.245 ± .011)[ac]	0.16 - 0.3 (0.22 ± 0.010)[cd]	0.21 -0.36 (0.282 ± 0.009)[b]	0.14 - 0.25 (0.20 ± 0.008)[d]
Total Nitrogen (%)	(0.0273 ± 0.012)[a]	(0.0278 ± 0.011)[a]	(0.297 ± 0.015)[ab]	(0.0307 ± 0.017)[b]	(0.033 ± 0.016)[b]

The values in parentheses are average of 3 replicates with standard error.
The average values with same alphabet as superscript are not significant (p>0.05).

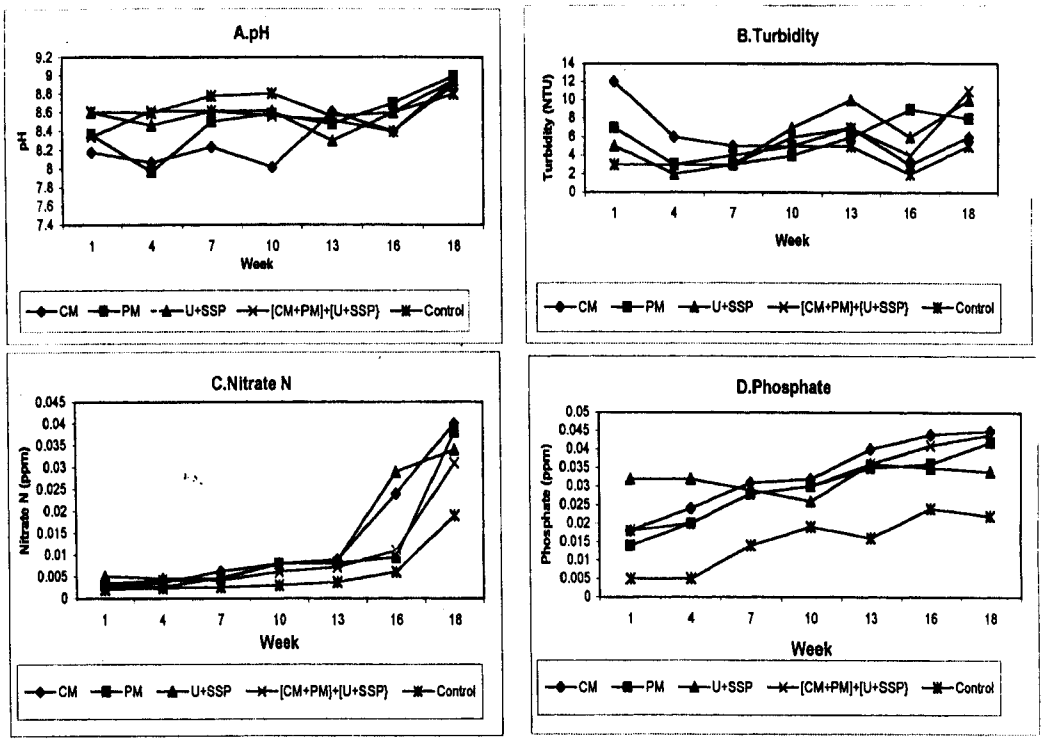


Fig. 2. Weekly changes in water quality parameters during culture period

have been observed by research workers (Rao *et al.*, 1982; Singh and Sharma, 1999) with the application of manures. Ayyapan *et al.* (1991) reported low primary production as the ponds did not receive any external nutrient input.

Water pH did not vary widely among the treatments after stocking shrimp during whole culture period (Fig. 2A). Subosa and Bautista (1991) observed no change in water pH in the experiment with manures and fertilisers. The turbidity in water (Fig. 2B) did not show a definite increasing or decreasing trend with progress of time. Nitrate nitrogen concentration was significantly more in treatments wherever chemical fertiliser was applied. Average nitrate N values of 0.0083 to 0.0089 ppm were observed with chemical fertilisers as compared to 0.0012 ppm with

manure treatments (Fig. 2C). Poultry manure rich in nutrient concentration, fertiliser and manure plus fertiliser combination treatments were significantly superior over cattle manure in increasing phosphate concentration (Fig.2D).

One of the major water quality problems in intensive aquaculture systems is the accumulation of the inorganic N species (NH_4^+ -N and NO_2^--N) in water. Ammonia N values did not vary among the treatments. All the treatments showed an increasing trend after 10 weeks (Fig.3A). The lack of significance may be attributed to the uniform application of urea and SSP to all the tanks every month as a booster dose. Nitrite-N concentration was more in poultry manure and fertiliser tanks as

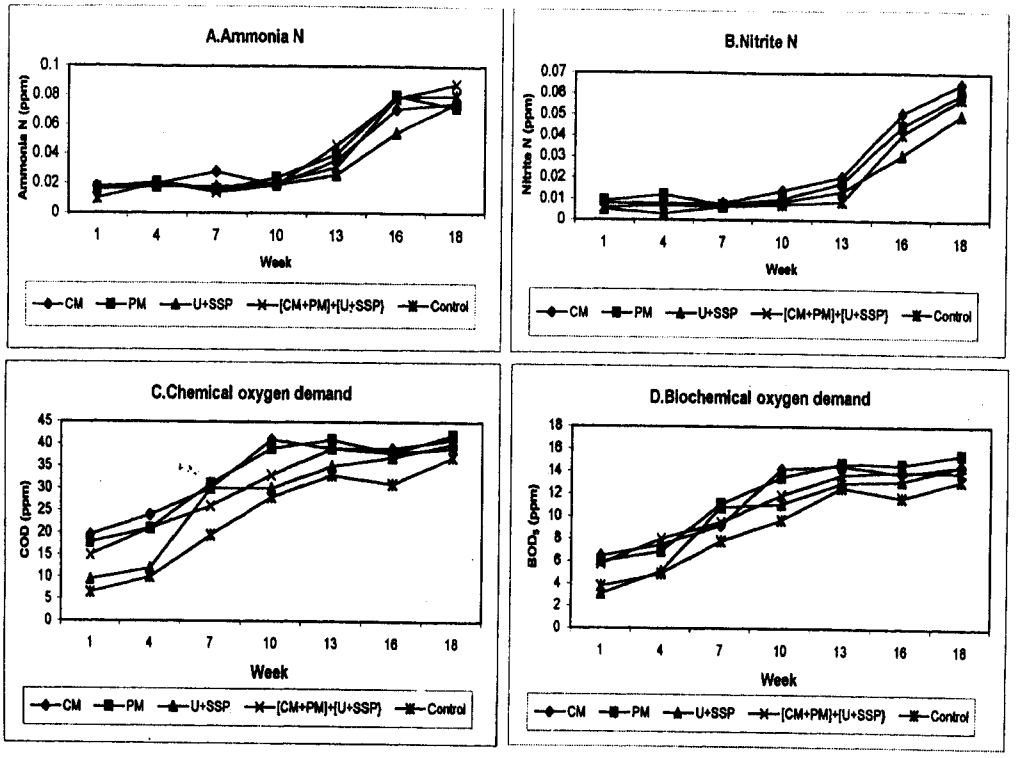


Fig. 3. Weekly changes in metabolites concentration during culture period

compared to cattle manure and manure plus fertiliser treatments. An increasing trend was observed after 13 weeks (Fig.3B). Chemical oxygen demand (COD) was significantly more in treatments, wherever manures were applied. Increase in COD was recorded upto 10 -13 weeks with all the treatments (Fig.3C). Manure plus fertiliser combination treatment registered low biochemical oxygen demand (BOD_5) values of 9.92 ppm as compared to other treatments. BOD_5 values showed an increasing trend upto 13 weeks and then more or less same with the progress of time (Fig.3D). BOD_5 value being an indicator of organic pollution indicates that manure treatments exert maximum load. All the water quality parameters during experiment are within normal range. The environment comprising of soil and water quality parameters in the experiment with manures and fertilisers are in normal range (Mohan *et al.*, 1995).

Manures when applied alone were superior in making soil more reduced. The decomposition of organic matter can lead to anaerobic conditions in pond soil. Anaerobic zones in soils have low redox potential. Average E_h values of -134 mV and -136 mV have been observed with cattle manure and poultry manure treatments, respectively. More reduced soil condition has been observed during harvest time (Fig.4A). Soil pH and electrical conductivity did not vary among the treatments. Manures and fertilisers combination treatment registered more average organic carbon (Fig.4B). Total nitrogen content in soil was more or less same with all the treatments.

Shrimp growth and production

Manure plus fertiliser combination followed by poultry manure registered more mean increase in shrimp weight of 4.82 and 4.42 g, respectively (Fig.5), which resulted in extrapolated shrimp production of 366 and 333 kg/ha (at 95% survival rate). It has been observed from

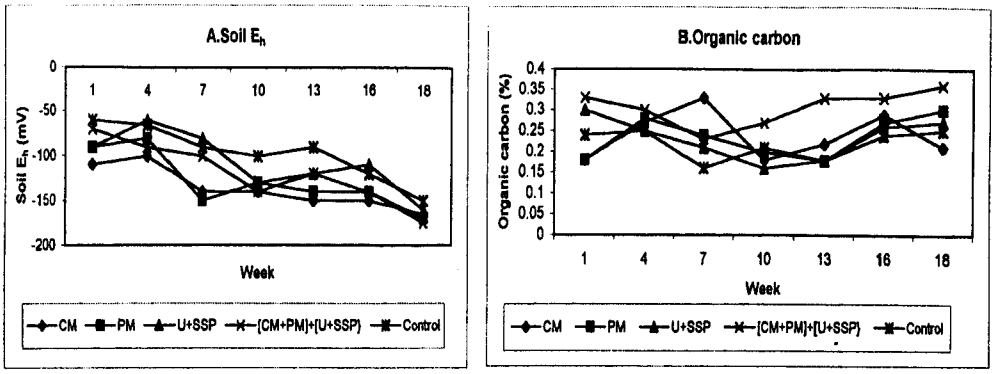


Fig. 4. Weekly changes in soil redox potential and Organic carbon during culture period

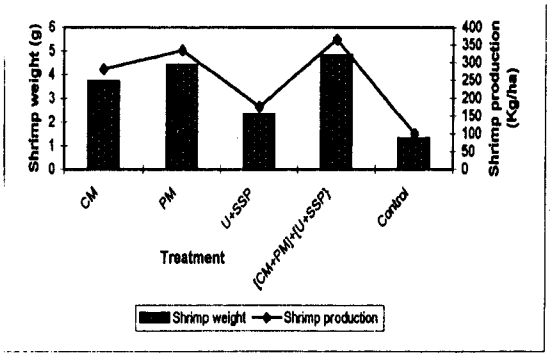


Fig. 5. Effect of manures and fertilisers on increase in shrimp growth and production.

Fig.5 that chemical fertiliser treatment registered low shrimp production (176 Kg/ha) next to control (100 kg/ha). Subosa and Bautista (1991) reported more mean increase in shrimp body weight at harvest in fertilised ponds along with manure. Fertilisation in some cases may have desired effect of increasing primary productivity, but shrimp production may not likewise affected (Teichert Coddington and Rodriguez, 1994). Maclean *et al.* (1994) observed similar findings.

Generally the productivity of water bodies like confined ponds

has been observed less where inputs are not supplemented. The application of manures plus fertilisers in combination gave high shrimp production in the present experiment. Manures and fertilisers either singly or in combination did not pose any negative impact on environment comprising of soil and water quality.

Acknowledgement

The authors are grateful to Dr.G.R.M.Rao, Director, CIBA, Chennai for providing facilities and encouragement throughout this work.

References

APHA 1989. *Standard methods for the examination of water and wastewater.* 16th ed. American Public Health Association, Washington, D.C., USA.

Apud, F.D., K.Gonzales and N. Deatras, 1981. Survival, growth and production of *Penaeus monodon* Fabricus at different stocking densities in earthen ponds with flow – through system and supplemental feeding. *Fish. Res. J. Philipp.*, 6: 1-9.

Ayyappan, S., C.S. Purushothaman, P.K.Saha and K.C.Pani 1991. Heterotrophic bacterial populations and primary production in fish ponds under different management practices. *J. Aquaculture*, 1: 11-29.

Bhimachar, B.S. and S.D.Tripathi 1966. A review of culture fisheries activities in India. F.A.O. *World Symp. on Warm Water Pond Fish culture*, Rome.

Chen, T.P. 1972. Fertilisation and feeding in coastal fish farms of Taiwan. In: *Coastal Aquaculture in the Indo-Pacific Region, Fishing News* (Books) Ltd., London.

Das, S.K. and B.B. Jana 1996. Pond fertilisation through inorganic sources: an overview. *Indian J. Fish.*, 43(2): 137-155.

Gomez, K.A. and A.A.Gomez 1984. *Statistical Procedures for Agricultural Research,* 2nd ed. An International Rice Research Institute Book. Wiley, New York, NY, 680 pp.

Jackson, M.L. 1967. *Soil chemical Analysis.* Prentice-Hall of India Pvt. Ltd., New Delhi, 498 p.

Lin, S.Y. 1968. Milk fish farming. In: *Taiwan Fish Culture Report.* Taiwan Fish. Res. Inst., 3.

Maclean, M.H., K.J. Ang, J.H. Brown, K.Jauncey and J.C.Fry 1994. Aquatic and benthic bacteria responses to feed and fertilizer application trials with the freshwater prawn, *Macrobrachium rosenbergii* (de Man), *Aquaculture,* 120: 81-93.

Mohan. D., S.Sundaramoorthy and S.Ramachandran 1995. Water management and water quality control in aqueous farms. In: Ramachandran,S.(Ed) *Proceedings of National Conference on Sustainable Aquaculture,* pp. 116-137.

Moore, L.B 1986. Input of organic materials into aquaculture systems: emphasis on feeding semi- intensive systems. *Aquacult. Engg.,* 5:123-133.

Moriatry, D.J.W. 1986. Bacterial productivity in ponds used for the culture of penaeid prawns. *Microbial Ecol.,* 12: 259 -269.

Piper, C.S. 1966. *Soil and Plant Analysis.* Hans Publishers, Bombay, 368 p.

Rao, P.B., A.P.Sharma, and J.S.Singh, 1982. Limnology and phytoplankton production in high attitude lake. Intl. *J. Ecol. Environ. Sci.,* 8: 39-51.

Schroeder, G.L. 1978. Autotrophic and heterotrophic production of microorganisms in intensely manured fish ponds, and related fish yields. *Aquaculture,* 14: 303-325.

Singh, V.K. and A.P. Sharma 1999. Hydrological characteristics and primary production in fish ponds manured with different organic manures. *Indian J. Fish.,* 46(1): 79-85.

Strickland,T.D.H. and T.R.Parsons. (1972). A practical handbook of seawater analysis. *Bull. Fisheries Research Board of Canada,* Ottawa, No.167, 310 pp.

Subosa, P.F. and M.N.Bautista 1991. Yield of *Penaeus monodon* Fabricus in brackishwater ponds given different fertilizer combinations. *Aquaculture* 94 (1): 39-48.

Tacon, A.G.J. 1990. *Standard methods for the nutrition and feeding of farmed fish and shrimp.* Argent Laboratories press, Redmond, Washington, USA.

Teichert – Coddington, D.R. and R. Rodriguez 1994. Inorganic fertilisation and feed reduction as commercial production of *Penaeus vannamei* during wet and dry seasons in Honduras, pp. 136-146. In: H.S.Egna, J-Bowman and N.Weidner, 12th *Annual Technical Report. Pond Dynamics / Aquaculture Collaborative Research Program* 1990, International Research and Development, Oregon State University, Corvallis, Oregon, USA.

A study of antibacterial activity of *Annona squamosa* seed oil

K.K. Krishnani, L.S. Azad,
K.V. Rajendran, B.P. Gupta,
K.O. Joseph, M. Muralidhar
and J.J.S. Rajan
Central Institute of Brackishwater Aquaculture,
Chennai - 600034.

ABSTRACT

In order to introduce natural drugs and pesticides, instead of synthetic ones, the antimicrobial properties of custard apple (Annona squamosa) *was tested against shrimp pathogenic bacteria and probionts. The oil extracted from custard apple seeds using hexane solvent was tested against pathogens and probionts by Kirby-Bauer method. Three different concentrations of custard apple oil in hexane solvent, were applied on bacteria. At the end of incubation period the diameter of zone of inhibition was measured and the results were recorded. Only* Vibrio alginolyticus *was found to be susceptible up to 20 ul of mixture of custard apple oil and hexane (1:3) while other pathogens i.e.* Vibrio harveyi, Vibrio anguillarum *and* Aeromonas hydrophilla *and probionts such as* Bacillus subtillus *and Pseudomonas sp. were resistant to the oil at different concentrations used in the experiment.*

Introduction

Intensification of culture systems has led to increased health related problems. (Lightner, 1983 ; Krishnani *et al.*, 1997). There have been heavy mortalities of shrimp either in growout farms or in hatcher-

ies. Bacterial infection caused by *Vibrio* species is one of the major problems (Lightner, 1988). There is also concern that the use of persistent antibiotics and other chemicals in the aquatic environment may not only cause adverse effects on the ecosystem (Jacobsen and Berglind, 1988; Kumar and Chauhan, 1991) but also resistance among microbial population. (Jones, 1986; Bjorklund *et al.*, 1991; Shome and Shome, 1999). To escape the hazards of synthetic drugs and pesticides use, considerable investigations are being carried out to develop safer sources of disease control, notably from plant sources (Deshmukh *et al.*, 1982 ; Kumar and Chauhan, 1991 ; Raman *et al.*, 1997). It has been reported that leaf extract of custard apple (*Annona squamosa*), a common tropical plant exhibited bactericidal activity(Annapurna *et al.*, 1989). In this study, an attempt has been made to find out the antibacterial properties of custard apple seed oil against shrimp pathogenic bacteria and probionts. Oil extracted from custard apple seeds was tested against shrimp pathogenic bacteria i.e. *Vibrio alginolyticus, Vibrio harveyi, Vibrio anguillarum* and *Aeromonas hydrophilla* and probionts *ie. Bacillus subtillus* and *Pseudomonas species.*

Materials and methods

Custard apple oil was extracted from custard apple seeds using hexane solvent. Shrimp pathogenic bacteria ie. *Vibrio alginolyticus, Vibrio harveyi, Vibrio anguillarum and Aeromonas hydrophilla and* probionts *ie. Bacillus subtillus* and *Pseudomonas* species were used for assaying antimicrobial activity. The bactericidal activity was assayed by Kirby-Bauer method (Baeur *et al.*, 1966). Different concentrations of custard apple oil in hexane solvent (1:3), ranging from 10 μl to 30 μl were applied on the sterile discs and were placed on agar medium already spread with test organism. They were subsequently incubated at room temperature for 1 day. At the end of incubation period, the diameter of zone of inhibition was measured.

Results and discussion

The results of the experiment with custard apple oil against different bacteria are presented in Table-1. From this, it is evident that only *Vibrio alginolyticus* was susceptible up to 20 µl of mixture of custard apple oil and hexane (1:3). In further dilution it became resistant. However, probionts such as *Bacillus subtillus* and *Pseudomonas* were found to be resistant to the oil at all different concentrations used in this experiment.

Table 1. Antibacterial activity of custard apple oil against shrimp pathogenic bacteria and probionts

Test organism	Diameter of inhibition zone in mm			
	Control (Hexane) (20µl)	µl of oil : hexane (1:3)		
		30	20	10
Pathogens				
V. alginolyticus	R	12	11	R
V. harveyi	R	R	R	R
V. anguillarum	R	R	R	R
A. hydrophilla	R	R	R	R
Probionts				
Bacillus subtillus	R	R	R	R
Pseudomonas sp.	R	R	R	R

R - Resistant

The control of bacterial infection in aquaculture with natural plant extracts has been reported earlier. In our previous study (Krishnani *et al.*, 1998), neem oil was used as such and it was found to be active against only *Vibrio alginolyticus*. Neem oil extract which was prepared by partitioning the neem oil between hexane and methanol was effective against *Vibrio alginolyticus* and *Vibrio anguillarum* (Krishnani *et al.*, 2000).Aqueous extract of green leaves of neem and turmeric rhizomes (*Curcuma longa*)

was used to control bacterial infection in African catfishes (*Clarias gariepinus*) (Chakraborty and Chattopadhayay, 1998). Neem was also used to control infection of *Macrobachium malcolmsonii* by *Aeromonas hydrophilla* (Behera *et al*, 1997).

Conclusion

Antibiotic resistance has been shown to occur more frequently in certain bacterial species. This deserve attention for appropriate health care to make aquaculture activity successful. The use of chemicals and commercial products has not been recommended for sustainable shrimp culture (Boyd, 1995). Conversely, the use of herbal products and medicinal plants is eco-friendly, economical and effective alternative to antibacterial drugs and chemicals.

Acknowledgement

Authors are grateful to Dr. G.R.M.Rao, Director, Central Institute of Brackishwater Aquaculture, Chennai for facilities provided and his encouragement.

References

Annapurna, J., D.S. Iyengar, S. Nagabhushan Rao and U.T. Bhalerao 1989. Antimicrobial activity of leaf extract of *Annona squamosa*. *J. Pest.* **1**, 43-44.

Bauer, A.W., W.M.M. Kirby, J.c. Sherris and M. Truck 1966. Antibiotic susceptibility testing by a standardised single disc method. *Am. J. Clin. Path.*, **45**, 493-496.

Behera, S. 1997. Studies on experimental infection of *Macrobrachium malcolmsonii* by the bacteria *Aeromonas hydrophilla* and its control by the application of neem. Abstract, National Workshop on Fish and Shellfish Health Management, CIFA, Bhubaneswar, **47**.

Bjorklund, H., C.M.I. Rabergh and G. Bylund. 1991. Residues of oxolinic acid and oxytetracycline in fish and sediments from fish farms. *Aquaculture*, 97, 85-96.

Boyd, C.E 1995. Soil and water quality management in aquaculture ponds. *Infofish International*, **5**, 29-36.

Chakraborty, C. and A.K. Chattopadhyay 1998. Turmeric (*Curcuma longa*) and Neem leaf *(Azadirachta indica)* extract in the management of bacterial infection in African catfish, *Clarias gariepinus* (Burchell), *Fishing Chimes*, **18**(9), 17-18.

Deshmukh, P.B., S.R. Chavan and D.M.Renapurkar 1982. A study of antibacterial activity of twenty indigenous plants. *J. Pes.* **12**, 7-10.

Jacobsen, P. and L. Berglind 1988. Persistence of oxytetracycline in sediments from fish farms. *Aquaculture*, **70**, 365-370.

Jones, J.G 1986. Antibiotic resistance in aquatic bacteria. *J. Antimicrob. Chemother.*, **18**, 149-154.

Krishnani, K.K., K.V. Rajendran, B.P. Gupta and K.O. Joseph 1997. Soil and water characteristics of shrimp ponds affected with viral disease. *J. Inland Fish. Soc. India*, **26**, 37-42.

Krishnani, K.K., I.S. Azad, K.V. Rajendran, B.P. Gupta, K.O. Joseph, M. Muralidhar and J.J.S. Rajan. 1998. Efiicacy of various neem extracts on pathogenic bacteria and probionts. Abstract 9th International Congress on Pesticide Chemistry, UK, A-045.

Krishnani, K.K., I.S. Azad, M. Muralidhar, B.P. Gupta, K.O. Joseph and J.J.S. Rajan 2000. Antibacterial activity of natural products of *Azadirachta indica* and sòme commercial products. 5th Indian Fisheries forum, CIFA, Bhubaneswar, YS-9, pp.156.

Kumar, M.S. and U.K.Chauhan 1991. A study of antimicrobial activity of *Zizyphus nummulria* leaf extract. *J. Environ. Biol.* **12**(3), 273-277.

Lightner, D.V. 1983. Diseases of cultured Penaeid shrimp. In; J.P. Mcvey(Ed.), *CRC handbook of Mariculture*, Vol. 1. Crustacean aquaculture, CRC Press. Boca Baton, FL, pp. 289-320.

Lightner, D.V. 1988. Vibrio diseases of penaeid shrimp. In : *Disease Diagnosis and control in North American Marine Aquaculture.* Developments in Aquaculture and Fisheries Science, Vol. 6, Elsevier, Amsterdam, pp. 42-47.

Raman, N. S. Nagendran, A. Sundaramahalingam 1997. Extractive, characterisation and antimicrobial activity of mango seed oil. *Asian J. Chem.*, **9**(2), 321-323.

Shome, R. and B.R. Shome 1999·Antibiotic resistance pattern of fish bacteria from freshwater and marine sources in Andamans. *Indian J. Fish.*, **46**(1), 49-56.

Effect of salinity level and manure dose on the release of nutrients into water from brackishwater

B. P. Gupta, K. O. Joseph, M.Muralidhar and K.K.Krishnani
Central Institute of Brackishwater Aquaculture, Chennai-600 034

ABSTRACT

Laboratory experiment was conducted with cattle manure @ 1% and 2% and chicken manure @ 0.3 % and 0.6%, respectively to study the changes in nutrient concentration in soil and water phase at different salinity levels for 37 days. Higher amounts of carbondioxide were registered with cattle manure 1% and 2% at all salinity levels. All manure treatments resulted in depletion of dissolved oxygen after application. Ammoniacal nitrogen content in water was higher than nitrate nitrogen on all days of incubation and salinity levels. Available nitrogen in water increased substantially with the progress of incubation. Chicken manure @ 0.6% resulted in higher amounts of available nitrogen and phosphorus release into water at 20 and 10 ppt, respectively. Manure treatments registered increase in organic carbon, available nitrogen and phosphorus as compared to initial values. Lower dose of manures (cattle manure 1% and chicken manure 0.3%) also resulted in favourable soil and water characteristics.

Introduction

The importance of organic matter in bottom soil of brackishwater ponds has been emphasized by many workers (Tang and Chen, 1967;

Mandal and Chattopadhyay, 1979). Lack of adequate fish food organisms is found to be one of the major causes of low productivity of brackishwater ponds (Bhimachar and Tripathi, 1966) and application of manures and fertilisers is suggested as a measure to increase their production (Lin, 1968; Djajadiredja and Poernoma, 1972). There is increasing awareness that bottom soil plays an important ecological role in fish ponds. Transformation of applied nutrient elements in brackishwater pond soil depends considerably on the widely fluctuating water salinities (Johnson and Guenzi, 1963; Chattopadhyay, 1978). Though some literature was available on the decomposition of cattle and chicken manures, still poultry droppings rich in manurial constituents than cowdung are less utilised as pond manures. Little information was available on the rate of decomposition of manures at different salinity levels. Hence, in the present investigation, decomposition of cattle and chicken manures have been studied at three salinity levels for 37 days and nutrients release from soil to water phase were also investigated which enhances the productivity of water and augment fish/shrimp production.

Material and methods

The surface saline soil collected from Mahabalipuram (Chengulpet district, Tamil Nadu) was air dried and powdered. The soil was passed initially through 2 mm and then through an 80 mesh sieve. Physico-chemical characteristics of experimental soil are presented in Table 1. The soil samples thus prepared were made into five equal portions for the purpose of treatment. The treatments are 1. Control (untreated) 2. Cattle manure @ 1% 3. Cattle manure @ 2% 4. Chicken manure @ 0.3% and 5. Chicken manure @ 0.6%. The manures were dried, powdered and applied based on soil weight. One Kg of soil under each treatment was placed separately in tall glass cylinders upto 10 cm height and submerged with different saline waters (30 cm water column).

Table 1. Physico-chemical characteristics of experimental soil

Sand (%)	51.0
Silt (%)	13.0
Clay (%)	36.0
pH (1:2.5)	7.8
Electrical conductivity (mmhos/cm)	13.2
$CaCO_3$ (%)	1.0
Organic carbon (%)	0.12
Available nitrogen (mg/100 g)	3.0
Available phosphorus (mg/100 g)	18.8

Saline water levels (10, 20 and 30 ppt) were prepared from clear seawater after filtering through a plankton net of bolting silk (No. 25) by mixing with required quantity of distilled water. The glass cylinders after filling with soil and water were kept in darkness to avoid the growth of photosynthetic organisms. The concentration of nutrients in water along with other hydrological characteristics was analysed upto 37 days (3, 6, 9, 12, 15, 22, 29 and 37 days). Soil condition and nutrient changes in soil were analysed at the end of experiment.

Water parameters have been determined following standard methods of water analysis (A.P.H.A, 1989). Soil characteristics were analysed following standard procedures given by Piper (1966) and Jackson (1967). The experiment was replicated thrice in completely randomised design.

Results and discussion

Hydrological characteristics

Average values of water temperature (28.0 - 30.6°C) did not show any significant changes during incubation period. The average free carbondioxide (CO_2) in control was nil under all salinity levels (Table 2). Cattle manure resulted in comparatively higher amount of CO_2 obviously due to large amount applied as compared to poultry manure in order to keep the same level of nitrogen under all manurial treatments. A maximum amount of 32 ppm free CO_2 was registered with 2% cattle

manure at 30 ppt saline water level (22 days). Maximum mean free CO_2 content of 14.25, 11.69, 10.75 and 8.88 ppm were observed with cattle manure 2% (30 ppt), cattle manure 1% (30 ppt), chicken manure 0.3% (10 ppt) and chicken manure 0.6% (10 ppt), respectively (Fig.1A), indicating a decreasing trend with increase in salinity level under chicken manure treatment. Chattopadhyay and Mandal (1980) also observed depression effect of higher salinity on the decomposition of poultry manure. Cattle and chicken manure treatments showed an increase in carbondioxide content till 22 and 15 days of incubation, respectively and becomes nil at the end of experiment (Fig. 2A). Three way interaction, manure dose X salinity level X days of incubation (M X S X D) and two way interactions manure dose X salinity level (M X S), manure dose X days of incubation (M X D) and salinity level X days of incubation (S X D) are significant in influencing CO_2 content. Application of organic manures caused a decrease in the concentration of dissolved oxygen (DO), the magnitude of decrease being generally more in cattle manure treated series (Table 3). The DO concentration decreased in all the treatments with days of incubation and becomes nil with cattle and chicken manures during 15 to 22 and 9 to 15 days of incubation, respectively (Fig. 2B). Three way interaction M X S X D and two way interactions M X S (Fig. 1B) and M X D were significant in influencing DO concentration. The heterotrophic microorganisms, which are responsible for the decomposition of organic matter, consumed oxygen from water environment causing a depletion of oxygen in the water phase.

Manure treatments showed a slight decrease in average pH values as compared to control probably due to the formation of free carbondioxide and organic acids from the decomposition of added organic manures. M X D interaction effect was significant in influencing water pH (Fig. 2C). Less variation in pH with salinity level may be attributed to buffering capacity of brackishwater (Reid, 1961).

Nutrient changes in water phase

The amount of available nitrogen (NH_4^+-N + NO_3^--N) in water increased significantly with the progress of incubation at all salinity levels. Among the interaction effects only M X S interaction was significant in increasing available nitrogen (Table 4). Ammoniacal nitrogen (NH_4^+-N) concentration in water was always higher than nitrate

Table 2. Changes in free carbondioxide in water as affected by manure dose, salinity level and days of incubation

Days	Carbondioxide (ppm)*														
	Control Salinity level			Cattle manure 1% Salinity level			Cattle manure 2% Salinity level			Chicken manure 0.3% Salinity level			Chicken manure 0.6% Salinity level		
	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt
3	Nil	Nil	Nil	Nil	2.0	2.0	Nil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
6	Nil	Nil	Nil	9.0	9.0	9.0	10.0	10.0	10.0	10.0	8.0	7.0	11.0	10.0	9.0
9	Nil	Nil	Nil	16.0	16.0	16.3	20.0	12.0	16.0	16.0	14.0	12.0	14.0	14.0	11.0
12	Nil	Nil	Nil	18.0	18.0	18.2	20.0	16.0	18.0	18.0	16.0	16.0	20.0	15.0	14.0
15	Nil	Nil	Nil	20.0	20.0	20.0	22.0	18.0	20.0	20.0	19.0	18.0	20.0	20.0	19.0
22	Nil	Nil	Nil	28.0	28.0	28.0	20.0	28.0	32.0	16.0	3.0	3.0	4.0	3.0	3.0
29	Nil	Nil	Nil	Nil	Nil	Nil	16.0	20.0	4.0	Nil	Nil	Nil	Nil	Nil	Nil
37	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

• Average value of three replicates

	Treatments (T)	Manure (M)	Salinity (S)	Days of Incubation (D)	MXS	MXD	MXDXS
C.D at 5 %	3.18	0.649	0.503	0.821	1.124	1.836	3.180

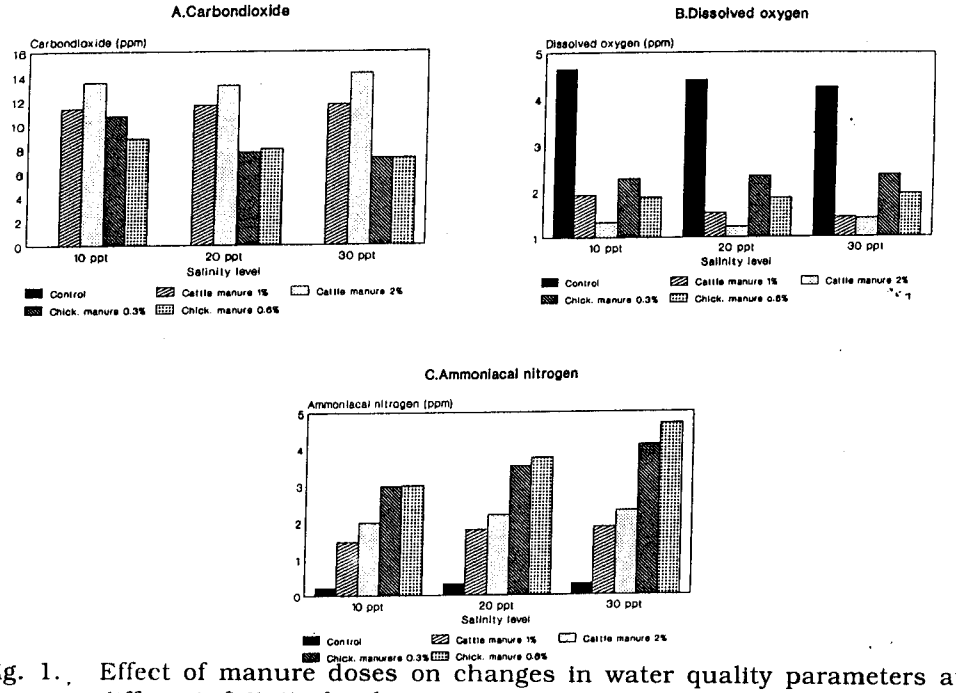


Fig. 1. Effect of manure doses on changes in water quality parameters at different salinity levels

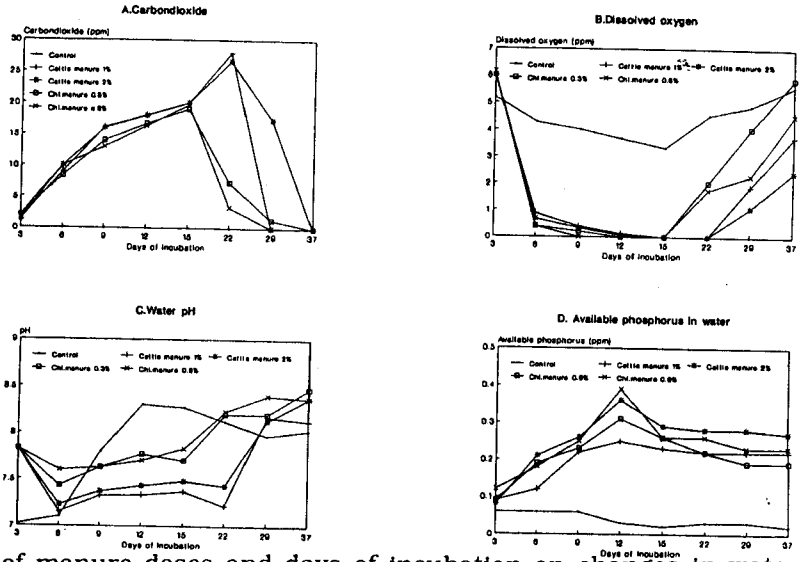


Fig. 2. Effect of manure doses and days of incubation on changes in water quality parameters

Table 3. Changes in dissolved oxygen in water as affected by manure doses, salinity level and days of incubation

Days	Carbondioxide (ppm)*														
	Col Salinity level			Cattle manure 1% Salinity level			Cattle manure 2% Salinity level			Chicken manure 0.3% Salinity level			Chicken manure 0.6% Salinity level		
	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt
3	5.6	5.0	5.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.4	6.0
6	4.6	4.2	4.0	1.0	0.8	0.8	0.8	0.6	0.6	0.4	0.4	0.4	0.4	0.4	0.4
9	4.3	4.0	3.8	0.4	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	Nil	Nil	Nil
12	4.0	4.0	3.0	Nil	0.2	0.2	0.2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
15	3.0	3.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
22	4.8	4.6	4.2	Nil	Nil	Nil	Nil	Nil	Nil	2.0	2.0	2.0	1.8	1.6	1.8
29	4.9	4.7	5.0	4.0	0.8	0.8	1.2	0.8	1.2	4.0	4.0	4.0	2.0	2.4	2.4
37	6.0	5.8	5.0	4.0	4.0	3.2	2.0	2.0	3.2	5.6	6.0	6.0	4.8	4.0	4.8

•Average value of three replicates

	Treatments(T)	Manure(M)	Salinity(S)	Days of Incubation(D)	MXD	MXS	SXD	MXDXS
C.D at 5 %	0.352	0.072	0.056	0.091	0.203	0.125	0.158	0.352

nitrogen. The maximum release of ammoniacal nitrogen at all salinities has been observed on 29th day. Higher water salinity level found to maintain slightly higher amount of NH_4^+-N in water. The results are in conformity with the observations of Mandal (1962) and Chattopadhyay and Mandal (1980). Chicken manure 0.6% registered highest amount of ammoniacal nitrogen (5.8 ppm) at 30 ppt salinity level. M X S interaction effect was significant in increasing the amount of NH_4^+-N (Fig.1C).

Table 4. Interaction effect of manure dose and salinity level on changes in available nitrogen.

	Available Nitrogen (ppm)				
Salinity level	Control	Cattle manure 1%	Cattle manure 2%	Chicken manure 0.3%	Chicken manure 0.6%
10 ppt	0.29	1.59	2.10	3.12	3.14
20 ppt	0.41	1.86	2.37	3.66	3.90
30 ppt	0.41	1.98	2.42	4.25	4.83
	Manure dose (M)		Salinity level (S)		MXS
C.D at 5%	0.319		0.247		0.552

Nitrate nitrogen NO_3^--N concentration was less than ammoniacal nitrogen obviously due to prevailing anaerobic conditions which are not favourable for the microbial transformation of NH_4^+-N to NO_3^--N. In waterlogged soils mineralisation ceases at the ammonium level and nitrification is very slow because of lack of oxygen supply (Goswami and Sahrawat, 1982; Laskar *et al.*, 1991; Senapati *et al.*, 1992). Changes in nitrate nitrogen did not show a definite trend and treatments registered a significant increase as compared to control. No interaction effect was significant in influencing nitrate nitrogen content. The irregular rate of release of nitrate N obtained with mineralisation of manures may be due to non-uniformity in the rate of nitrification (Sinha and Sharma, 1961; Banerjee *et al.*, 1979).

Available phosphorus in water increased in all the treatments as compared to control and the magnitude of increase was more with chicken manure at all salinity levels. A slight decrease in available phosphorus

was observed with the increase in salinity water level which may be attributed to the reason that at high salt concentration some of the available phosphorous might be fixed. The maximum release of phosphorus into water was noticed on 12th day with all the manure treatments at all salinity levels (Fig. 2D). Among the interactions only MXD interaction effect was significant.

Changes in soil nutrients

The changes in soil characteristics at the end of incubation period are presented in Table 5. A slight decrease in soil pH with all the treatments including control was observed as compared to initial value. Russel (1975) described the zeta potential of soil colloids to decrease with increase in salt concentration, which might result in low pH value under high saline conditions.

Manure treatments recorded a significant increase in organic carbon per cent from initial value and the magnitude of increase was more with cattle manure 2% (0.38% at 30 ppt). Higher dose of manures registered more organic carbon due to higher dose applied and slower rate of decomposition (Chattopadhyay and Mandal, 1980).

Available nitrogen content in soil increased significantly with all the manure treatments as compared to control at all salinity levels which might be due to mineralisation of organic nitrogen. Maximum amount of 36.2 mg/100 g was observed with chicken manure 0.6% at 20 ppt saline water level, obviously due to more per cent of nitrogen in chicken manure. Increased salinity after 20 ppt had some depressing effect on the release of N from organic manures, possibly due to adverse effect of higher salinity on microbial population responsible for the process of mineralisation (Paliwal, 1972).

Available phosphorus in soil increased in all the treatments including control and the increasing trend was more with chicken manure at all salinity levels. A slight decrease in available phosphorus being observed with the increase in saline water level, which might be due to fixation of available phosphorus at higher salinities.

Table 5 . Soil condition and amount of organic carbon, available nitrogen and phosphorus as affected by salinity level and manure dose at the end of incubation period

Treatment	Soil Parameters											
	Soil pH (1:2.5)			Organic carbon (%)			Available nitrogen (mg/100 g)			Available phosphorus (mg/100 g)		
	Salinity level			Salinity level			Salinity level			Salinity level		
	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt	10 ppt	20 ppt	30 ppt
Control	7.5	7.5	7.5	0.10	0.10	0.09	17.2	16.2	16.9	3.35	3.32	3.34
Cattle manure 1%	7.5	7.6	7.6	0.29	0.32	0.29	23.6	24.2	23.8	3.30	3.30	3.28
Cattle manure 2%	7.6	7.3	7.5	0.36	0.30	0.38	24.5	29.0	25.6	3.56	3.48	3.37
Chicken manure 0.3%	7.6	7.2	7.4	0.16	0.17	0.18	28.9	31.7	31.0	4.00	3.50	3.60
Chicken manure 0.6%	7.5	7.1	7.4	0.21	0.22	0.24	30.7	36.2	35.4	4.32	4.20	4.00
C.D at 5%												
Manure (M)		NS			0.38			1.773			NS	
Salinity level (S)		NS			NS			1.373			NS	
M X S		NS			NS			NS			NS	

Acknowledgement

The authors are thankful to Dr. G.R.M.Rao and Dr.K.Alagarswami, present and former Directors of Central Institute of Brackishwater Aquaculture for providing facilities and keen encouragement during the course of study.

References

A.P.H.A. 1989. *Standard methods for the examination of water and waste water* Ed. Taras, A.J., Greenberg, A.E., Hoak,R.D. and Rand, M.C. 16 th ed., APHA, Washington, DC, p 874.

Banerjee,R.K., P.Ray, G.S.Singit and B.R.Dutta 1979. Poultry droppings – its manurial potentiality in aquaculture. *J. Inland Fis. Soc. India* **2** (1): 94-97

Bhimachar, B.S. and S.D.Tripathi 1966. A review of culture fisheries activities in India. *F.A.O. World Symp. on Warm Water Pond Fish culture,* Rome.

Chattopadhyay, G.N. 1978. *Study on the chemistry of Brackishwater fish pond soils and water.* Ph.D Thesis, Bidanchandra Krishi Vidyalaya, India.

Chattopadhyay, G.N. and L.N.Mandal 1980. Inorganic transformation of applied phosphorus in brackishwater fish pond soil under different water salinity levels, *Hydrobiologia,* **17**:125-130.

Djajadiredja, R. ans A.Poernano 1972. Requirements for successful fertilisation to increase milk fish production. In: *Coastal Aquaculture in the Indo-Pacific Region.* Fishing News (Books) Ltd., London.

Goswami,N.N and K.L. Sahrawat 1982. Nutrient transformation in soils-micronutrients. In: *Review of soil Research in India, Indian Soc. Soil Sci.,* 123 pp

Jackson, M.L. 1967. *Soil Chemical analysis,* Prentice Hall of Indian Pvt. Ltd., New Delhi, pp 489.

Johnson, D.D. and W.D.Guenzi 1963. Influence of salts on ammonium oxidation and carbondioxide from soils. *Proc. Soil Sci. Soc. Amer.* **27**: 663 - 666.

Laskar,B.K., N.C.Debnath, G.K.De and K.K.Basak 1991. Nitrogen mineralisation in potential acid sulphate soils. *J. Indian Soc. Soil Sci.*.**39:** 564566.

Lin, S.Y. 1968. Milk fish farming. In: *Taiwan Fish Culture Report.* Taiwan Fish. Res. Inst., 3.

Mandal, L.N. 1962. Effect of salinity on the transformation of nitrogen in the brackishwater fish farm soils. *J. Indian Soc. Soil Sci.*, **10**(4): 255 - 261.

Mandal, L.N. and G.N. Chattopadhyay 1979. Effect of organic manuring on the availability of phosphorus in brackishwater fish pond soils under different levels of water salinity. *Symp. Inland Aquaculture*, Central Inland Fisheries Research Institute, Barrackpore, India.

Paliwal, K.V 1972. *Irrigation with saline water.* Water Technology Centre, IARI, New Delhi, 198 p.

Piper, C.S. 1966. *Soil and plant analysis*, Hans publishers, Bombay, 368 pp.

Reid, G.K. 1961. *Ecology of inland water and estuaries,* Reinhold publishing corporation, New York.

Russel, E.W. 1975. *Soil conditions and plant growth.* Longmans, London, 849 pp.

Sinha, S.B. and H.G. Sharma 1961. Mineralisation of nitrogen in organic manures in Gwalior soil. *J. Indian Soc. Soil Sci.* **9**(3):201-204

Senapati, H.K., A.K. Pal. and B. Behera, (1992). Nitrogen mineralisation in soil under submergence in relation to the population of ammonofiers and nitrifiers. *J. Indian Soc. Soil Sci.*, **40:** 198-201.

Tang, Y.A and S.H Chen, 1967. A survey of algal pasture soils of milk fish ponds of Taiwan, *F.A.O fish Rep.* **44** (3): 198-209.

Use of guar gum as binder in shrimp feed

A. Safiullah[1], C. Gopal[2], M.Natarajan[2], D.N. Swamy[2] and V. Bhagyalakshmi[2].

[1]*Department of Zoology, The New College, Chennai - 600 014*
[2]*Central Institute of Brackishwater Aquaculture, Chennai - 600 008*

ABSTRACT

Water stability of prawn feed depends on the type of binder used. Amongst different available conventional and non-conventional binders, guar gum assumes a prominence use in shrimp feed industry. This binder is having more binding capacity and is widely available at a nominal cost. In the present study guar gum was used at different levels (0, 1, 2 and 3%) along with different starches (tapioca, wheat flour, corn flour, rice flour and maida). Stability of the feed was evaluated by the nephelometric method (turbidity) and percentage loss in weight of the feed. The results indicated that guar gum at 2% level was able to keep the feed intact in water for more than 7 hours when used with any of the tested starches. Amongst the starches - wheat flour and tapioca in combination with guar gum gave the best results.

Introduction

There is an urgent need to develop shrimp feeds that are water stable so as to minimize disintegration and loss of nutrients upon exposure to water for which binders are used. The feed particles should be of high durability to withstand handling, transportation and longer shelf life. When introduced in water, the feed pellets should be stable for longer

duration till the shrimp consume its ration. Shrimp are supposed to be selective and slow eaters, so under such condition shrimp compounded feed should be well bound to withstand feed manipulation, minimize feed wastage and maintain good water stability. All these factors can be achieved provided the physical quality of pellet is maintained which depends on the composition of the feed mixture, moisture content, processing method adopted (Hastings, 1971) and extent of gelatinization during steam conditioning (Stivers, 1970). A number of binding agents are added to the formulae to reduce the amount of residual fine particles and improve the water stability of the pellets (Dominy and Lim, 1991). Some of these binders are of nutritional value and some effect feed consumption and growth in shrimps.

Several studies and reports on the evaluation of different types of natural, modified or synthetic substances as binding agents in shrimp feeds have been reported (Meyers and Zein-Eldin, 1972; Balazs *et al.*, 1973; Lim and Destajo, 1979; Castille and Lawrence, 1988; Huang, 1989; Dominy and Lim, 1991; Meyers, 1991). In addition to these, use of phospholipids have been shown to increase water stability (Briggs *et al.*, 1988) besides influencing growth (Chen and Jenn, 1992).

The binding potential of different binders reported are based on results of water stability tests using different formulations and preparation under laboratory conditions. Sometimes the feed processing conditions employed may not be optimum to activate some binders. Thus, the results expressed may sometimes be misleading. However, the effectiveness of different binders is expressed as percentage dry matter retained after 8 hours of immersion in fresh or sea water as the case may be. Number of methods have been adopted for evaluation of water stability of aquatic feeds.

The present study of use of natural binders in different concentration levels had been envisaged to evaluate the water stability of feeds for use in prawn *Peneaus monodon* culture. The evaluation was done based on the turbidity method and loss in weight on hourly basis.

Material and methods

Formulation and preparation : A basal feed was formulated using various plant and animal sources in addition to vitamin and minerals (Table 1). All dry ingredients were obtained fresh from local market, grounded and sieved through 200 micron mesh before mixing. All ingredients were mixed as per the formulation with different starches and levels of guar gum separately. The starch level were maintained constant and the guar gum levels ranged from 0, 1, 2 and 3% in the feed formulation. The vitamin and mineral mixtures were prepared in the laboratory using pure chemicals (AR grade) and were mixed along with yeast, cholesterol and *Spirulina* separately before adding to the basal mixture.

Table 1. Feed formulation and proximate composition of pelleted feed for testing water stability using different guar gum levels.

Ingredients	**g/100g of diet**
Fish meal	30.0
Squilla meal	5.0
Squid meal	5.0
Prawn head meal	5.0
Soyabean meal	25.0
Fish oil	2.7
Sunflower oil	1.2
Lecithin	1.2
Cholesterol	0.5
Vitamin - mineral mix	3.3
Yeast	1.0
Spirulina	0.2
Guar gum	0 - 3
a- Cellulose	4 - 1

Starches	**g/100g diet**
Tapioca	15.9
Wheat flour	15.9
Corn flour	15.9
Rice flour	15.9
Maida	15.9
Proximate composition	**% (Range)**
Moisture	9.2 - 10.6
Crude protein	39.8 - 40.1
Crude lipid	6.6 - 7.8
Crude fibre	2.8 - 3.1
Ash	18.9 - 19.9
NFE	27.7 - 28.8

The basal feed was kneaded well manually adding sufficient water (1.0 : 0.35) to each of the feed formulation and later all were steam cooked for 10 mins. After cooling the mixture of vitamin and others were added and kneaded again. Using a hand pelletizer with 2.0 mm dia. the noodles were drawn and dried at 50°C for 48 hours till the moisture content of the feed was less than 10%. The dried noodles were then manually broken into 5-10 mm length pellets and packed in polyethylene bags till further use.

Proximate composition : The formulated dry feeds were analysed for their proximate composition (Table 1). The moisture content was determined by heating the sample at 60°C for 24 hrs till constant weight was obtained. Total nitrogen was estimated using microkjeldahl method (AOAC, 1975) and the crude protein was calculated by multiplying N_2 content in the sample with factor 6.25. The crude fat content was estimated by extraction with petroleum either (BP 60-80°C) for 8 hrs in a Soxhlet apparatus. Crude fibre was estimated by the modified method of De Silva (1985). Ash content was determined by burning the sample at

550°C for 8 hrs in muffle furnace. The NFE was found out by the difference method (Hastings, 1976).

Water stability test : The pelleted diets were tested for water stability following turbiditometry method. A series of beakers (250 ml cap.) were arranged serially with 200 ml seawater. In each of the beaker 2g of the pellet feed of uniform size (5 mm length) were put. The beakers were moderately stirred at intervals with a glass rod. At the end of 1, 2, 3, 4, 5, 6, 7 and 24 hrs the tubidity of the water was recorded using turbidity meter. The turbidity was expressed in nephlons. In additions to this the condition of the pellet and loss in weight was also recorded. The sample was filtered through a filter paper and dried overnight at 60°C. The difference in weight was loss in weight of feed at that particular hour.

Statistical analysis: Difference in water stability of the formulated feeds based on the nephelometric and percentage loss in weight were tested for significance by ANOVA (Snedecor and Cochran, 1975) for the effect of guar gum levels on starches for different hours of immersion in seawater.

Results and discussion

When guar gum was not added along with the starches, feeds showed poor water stability in water. The nephelometric values at different percent guar gum levels ranged from 4- >100 nephlons and the percentage loss in weight ranged from 21.75 - 86.0% at different hours in various starches. The turbidity and percent loss in weight values showed positive correlation (Fig. 1 and 2) and exhibited similar trends.

The hourly readings of turbidity and percent loss in weight for all starches with and without guar gum showed that there was a steady rise in mean nephlons and percent loss in weight upto 24 hrs. Amongst the starches, corn flour with and without guar gum showed high nephlons and percent loss in weight at different hours (1-7, 24 hrs). This means corn flour is a poor starch and use of guar gum does not influence its water stability in feeds. Rice flour showed comparatively lower nephlons but high percent loss in weight from first hour onwards with and without guar gum. The low nephlons values could be due to heaviness of particles which tend to settle faster compared to other starches. The high percent loss in weight shows the insufficiency of rice flour to function as

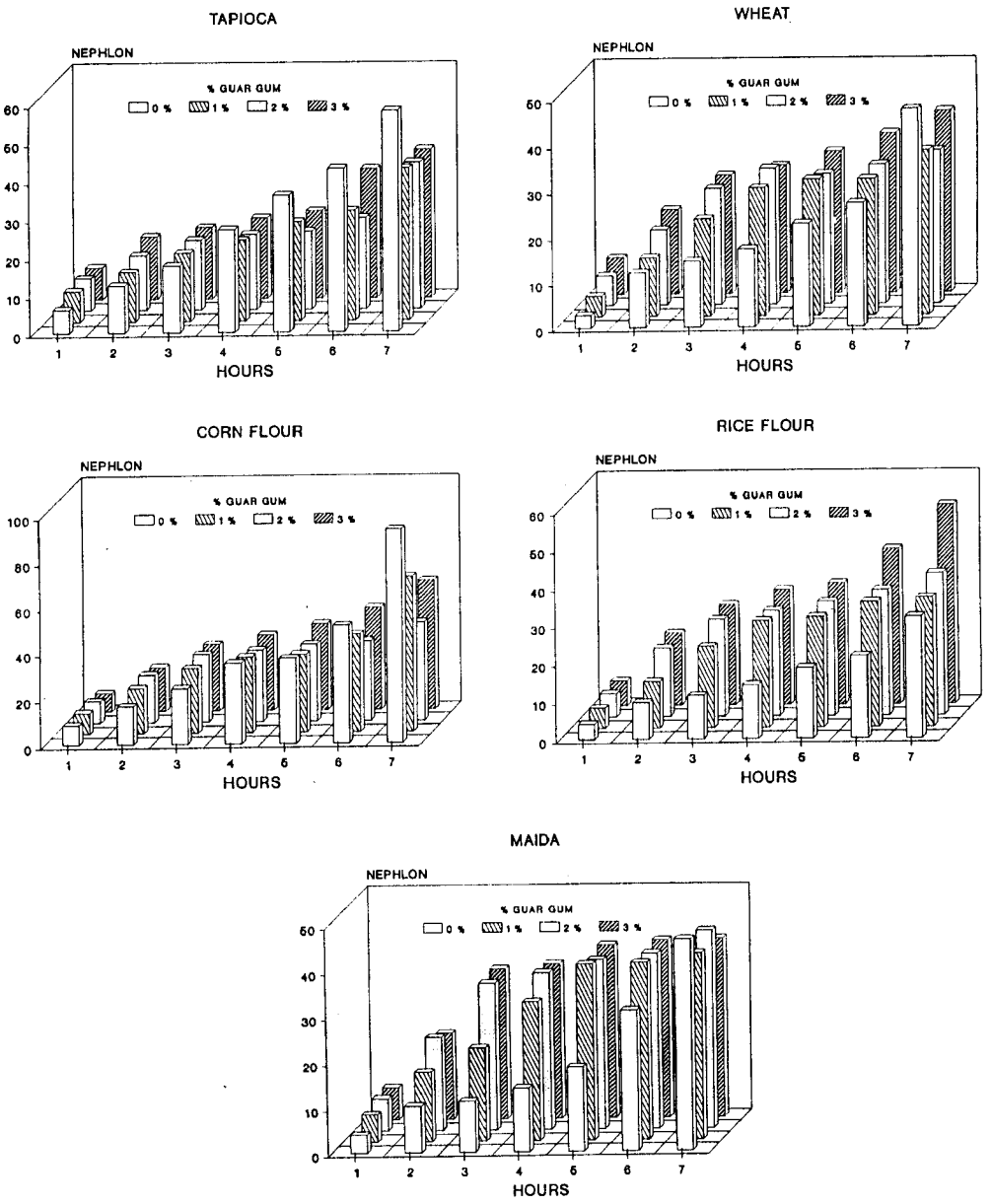


Fig. 1. Nephelometric value of feeds containing various starches and defferent levels of guar gum.

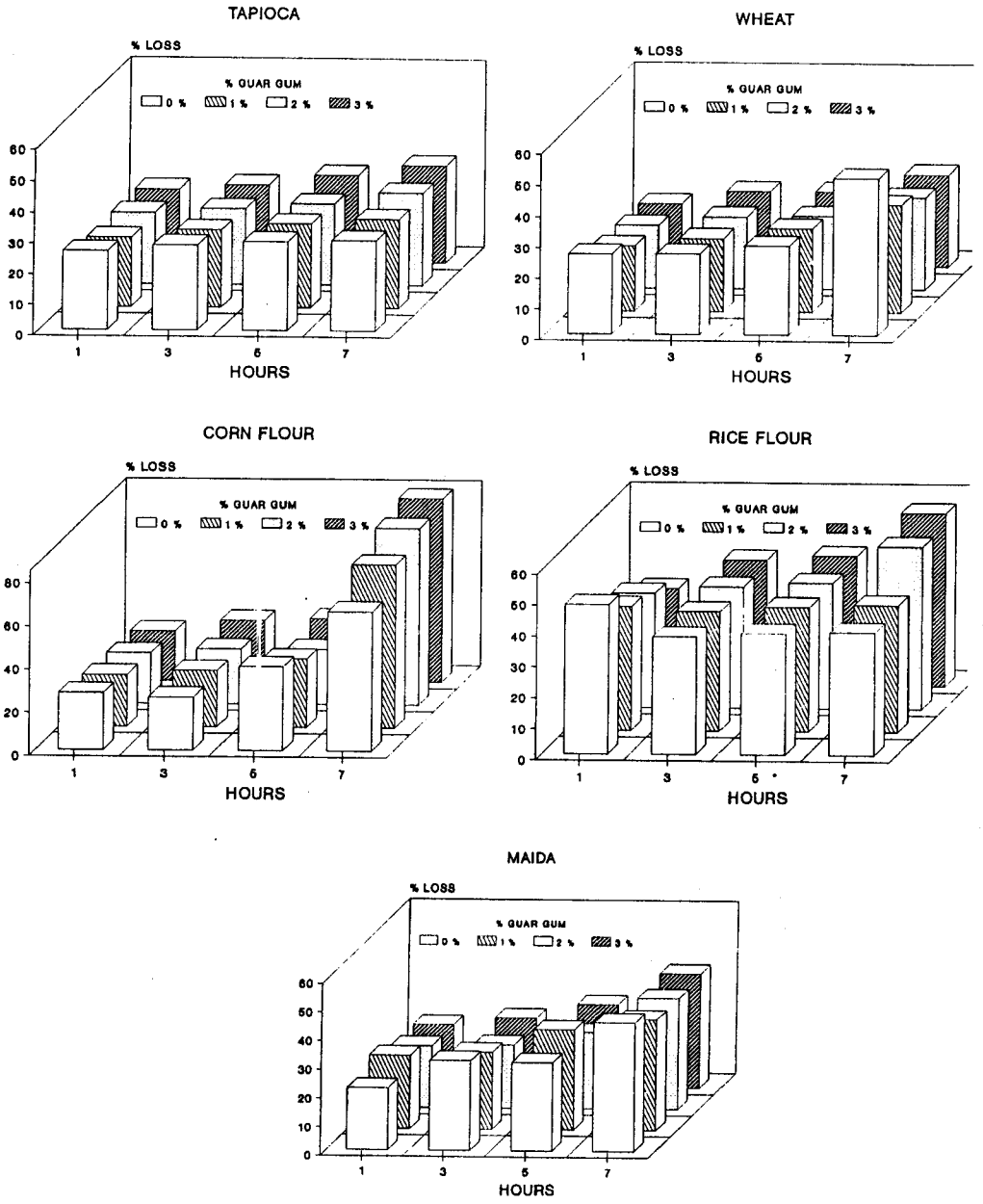


Fig.2. Precent loss in dry weight of feeds containing various starches and different levels of guar gum.

good starch in improving the water stability in feeds. Thus, both corn flour and rice flour were poor starches and the influence of guar gum to improve the water stability of feed was negligible.

Comparing the other starches - tapioca, wheat flour and maida, these starches showed lower nephlons and percent loss in weight with and without guar gum in the feed. The nephlons for tapioca (7.75-93.25), wheat flour (5.45-90.63) and maida (6.0-83.55) were showing similar trends when compared to percent loss in weight (23.73-38.06%; 21.94-44.88% and 22.60-48.56%, respectively) and it was observed that amongst these, wheat flour and tapioca starches are more water stable. Statistical analysis shows that in first three hours, significant variation ($p>0.05$) was observed irrespective if starches with and without guar gum use in the feed. Beyond the third hour, the feeds showed no significance. So, the use of guar gum and the type of starch significantly affects the water stability in feeds.

Comparing the level of guar gum influence on various starches in providing better water stability to feeds showed that corn flour and rice flour had no influence of guar gum at different levels in providing good water stability. Corn flour and rice flour even after usage of 3% guar gum (50.4/45.5% loss in weight) could not give good water stability to feeds. Compared to these starches - tapioca, wheat flour and maida had the effect of guar gum level in feed in improving the water stability. This improvement of water stability by these starches along with guar gum was because, these starches have more ability to gelatinize and bind (Stivers, 1970; Hastings, 1971) during feed processing.

Thus, the comparative study of different starches and guar gum levels suggests that tapioca and wheat flour are best starches which can be used in shrimp feeds along with 1-2% guar gum for good water stable feeds.

Acknowledgements

The authors thank the former Directors, Dr. K. Alagarswami, CIBA,

Chennai and Dr. G. Gopakumar CIFT, Kochi and the present Director, Dr. G.R.M. Rao, CIBA, Chennai for providing facilities and encouragement for the study. Also thanks to Department of Biotechnology, New Delhi for funding the project.

References

AOAC, 1975. *Official Methods of Analysis* 12th Ed. Association of Official Analytical Chemists Washington DC.

Balazs, G.H., E.Ross and C.C.Brooks, 1973. Preliminary studies on the preparation and feeding of crustacean diets. *Aquaculture* **2** : 369-377.

Briggs, M.R.P., K. Jauncey and J.H.Brown, 1988. The cholesterol and lecithin requirements of juvenile prawn (*Macrobrachium rosenbergii*) fed semipurified diets. *Aquaculture* **70** : 121-129.

Castille, F.L. and A. L. Lawrence, 1988. The effect of Bsafin on growth and tissue accumulation of formaldehyde residues in shrimp *Peneaus vannamei* Boone. Abstracts, 19th Annual Confr. and Exposition of the World Aqua. Soc., 2-9 Jan. 1988, Honolulu, Hawaii : 43

Chen, H.Y. and J.S. Jenn, 1992. Increased pellet water stability by the supplementation of phospholipid and its effects on shrimp growth. *Asian Fisheries Science*, **5** : 211-217.

De Silva, S.S., 1985. Methodological Approaches to research and Development. In *: Finfish nutrition in Asia* (Ed. Cho, C.Y., Cowey, C.B. and Watanabe, T.), p154. IRDC, Ottawa, Canada.

Dominy, W.G. and C.Lim, 1991. Performance of binder in pelleted shrimp diets. Proc. of Aquacult. Feed Processing and Nutr. Worksh., Thailand and Indonesia, 19-25 Sept. 1991. *Amer. Soybean Assoc., Singapore* : 149-157.

Hastings, W.H., 1976. Fish nutrition and fish feed manufacture. *FAO Technical Conference on Aquaculture*, Kyoto, Japan. FIR : AQ/Conf./76/R23.

Hastings, W.H., 1970. Study of pelleted fish food stability in water. In. : *Report of the 1970 Worksh. On Fish Feed Technol. and Nutr.* (Ed. J.L. Gaudet),

pp75-80. FAO/EIFAC and USID/BSFW, U.S. Govt. Printing Office, Washington D.C.

Huang, H.J., 1989. Aquaculture feed binders. Proc. of the People Rep. of China Aquacult. and Feed Worksh., 17-30 Sept. 1989. *Am. Soybean Assoc., Singapore* : 316-319.

Lim, C. and W.H. Destajo, 1979. Effects of crude, semipurified and purified starch of sago (Metroxylon sago Rottb) on the water stability of pelleted shrimp diets. *Fish. Res. J. Philippines* IV : 19-23.

Meyers, S.P. and Z.P. Zein Eldin, 1972. Binders and pellet stability in development of crustacean rations. *Proc. World Maricult. Soc.* **3**: 351-364.

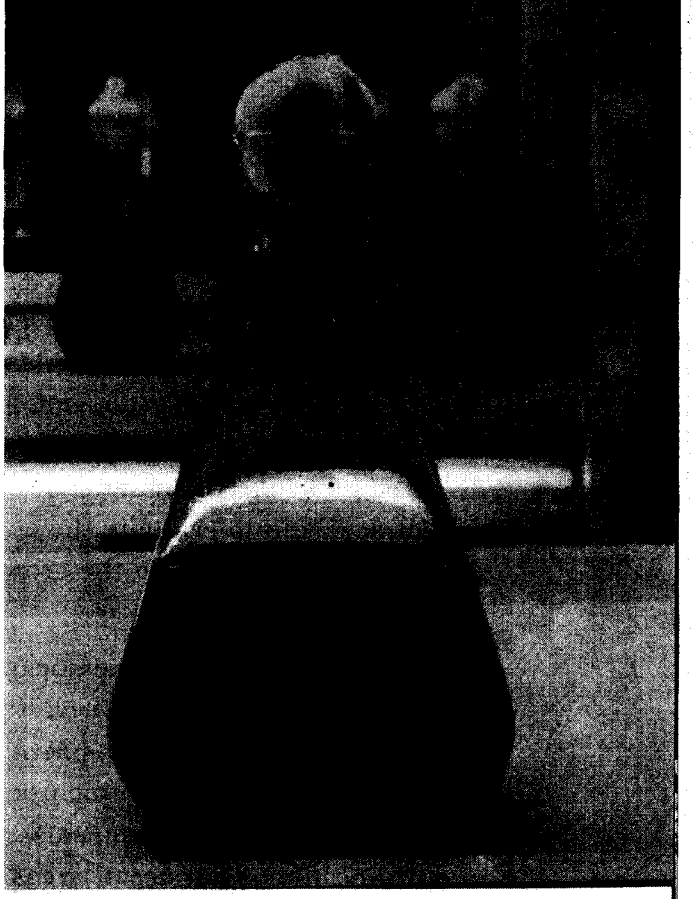
Meyers, S.P., 1991. Water stability in pellets. *Feed Management* **42**: 13-16.

Snedecor, G.W. and W.G. Cochran, 1975. *Statistical methods.* Oxford and IBH Publishing Co., New Delhi.

Stivers, T.E., 1970. Feed manufacturing. In. : *Report of the 1970 Worksh. on Fish Feed Technol. and Nutr.* (Ed. J.L. Gaudet), pp14-42. FAO/EIFAC and USID/BSFW, U.S. Govt. Printing Office, Washington D.C.

Dunaliella salina - an unconventional live feed

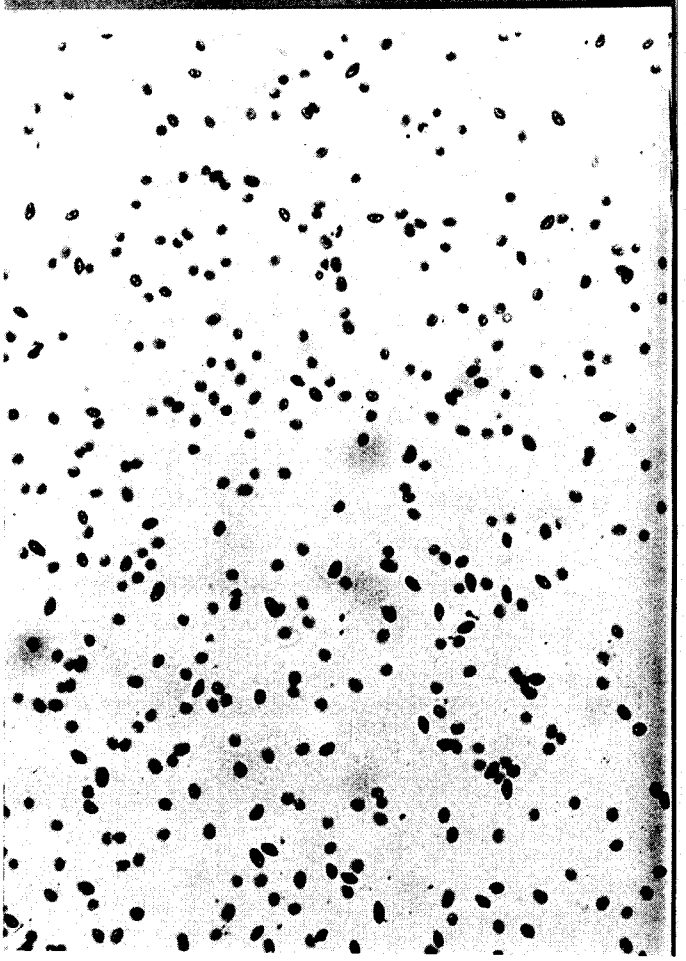
R. Gireesh, K.S. Smitha, K.B. Bindu and C.P. Gopinathan
Central Marine Fisheries Research Institute , Cochin-682 014



ABSTRACT

Live feeds play an important role in aquaculture operation. Presently, the groups of live feeds employed in the culture systems are limited to a few species of phyto and zooplankters. Dunaliella salina, *(greenmicroalga), a member of Chlorophyceae, is an unconventional live feed. The culture of this species is presently limited to laboratory experimental stage only.*

In view of the paucity of studies on the culture and utilization of Dunaliella salina, *the present investigation was undertaken to explore and estimate the potential use of this species. The results of rearing of juvenile clams with* Dunaliella *as live feeds are presented and discussed.*



Introduction

In aquaculture systems, several live feeds are used presently such as *Chaetoceros, Isochrysis, Skeletonema,* etc. The hatchery rearing of prawn/ mollusc larvae is dependent on these live feeds. There are innumerable species of phytoplankton in our waters; but only very few of them are used as live feeds in aquaculture. In view of this, the present study was undertaken to evaluate the culture possibilities of *Dunaliella salina* and to study its effect on the growth, survival rate and performance in juvenile clams.

Dunaliella salina is a member of Chlorophyceae; a green halotolerant (ie., thrives in media with a very board range of salt concentrations) microalga. The dominant pigment - chlorophyll, is masked by the presence of a pigment - haematochrome. It accumulates large amounts of commercially valuable chemicals - glycerol b and - carotene. *Dunaliella* is cultured in coastal ocean areas in large outdoor ponds in regions of high solar radiation and moderate temperatures. An attempt has been made in this brief work to evaluate the performance of *Dunaliella salina* in laboratory / hatchery conditions.

Materials and methods

Four live feeds *viz, Dunaliella, Chaetoceros, Tetraselmis* and *Nanochloropsis* were cultured in laboratory conditions using Walne's medium in sterilized seawater at 30 ± 2ppt salinity.

Juvenile clam of *Villorita cyprinoides* (7.52g average initial weight) were brought from a local farm off Cochin and reared in 8-10 ppt salinity under labouratory conditions. The clams were acclimatized for rearing in 8-10 ppt salinity under laboratory conditions. The clams were acclimatized to the rearing conditions for over a period of one week. Prior to starting of the experiment, the clams were starved for 48 hrs. The clams were then grouped into four goups; each group containing 10 animals (Table 1). Each group was fed on separate feeds to study the comparative efficiency of *Dunaliella* with respect to the other conventional live feeds.

Table 1. Groups, feeds and feeding rate used in the experiment

Groups	Feeds	Feeding rate
Group I	*Dunaliella*	500-700 cells/ml
Group II	*Chaetoceros*	-do-
Group III	*Tetraselmis*	-do-
Group IV	*Nanochloeopsis*	-do-

The animals were maintanined in separate tubs with 3 l of filtered seawater. The feed ration was divided into two and given at intervals of 8hrs. 100% water exchange was done every day. The feeding rate (ingestion rate) was determinded as :

$$\text{Feeding rate or ingestion rate} = \frac{C1 - C2}{nt} \times V \times 60$$

where, C1 - initial cell concentration (cells/ml)

C2 - final cell concentration (cells/ml)

V _ Volume of water (l)

t _ duration of the experiment

n _ no. of animals.

The animals were reared with the respective feeds for a period of 15 days. At the end of the experiment, the animals were sacrificed and their biochemical composition estimated. Growth and survival rate was also studied. Biochemical assays were carried out to estimate the protein, carbohydrate and lipid contents.

- Protein was estimated following the procedure of Lowry *et al.* (1951). The values were obtained by comparing with standard graph.
- Carbohydrate was estimated using Glucose as standard.
- Fat (lipid) was determined using gravimetric method.

Simultaneously a group of 10 clams were maintained as control for the same length of period as that of the experimental only. All conditions were the same for this group also, except that they were not fed any feed.

Culture of *Dunaliella* : The candidate species, *Dunaliella salina*, was cultured in three different media for a period of 15 days at 30 ppt salinity and 24°C.

Medium I - Walne's medium

Medium II - Miquel's medium

Medium III - Enriched seawater medium (modified 'F' medium).

20 ml of *Dunaliella salina* was inoculated into 500 ml of filtered and sterilised seawater containing the respective medium. The culture was carried out in triplicates. (1 ml of inoculum contains approximately 500 cells/ml).

Ressults

Feeding experiment: The performance of each group of clams was monitored. The efficiency of the feed was determined in terms of growth, survival and biochemical changes of the clams. A summary of the results obtained is shown in Table 2. No mortality was reported with any of the feeds. However, the results were discouraging with respect to *Dunaliella* fed clams. Clams fed on *Dunaliella* showed comparatively less protein and carbohydrate contents. However, they showed better lipid profiles, as explained elsewhere. The growth rate was more with *Dunaliella* fed clams as seen in Table 2; but biochemical estimation gives comparatively lower values for *Dunaliella* fed clams.

Table 2. Growth and biochemical composition of the differet groups of clams

Group	Feed fed	Av. initial weight(g)	Av. final weight(g)	% growth	Protein (/g)	Carbohydrate(/g)	Lipid*
I	*Dunaliella*	8.69	8.86	1.96	21μg	0.55μg	0.249
II	*Chaetoceros*	11.42	11.45	0.263	30μg	1 μg	0.249
III	*Tetraselmis*	9.73	9.79	0.617	28μg	0.8μg	0.109
IV	*Nanochlor*	12.28	12.30	0.163	10μg	0.4μg	0.135

* Expressed as mg lipid/ gm tissue.

Culture of *Dunaliella* : The performance of each media was determined taking into consideration the following facts:

incubation period, ie, the time taken for the culture to start growth. - exponential period - the duration for which the bloom lasts.

final maximum no. of cells/ml at the time of harvest.

Accordingly, the medium with the least incubation period and highest exponential period along with better biochemical results is recommended as the ideal one. The results of culturing *Dunaliella* with the different media is given in Table 3.

Table 3. Results of *Dunaliella* culture with different media

Media	Initial no. of cells/ml	Final no. of cells/ml	Incubation period (days)	Exponential period (days)	Biochem. compostion		
					Protein µg/g	Carbo-hydrate. µg/g	Lipid mg/g
Walne's	500	13x103	3 to 4	one month	17.5	14	0.773
Miquel's	-do-	*	two weeks	one week	-	-	-
Modified F	-do-	*	-	-	-	-	-

* not determined as culture did not develop.

In the case of Walne medium blooming started within three to four days after inoculation. With Miquel's medium, blooming took ten to eleven days and declined in about two days time. With modified 'F' medium, there was no blooming at all. Since, the amount of sample that could be obtained from these two media ('F' and Miquel media) were very less, no biochemical estimation of these could be carried out. However, biochemical studies were carried out with *Dunaliella* cultured in Walne medium.

Discussion

Clams fed with *Danaliella* showed a higher percentage of growth (1.96%) compared to the other groups. Also, *Dunaliella* fed clams showed an increased lipid content (0.2488 mg/g) compared to other groups. This is attributed to the high levels of glycerol accumulated by *Dunaliella* in culture conditions (Della et al. 1995). Studies on feeding *Artemia* with *Dunaliella* have given large scale mortality. Similarly, with shrimps also mortality and collapse of culture has been recorded (C.P. Gopinathan, unpublished).

The results indicate that *Dunaliella* is not a substitute for the al-

ready widely used live feeds such as *Chaetoceros* and *Tetraselmis*. But, it can be incorporated as a supplementary live feed in molluscan culture. The study also indicates that *Dunaliella* is not toxic to juvenile and adult calms. The effect of rearing larval molluscs with *Dunaliella* remains to be studied. As regards the lower biochemical values obtained with *Dunaliella*, it can be mentioned that *Dunaliella* uses a lot of its energy on photoconversion - where the dominant green pigmet is converted into Xanthophyll. Also the higher lipid values explains the lower protein and carbohydrate values.

The results indicate that Walne medium is the ideal one for culturing *Dunaliella*. With this medium a good bloom of the culture and excellent exponential growth is obtained.

References

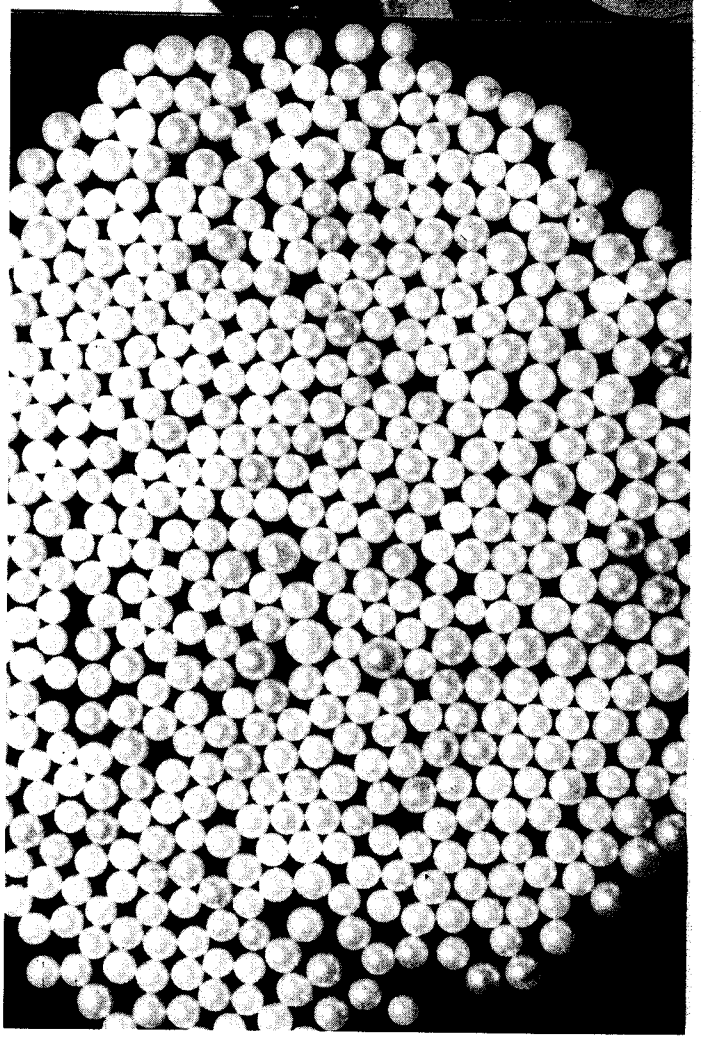
Della Croce, N; S. Connell; R. Abel (Eds.) 1995 In: *Coastal Ocean Space Utilization*:3; pp. 433-436.

Lowry, C.H., N.J., Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. *J.Biol. Chem.*, 193: 265-275.

Results of the mother oyster culture and pearl production in *Pinctada fucata* (Gould) in the inshore waters of the Gulf of Mannar and Palk Bay

A.C.C. Victor, D. Kandasami, I. Jagadis, Baby Ignatius, A. Chellam, G. Chitra, P. Villan and M. Rajkumar

R.C. of Central Marine Fisheries Research Institute, Mandapam Camp.



ABSTRACT

It is established experimentally that the inshore areas of the Gulf of Mannar and Palk Bay bordering Mandapam Camp can profitably be used for mother oyster culture and cultured pearl production.

The depth of the inshore waters of the Gulf of Mannar is comparatively deeper where the raft culture can be adopted from November to May, whereas, the

nearshore waters of Palk Bay is shallow where rack can be used to farm the oysters from June to October, thus a continued farming is possible in all months in a calender year.

Though the hydrological and environmental conditions of both the seas are almost same, it is found that Gulf of Mannar is better suited for mother oyster culture as indicated by the better growth rates of spat and oysters.

If farming is done properly, high survival rates of spat, mother oysters and implanted oysters can be achieved with an enhanced rate of production of better quality cultured pearls.

Introduction

Pearl oyster *Pinctada facata* Gould occur in large numbers on paars lyeing between Kilakarai and Kanyakumari in the Gulf of Mannar. These oysters are known for their best quality pearl production. CMFRI has developed technologies for pearl production in 1973 and Hatchery production of seeds in 1982. However, inspite of the technologies readily available, enterpreneurs are to venture into this industry. In Tamilnadu, in 1983 a joint venture project between Tamil Nadu Fisheries Dvelopment Corporation and Southern Petrochemical industries was started and a pearl culture farm was established in Krusadai Island, Gulf of Mannar. Except for this no other enterpreneurs had come forward to establish pearl culture farms for reasons best known to them. Hence an attempt was made by Mandapam Regional Centre of CMFRI to carry out experimental culture of pearl oyster spat to operatable sized oysters and production of cultured pearls in *Pinctada fucata* in the inshore areas of Gulf to assess the suitability and redemonstrate the eonomical viability of pearl culture in the inshore areas of Gulf of Mannar and Palk bay sea during 1996-97. The results of these experiments on rearing spats to mother oyster size and pearl production was encouraging. The salient features of the experiments, problems encountered and the need for propagation of pearl culture in these area were discussed in this paper.

Results

Topography and environmental conditions of the culture sites

Gulf of Mannar : Gulf of Mannar sea is calm during the period

November to April. The seawater salinity ranged from 29.0 to 36.0 ppt, temperature from 25.0 to 32.0 C, pH from 8.0 to 8.4 and dissolved oxygen content from 3.6 to 5.5 ml/l in general throughout the year. The present culture site is sandy intermittent with rocks here and there.

Palk Bay : The Palk Bay sea is very shallow and calm during May-October. The culture site sea bottom is sandy and has abundant seagrass growth in places. It also has natural bed of pearl oyster *Pinctada suqillata* and rock oyster *Saccostrea cuculata. Pinctada fucata* population in this bed is very sparse. The sea water temperature ranges from 25.7 to 33.0°C salinity from 26.9 to 35.6 ppt, pH from 8.0 to 8.8 and dissolved oxygen from 2.8 to 6.0 ml/1.

Observations on spat rearing

In the present experiments, oyster spats were reared both in the Gulf of Mannar and Palk Bay inshore waters to assess the suitability of these areas for pearl culture. Spats produced from the Tuticorin Research Centre hatchery of CMFRI were transported to Mandapam Camp by adopting 'wet' method, and also spats produced out of the larvae (veliger stage 70μ) transported from TRC of CMFRI and subsequently reared at MRC of CMFRI were used for the culture experiments. The growth rate were monitored separately upto the oyster reaching operatable size (45mm) or till the time the oysters had to be transferred to the other sea due to the inclement sea conditions. The oysters were also allowed to grow in both the seas by sifting them to access the cumulative growth rates of pearl oysters in both the inshore areas.

Gulf of Mannar : **Experiment no.1.** In this experiment laboratory produced pearl oyster spats transported from TRC of CMFRI was used (16,500). In July 96 the spats were stocked in conventional box cages of 40x40x10 cm size. Uniform stocking of 1500/cage was maintained and the cages were suspended from floating rafts made out of the palmyrah rafters and moored in the inshore area at an average depth of 2 1/2 m. The rafts hold an average of 80 cages each. Periodical observations were made on the condition of the cages, condition of oyster spats, fouling and mortality. The initial size of the oysters spat ranged from 3.2-12.3mm (Ave.7.2mm). The oysters were periodically thinned as they were growing. At the end of the sixth month the spats had grown to operatable size of 34.6-51.6mm(44.9mm). The monthly growth rate worked out was

6.3mm. The survival percentage of the oysters under culture was 50 % (Table 1).

Experiment no. 2. In the second experiment a total of 50,000 spats of size ranging from 7.7 to 21.8mm (16.6mm) were used. The average stocking density per cage was 1500 nos initially. The culture was carried out for only 1 1/2 months as the sea has become rough. The thinning was carried out and the density of the oysters had grown to 15.2-34.5 mm (24.9mm) registering an average growth rate of 5.5. mm/month. The survival rate at the time of shifting it to the Palk Bay was 80% (Table 1).

***Palk Bay* : Experiment no. 1.** In the experiment a total of 14,000 laboratory produced spats were used. As the Palk Bay sea is very shallow a rack was constructed using casurina poles. The size of the rack was 30sq m. The average depth of the sea in the area was 1 1/2-2 m. The density of the spats maintained in each cage was 1000 nos. The initial size of the spat were from 6.1 to 10.4 mm (8.0m). These spats were regularly moniotored for their growth, fouling and periodical thinning was also carried out. At the end of the five month culture period each cage had 400 nos after the periodical thinning. The oysters had grown to the size ranging from 27.0-39.1mm(31.0mm) showing average growth of 4.6mm/month. The percentage survival was observed to be very high (92.0%). Subsequently the oysters had to be shifted to the Gulf of Mannar due to rough weather. The details of the experiments are presented in Table 1.

Table 1. Growth of pearl oyster *(Pinctada fucata)* spat in cages at the Gulf of Mannar and Palk Bay 1996-1997.

Experiment no/ location	Initial size/ (Ave) mm	Final size/ (Ave) mm	Growth/ month	Duration (Month)	% Survival	Remarks
Gulf of Mannar						
Experiment 1	3.2-12.3 (7.2)	34.6-51.8 (44.9)	6.3	6	50	Moderate fouling
Experiment 2	7.7-21.8 (16.6)	15.2-34.5 (24.9)	5.5	1½	80	""
Palk bay						
Experiment 1	6.1-10.4 (8.0)	27.0-39.0 (31.0)	4.6	5	92	Less fouling

Gulf of Mannar and Palk Bay : Experiment no 1. Under this experiment the oysters were allowed to continuously grow in both the seas by shifting the oysters as and when required. The oysters shifted to Palk Bay during June '97 had the size range of 15. 2-34. 9. (24.9mm) and were reared up to middle of October '97 and had reached a size of 29.3-40,0 mm (34.3mm) showing an average monthly growth rate of 2.1mm. Subsequently they were shifted to the Gulf of Mannar and reared, the oysters reached operatable size of 36.2-40.3mm during December 97 by registering a monthly average growth rate of 4.0mm. However, the cumulative average growth rate of oysters in these seas was found to below 2.6mm/ month. The collective survival rate of the oysters in general was high. The details are given in Table 2.

Table 2. Observations on the growth rate of pearl oyster *Pinctada fucata* reared continuously in the Gulf of Mannar and Palk Bay.

Location/Date	Initial size (Ave) (mm)	Final size (Ave) mm	Growth/ month	Duration Month	% survival	Cumulative growth(mm)
Palk bay						
June 97 to	15.2-34.5	29.2-40.0	2.1	4.5	High	
October 97	(24.9)	(34.3)				2.6
Gulf of Mannar						
October 97 to	29.3-40.0	36.2-45.5	4.0	1.5	High	
December 97	(34.3)	(40.3)				

Results of the nucleus implantation and pearl production

Experiments were conducted in *Pinctada facata for* nucleus implantation and pearl production at the Gulf of Mannar during 1997. A total of 4350 oysters were nucleated during July-December 97 (Table 3). The nucleus used were 3-5mm depending upon the size of the oysters. The nucleated oysters were placed in netlon trays and suspended in an one ton FRP tank where a mild flow of sea water was ensured. The oysters were kept in the tank for a period of 3 days for observation in the post operative convalescence, rejection of nuclei and mortality. Later, the oysters were placed in box type cages and were suspended in the Gulf of Mannar at 2 m depth. The average depth of the culture area was about 2.5m. The stocking density of the oysters ranged from 60-85 nos/box/ cage of 40x40x10 cm size. Inititally to observe the rejection, a fine mesh

velon netting was provided in the cage. Periodical monitoring of the oysters were carried out and the health of the oysters, mortality, predation and damage to the cages were noted. Nucleus rejection was observed upto a period of 2 months from the implantation. To enhance free flow of water the velon netting was removed after a period of 1 month and the culture was continued. After a post operative culture period ranging from 6-9 months, the oysters were brought to the laboratory and the pearls were retrieved by teasing out from the gonad of the oyster. The harvested oysters were placed in cages and once again transfered to new cages and taken to farm for the healing of the incision wound. Out of 4350 oysters monitored, about 2950 survived at the end of the harvest accounting for 67.8% of the initial stock. Of this 2034 oysters were found to retain the nucleus implanted (68.9%). The harvest yielded a total of 1809 pearls comprising different grades of pearls accounting for 61.3% pearl formation in the surving oysters. Among the pearls, premium quality pearls of 'A' grade was 523 (17.7%); 'B' grade pearls was 550 (18.6%) and 736 nos (24.9%) were 'C' grades and the rest being the poorly coated pearls of no commercial value (217 nos; 7.4%).

Table 3. Results of pearl production experiments in the Gulf of Mannar 1996-1977

Particulars	July 97	Aug.	Sep.	Oct.	Nov.	Dec.	Total
No. of oysters operated	549	945	115	380	1202	1159	4350
Nos.surviving at harvest	327	682	85	322	897	637	2950
% survival	59.6	72.2	73.9	84.7	74.6	55.0	67.8
Nos. retained nucleus	231	494	64	235	590	420	2034
% retention in surviving oysters	70.6	72.4	75.3	72.9	65.8	65.9	68.9
No. of quality pearl/	172	448	61	214	547	367	1809
*percentage	52.6	65.6	71.7	66.5	60.9	57.6	61.3
Grade 'A'	43	125	21	101	167	66	523
%	13.1	18.3	24.7	31.4	18.6	10.4	17.4
Grade 'B'	36	149	20	64	163	118	550
%	11.0	21.8	23.5	19.9	18.2	18.5	18.6
Grade 'C'	93	174	20	49	217	183	736
%	28.4	25.5	23.5	15.2	24.2	28.7	24.9
Ungraded pearls	59	46	3	21	46	42	217
%	18.0	6.7	4.7	6.5	5.1	6.6	7.4

* *% in terms of surviving oysters.*

Discussion

From the forgoing account it can be seen that the inshore waters of the Gulf of Mannar and Palk Bay seas offer ideal conditions for farming pearl oyster in the open sea. Comparatively deep the Gulf of Mannar offers ample scope for mooring offshore rafts for pearl culture while Palk Bay being very shallow is ideal for rack construction. Culture can be taken up at the Gulf of Mannar during November to April and in Palk Bay from May to October.

Although the physico - chemical characteristics of both the Gulf and the Bay are almost same, the Gulf of Mannar is more suitable for pearl culture than Palk bay as indicated by better growth rate in the former area. (Jeyabaskaran *et al.*, 1983).

In both the farms, predation is found to be negligible. While fouling on the oysters was moderate in the Gulf of Mannar and it was less in Palk Bay. The major fouling organisms were sponges, barnacles and ascidians. Alagarsami and Chellam (1976) has described the fouling on the oysters cultured at Veppalodai. Barnacles, bryozoans, spats of other molluscs *Avicula* and *Crassostrea* sp are the dominant foulers and spionid polychate *Polydora* sp and the Clionid sponge *Cliona cellata* were important borers.

Mortality in general were observed in the cages which were either over stocked or those which were not periodically monitored due to logistic problems. Mortality of oysters, in cages where small crabs and predatory molluses have entered, were also observed.

The optimum size of floating rafts and rack could be 3x3 m and 30 sq m which can hold about 80 and 240 cages respectively. A unit of 3 such rafts and one rack can be established in the Gulf of Mannar and Palk Bay respectively on which year round pearl culture could be successfully carried out.

The model unit suggested has a production rate of about 5400 pearls/ 6-9 months if exclusively used for rearing nucleated oysters (Table 4).

Table 4. Production potential of a model pearl culture unit.

No. of unit/ size	Holding capacity	Stocking density/ cage	Total oyster under rearing	Survival %	Pearl formation %	Production (nos)	Gross + Revenue (Rs. Lakhs)
One unit of Three rafts of 3x3 each & a rack of 30 sq m.	240 cages	75	18,000	60	50	5,400	2.16

* *Calculation based on the results given in this paper.*

\+ *at average price of Rs.40/pearl.*

From the experimental result, a good survival rate ranging from 72.2 (Aug) to 84.7% (Oct) was observed. The moderate survival during December and July (55.0 and 59.6%) may be attributed to the health of the oysters implanted during that period. The overall nucleus retention in the implanted oysters were good ranging from 66 to 75% in different months.

The production of premium quality pearls (Grade A) ranged from 10.4 (Dec) to 31.4% (Oct) and the average was 17.7%; good quality (grade B) pearls ranged from 11.0 (July) to 23.5% (Sept) and the average being 18.6% of the surviving oysters.

Alagarsamy (1974) reported 55.8 to 62.8% pearl formation in the Veppalodai farm and the results obtained in this experiments (52.6 to 71,7%) are fairly comparable with his observations and an average of 50% of pearl formation out of the total implanted oysters is quite possible in the inshore areas of this region.

Acknowledgements

The authors wish to thank the Director, CMFRI, Kochi for inspiring the team and for facilities extended. They also thank the Head, Molluscan Fisheries Division, CMFRI, Kochi, for his constant guidance.

References

Alagarsami, K. 1974 Development of cultured pearls in India, *Curr. Sci.* **43** (7) : 205-207.

Alagarsami, K and A. Chellam 1976. On the fouling and boring organisms and mortality of pearl oysters in the farm at Veppalodai, Gulf of Mannar. *Indian J. Fish.*, **24** (1&2) : 1-14.

Alagarsami, K., A. Chellam, A.C.C. Victor, S. Dharmaraj, T.S. Velayudhan and A.D. Gandhi. 1987. "Pearl oyster resources of India". *CMFRI, Bulletin* No. **39.** 37-48.

Jeyabaskaran Y., Daniel Sudhandra Dev, I. Nalluchinnappan and N. Radhakrishnan, 1983. On the growth of the pearl oyster *Pinctada fucata* (Gould) under farm conditions at Tuticorin, Gulf of Mannar. *Proc. Symp. Coastal Aquaculture*, Pt.2: 587-589.

Ecofriendly onshore marine pearl culture – an overview

G. Syda Rao
V.R.C. of Central Marine
Fisheries Research Institute,
Visakhapatnam – 530 003

ABSTRACT

The onshore marine pearl culture technology developed by CMFRI at Visakhapatnam has been taken up on a commercial scale in the vicinity of Visakhapatnam. Hatcheries for the pearl oyster Pinctada fucata *have been established. The pearl oysters were grown in cement tanks of 75t capacity from 5 mm to 50 mm DVM. The growth rates of pearl oysters fed with different species of diet* Chaetoceros, Nanochloropsis *and* Isochrysis *at different cell concentration showed wide variations. The growth rate of oysters reared at a density of 100 nos./sq.m is better compared to that at higher densities. The depth of seawater and rate of water exchange per day have been standardized. Raw seawater filtered through a specially designed slow sand filter is being used in hatchery and culture tanks. The filtered seawater is directly used for all purposes without any treatment with chemicals or antibiotics. Usage of chemicals is undesirable even for cleaning purposes. The algal cell concentration up to 1.2 million/ml was achieved on a sustained basis in outdoor mass culture tanks. Use of only live feed and avoiding chemicals make this technology ecofriendly. Several factors related to growth and maintenance of hygiene of pearl oysters in onshore culture tanks are discussed.*

Introduction

The marine pearl culture is one of the oldest sustained mariculture technologies. The Japanese are practicing this in shallow sheltered areas. Due to the use of the same site continuously over a prolonged period, there have been reports of some problems associated with the accumulation of waste matter in these grounds. There are no efficient mechanisms to completely eliminate this, resulting in shifting of the rafts to new culture sites. The problem has also affected the growth of oysters and quality of pearls (Matsui, 1958; Algarswami, 1970; Algarswami, 1983).

It has been mentioned by Daniel (1993) that none of the locations in the Indian main land are suitable for raft culture. Some of the suitable locations are Lakshadweep Islands and Andaman and Nicobar Islands, while Gulf of Mannar is partially suitable. This is one of the main reasons for the country lagging behind in the establishment of commercial pearl culture industry, inspite of the availability of technology, for the past two decades. Onshore marine pearl culture technology recently developed by Rao and Devaraj (1996) is mostly free from pollution problems. The technology with particular reference to its eco-friendly nature and associated advantages are presented and discussed here.

Materials and methods

The pearl oysters *Pinctada fucata* were originally transported from Tuticorin. They were reared in the CMFRI laboratories, in private shrimp hatcheries and two private onshore (pearl) culture farms. The rearing tanks varied in size from one tonne FRP tanks to 120 t cement tanks. The pearl oysters were specially reared for maturation and used as broodstock for further propagation. Thus continuous availability of pearl oyster spat became possible and they were reared to adult size in onshore tanks. The salinity and temperature are measured on daily basis with the help of a refractometer and thermometer respectively. For the hatchery rearing, the methodology developed by Algarswami *et.al.*(1983) is being adopted. The microalgal feed required for feeding is produced at

the rearing sites by following the standard phytoplankton culture methods, with suitable local modifications.

Technology

The seawater is drawn from a clean location near the shore by laying a pipeline of suitable length and diameter. At the sea end the pipeline is connected to a filter buried in the sand. Thus semi-filtered seawater is pumped to the culture site. Seawater outlet is also connected to slow sand filter. Here the filtration rate is regulated by the second chamber (coal chamber) where the filtered seawater travels upwards before entering the storage chamber. The filtered water is mainly utilized for phytoplankton culture and hatchery operations, without any chemical or antibiotic treatments.

Feed production: The ciliate free inocculum of the required phytoplankton species are maintained in sterile, filtered water. From the inocculum level, the cultures are enhanced to desired levels up to 10 t tanks through different stages. The filtered seawater is enriched with Conway medium (Walne, 1974). This gives a concentration of about 1.2 million cells/ml on a sustained basis throughout the year, in outdoor conditions in about 48 hours.

Onshore tank: The onshore cement tanks size varies from 20 t onwards, depending on the culture site. It has a sloppy bottom and side walls of 1.2 m height. At 1 metre level all the walls are provided with ventilator holes to flush out hot air. The tanks are provided with aeration facility. The seawater intake system is connected to each tank, so that raw seawater can be let into tanks at any time. The tanks are totally covered with black covering to avoid growth of filamentous algae and blooming of unwanted phytoplankton. 10% of seawater from the tank is replaced daily.

Oyster stock: The pearl oyster *Pinctada fucata* are stocked at suitable densities from 500 to 75/sq.m depending on the size. Granite stones are

keft at the bottom of the tank and the pearl oysters will attach themselves to the stones. The stones are adjusted to desired density of oysters at regular intervals. The growth rate recorded at 100 no./sq.m. of 30 mm DVM is better compared to higher densities. Each tank is connected to a feed tank from which phytoplankton feed drips throughout the day, ensuring constant supply of feed. The flow rate is adjusted in such a way so as to ensure the maintenance of cell concentration at desired level in relation to the size of the pearl oysters.

Feed species: Several species of phytoplankters were tried as feed, individually and in combination. The species are *Chaetoceros calcitrans*, *Chaetoceros* sp., *Skeletonema* sp., *Tetraselmis* sp., *Chlorella* sp., *Isochrysis galbana and Nanochloropsis salina.* Among them, a combination of *Chaetoceros calcitrans, Nanochloropsis salina* and *Isochrysis galbana* gave the best growth rates in terms of length and weight of pearl oysters. A concentration of 20000 to 80,000 cells/ml is being maintained to get better results depending on the size and density of pearl oysters.

Growth : Under the prevailing temperature and salinity conditions (Fig.1) 5 mm pearl oyster spat will attain a dorsoventral measurement of 50 mm and a weight of 10-12 grams, in about 6-8 months. This may be considered as a proper size for the first implantation with 3-4 mm nuclei. It takes about 6 months for the pearl formation. A minimum of 25 % pearl harvest can be achieved from the implanted oysters.

Survival: The mortalities from 5 mm to 50 mm in about 6 to 8 months is low and will be less than 10 %. Under the onshore conditions post implantation mortalities are also least and are less than 10 %. The tanks and stones need to be cleaned occasionally with salt. Cleaning with detergents and chemicals is totally avoided. As there is no significant settlement of fouling and boring organisms like algae or epifauna in onshore system the oysters need to be cleaned rarely. Only ascidians are occasionally found over the pearl oysters, which can easily be removed.

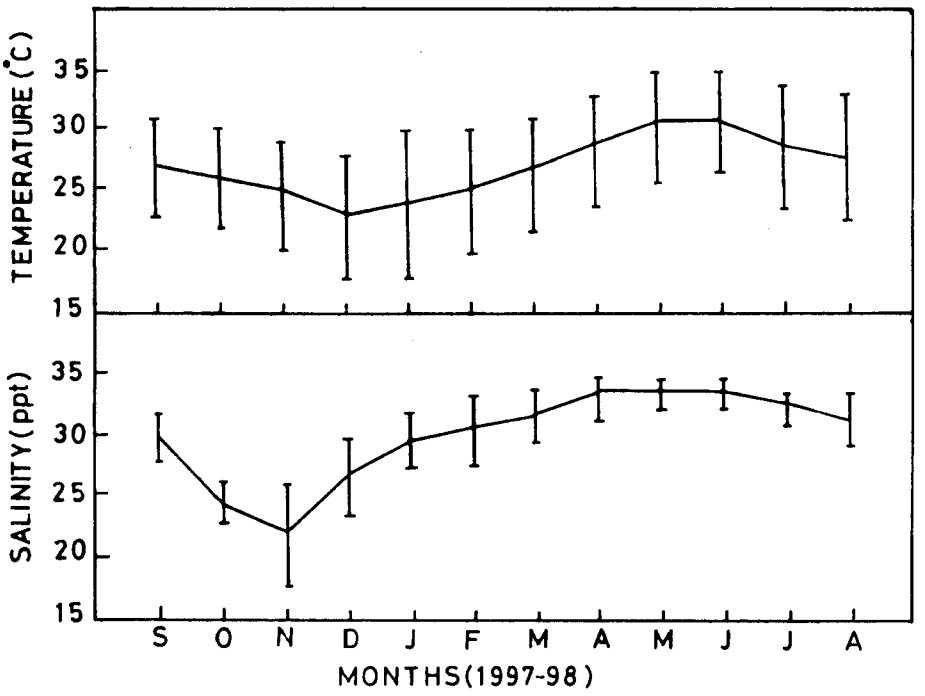


Fig. 1. Salinity and temperature profile of pearl oyster culture tanks.

Discussion

According to Dharmaraj *et al.* (1987), fouling is a major problem in the sea based pearl culture operations. The foulers also adversely affect the growth of pearl oysters and pearls (Nishi, 1961). The near absence of foulers, borers and predators is a very good advantage in onshore pearl culture, which will enhance the growth rates. Chellam (1987) and Daniel and Durairaj (1993) recorded high adult pearl oyster mortalities of about 45% in the sea due to various problems. The minimum mortalities occurring in onshore culture from spat to pearl production stage will enhance the rate of gross pearl production and adds to economic sustainability. Better growth of pearl oysters in onshore system than in inshore culture is achieved by the constant availability of live feed species at desired concentrations throughout the period and maintenance of hygiene in the culture tanks. Thus the use of untreated seawater,

nonuse of chemicals, darkening of the tank and use of only live feed makes onshore pearl culture ecofriendly, resulting in much better growth and low mortality.

References

Algarswami, K. 1970. Pearl culture in Japan and its lessons for India. *Symp. Mollusca* Pt.*III*, *Mar. Biol.Ass.India*, 975 - 998

Algarswami, K., S.Dharmaraj, T.S.Velayudhan,A.Chellam,A.C.C.Victor and A.D.Gandhi,1983. Larval rearing and production of spat of pearl oyster *Pinctada fucata* (Gould). *Aquaculture* , 34. 287 - 301

Chellam, A. 1987. Biology of pearl oyster *Pinctada fucada* (Gould). *Bull. Cent.Mar.Fish.Res.Inst* 39: 13 - 20

Daniel S.Dev and S.Durairaj 1993. On commercial aspects of cultured pearl production from pearl oyster *Pinctada fucata* (Gould) in India. *Sea food Export Jour.*, 25(8): 25 - 38

Dharmaraj.S, A.Chellam and T.S.Velayudhan, 1987. Biofouling, boring and predation of pearl oyster. *Bull. Cent. Mar. Fish. Res. Inst*, 39: 92 - 97

Matsui, Y. 1958. Aspects of environment of pearl culture ground and the problems of hybridization in the genus *Pinctada*. In : *Perspective in Marine biology* (A.A.Buzzattra verso.ed) Univ. Calif. Press, Burkeley:519 - 531

Nishi, T. 1961. The influence of sessile organisms on the growth of pearl oyster and quality of cultured pearls. *Bull. Natl.Pearl Res. Lab*, 6: 684 - 687.

Rao, G. Syda and M. Devaraj. 1996. Prospects of large scale onshore marine pearl culture along the Indian coasts. *Mar.Fish.Infor.Serv.,T&E Ser.*No: 143: 1 - 7

Walne, P.R. 1974. *Culture of bivalve molluscs*. Fishing News (Books) Ltd. Surrey. 173 pp.

Mariculture in India - an opportunity as vast as the sea

K.O. Isaac
ABL Biotechnologics Ltd. Chennai

ABSTRACT

The magnitude of the Indian Ocean with its distribution of over 7,40,00,000 sq.kms. an average depth of 4000 meters and an 8000 km perimeter along India's coastline, underlines its importance. What is even more important is the fact that while fishery catches and productivity have collapsed in all the major oceans, the Indian Ocean has been indicating an increase. Unbalanced strategies of exploitation have reduced numerous fish populations to levels of extinction apart from destabilising marine ecosystems and impoverising coastal communities. What has been overlooked is that since wild fish reproduce at rates determined by nature, attempts at increasing market supply will eventually run into limits.

In addition, fishermen inadvertently capture numerous forms of marine life collectively known as bycatch, most of which is discarded. Thus one out of every four animals taken from the sea is unwanted. The United Nations has estimated that in 1990 the high sea drift nets entangled 42 million animals that were not targeted, including diving sea birds and marine mammals.

The biospheres in the Bay of Bengal, Indian Ocean and the Arabian Sea need to be seen for the treasure trove they are, as the biodiversity in this entire area is unmatched for its potential. The fact is that since fishery and other marine resources in these areas remain under exploited, there is yet time to institute a proper strategy for sustainable exploitation underlined by the need for conservation.

Under-utilised opportunities include ocean based mariculture of finfish, fattening of marine organisms, pond based aquaculture of penaeids and finfish, microbial metabolites, ornamental fisheries and macroalgal derivatives.

Since the focus of this paper is restricted to mariculture technologies for the open seas, the content will provide an insight into the equipment, systems, processes, problems and potential of open sea mariculture of food finfish. The objective will therefore be to evolve a strategy for seafarming as a credible and sustainable option to fill the huge deficit in demand and supply for food fish.

Commercial mariculture today - the Indian scenario

In India, the principal maricultured organism has been the penaeid shrimp *Penaeus monodon*, accounting for over Rs.2000 crores of foreign currency earnings to the country last year. However, excepting this species, there is no production of any other marine species on a commercial scale in this country. Of late, there are some stray efforts to produce cultured pearl oysters with limited success. However on the whole, India ranks second in global aquaculture production due to its efforts with fresh water fish i.e. approximately 850000 MT of carp.

With around 1.5 lakh hectares utilised out of an estimated 1.2

million hectares available for coastal aquaculture, India still has about 90% of its culture potential left fallow. Considering that the principal species cultured is the black tiger shrimp, due to the problems and risks associated with coastal aquaculture, expansion of this industry will be considerably slower than required. While it has been proved worldwide that scientific and properly managed shrimp culture activity has a limited impact on coastal eco systems, the impact of polluted coastal waters on the aquaculture industry is far more serious. With its coastline of over 8000 kms and the dire need to establish diversified products in aquaculture, it is perhaps time we looked seriously at sea cage mariculture.

Marine resources and opportunities

The biospheres in the Bay of Bengal, Indian Ocean and the Arabian Sea need to be seen for the treasure trove they are, as the bio-diversity in this entire area is unmatched for its potential. The fact is that since fishery and other marine resources in these areas remain under exploited, there is yet time to institute a proper strategy for sustainable exploitation underlined by the need for conservation.

Under-utilised opportunities include :

1) Ocean based mariculture of finfish and other organisms

It is indeed tragic that India does not produce any edible finfish through mariculture. Considering the limitations in the economics of pond based mariculture and the attendant problems with environment and disease, it has been found that seafarming of finfish in open cages is the better option. We have a number of excellent candidate species including breams, bass, groupers, snappers, etc., apart from brackishwater options like milkfish, mullet and *Etroplus*. Also rope and tray/cage culture of mulluscs are yet other viable options.

2) Fattening of marine organisms

This is particularly relevant for those organisms whose breeding methodologies in captivity are still under various stages of development. Considering FAO's estimates that around 25% of fishery catches are untargetted, mainly due to the fact that sizes captured are too small for market viability, it would perhaps be more advantageous to stock finfish and other juveniles from this by catch, in cages for rearing upto adulthood.

3) Pond based culture/fattening of other crustaceans and finfishes

Organisms like crabs, lobsters, non-penaeid shrimp and fish like *Etroplus*, tilapia, milkfish, mullet, etc., are excellent candidates for product diversification in present shrimp farms.

4) Microbial metabolites

These include micro algal, fungal and zooplanktonic bio-diversity which has dramatic potential in nutrition, pharmacutical, cosmetic and industrial applications. With the right kind of research, products including vitamins, polyunsaturated fatty acids, biopolymers, antifoulants, sterols, bactericides, antifungals, antivirals, anticancer products, pigments and such others can be commercially exploited.

5) Ornamental fisheries

This industry worldwide has a market of around $ 2.5 billion per annum. There already exists a thriving illegal ornamental fishery in India for exports. However, India's contribution is marginal. If capture can be replaced by culture, this could provide a high return business for most families along the coast.

6) Macroalgal derivatives

The seaweed collection industry along the Gulf of Mannar has become a preserve of women, where they dive without any gear and harvest seagrasses at random. However this activity has irrepaiably

damaged the seaweed beds leading to further destruction of other marine life forms. These industrially important species of seaweed can be cultivated along the seashore and can offer immense employment opportunities for women in coastal communities. Inspite of around 45000 MT of seaweed harvested per year and around 13 units manufacturing alginates, it is tragic that the agar and alginates produced in this country are classified as third grade. Of the approximately Rs.50 crores worth of carrageenan used in India annually, not one gram is produced locally.

This paper focuses at mariculture technologies for the open seas, with an insight into the equipment, systems, processes, problems and potential of open sea mariculture of food finfish.

The seafarming industry

Systems : As in agriculture and coastal aquaculture, principles of seafarming follow the life cycle of the organisms to be cultured. This process begins with healthy parent selection and maintenance. Due to the often seen problems of bacterial and viral disease infestation in densely packed populations, disease resistant and immunised parent stock are the foundation of a successful seafarming industry. Thus the salmon industry in Europe works with a number of indigenously developed strains which exhibit specific characteristics in growth rates, colour, body weight, texture, immunity and maturation. These strains then become the first input into the land based hatchery modules.

The hatchery : Due to the need for controlled environmental standards in reproduction and captive breeding programmes, it is necessary to establish hatchery facilities on land, near the sea. Once broodstock have been selected and acclimatised, the process of induced maturation using either specifically developed photoperiod methodologies, hormonal induction or a mixture of the two are

instituted. In most cases roe or eggs are manually removed from mature females and milt from males. Fertilisation is done *in vitro* and the eggs are hatched in a separate section. From here, the larvae are reared indoors in tanks with treated recirculated water and automated batch feeding systems. The recycling also provides a continuous laminar flow in the tank, essential for proper larval growth. Quality maintenance is on the basis of a statistical control exercise with specimens being evaluated constantly by histopathologists. On achieving a certain size, depending on the species cultured, the larvae are moved into nursery tanks outside to acclimatise them to open conditions. They are now grown from larval to smolt or fingerling size before being shifted to the cages for culture. The growth of larvae to smolts can also be controlled by photo regulation so as to ensure that fingerlings are available throughout the year for culture. These hatcheries follow strict sanitation and quarantine conditions so as to minimise infections or stress to the animals. Larval nutrition is an extremely important area where dietary requirements are strictly followed and ensured by a combination of phytoplankton and zooplankton and formulated diets. In addition, these fingelings are vaccinated against a variety of bacterial and viral diseases to ensure required immunity levels and survivals during culture. These fingerlings are now transported to sea cages for stocking and rearing.

The sea cage system : The principle behind design of sea cage systems is to provide natural marine conditions while ensuring sufficient stocking densities that make culture viable. This is principally achieved by the concept of a floating bag or net in the sea where the cultured organism is held captive even while experiencing the quality of natural sea water. Technology has advanced to such an extent that while achievable biomass in shrimp and fish in Indian ponds range around 800 gms to one kg per cubic meter, sea cage systems offer productivity between 25-100 kg per cubic meter. However as in

the case of over doing anything, it has been found that production levels exceeding 25 kg per cubic meter, in Europe, tend to create environmental and other problems. Thus sustainability has been ensured by the introduction of a law that prohibits production levels exceeding 25 kg per cubic meter for salmon.

The cage facility essentially consists of square or round frames made of galvanised steel or polymer from which nets are hung up to a depth of 30-50 m depending on the conditions of the sea. These frames are suitably ballasted by floats specially designed for the sea. The structure and construction are normally designed to cater to tidal amplitudes and conditions in the sea. Adequate walk ways are also provided for easy movement between cages and to facilitate transport of personnel and material. Cages are linked through a system of hinges that move with the wave motions of the water. These floating cages have an attached housing unit to provide sufficient infrastructure for technical personnel assigned to the sea cage system. This is absolutely essential as a sea farming system requires constant monitoring and response. Animals need to be constantly inspected for disease, stress and growth rates. Also water quality at different levels in the cage needs to be tested on a daily basis to ensure that flushing of water through tidal action is effective. Other infrastructural requirements include boats as well as a radio telephone facility.

Feeding of these fish is either done manually or by automated feeding systems. Due to the higher densities stocked, sea cage farmers find formulated diets a better and coast effective feeding alternative. On occasion, fish are transferred from one cage to another depending on the problems noticed. Also if necessary, these fish are vaccinated again to ensure necessary immunity levels. Due to the large biomass handled, these fish are normally transferred or harvested using large fish pumps. Sometimes, processing facilities

which own the fish farms merely detach the net cages from the floating structure and tow them to onshore anchor areas from where they are pumped directly into processing plants.

Processing facility : In order to ensure minimum handling and prevent damage, most finfish processing facilities are automated. Fish are pumped from cages into a wash tank where water is constantly recirculated. The temperature in these tanks is reduced to around freezing levels so that fish are killed instantly due to thermal shock. These fish are now collected and sent to the processing line where they are gutted, deheaded and filleted to suit market requirements.

Opportunities and prospects

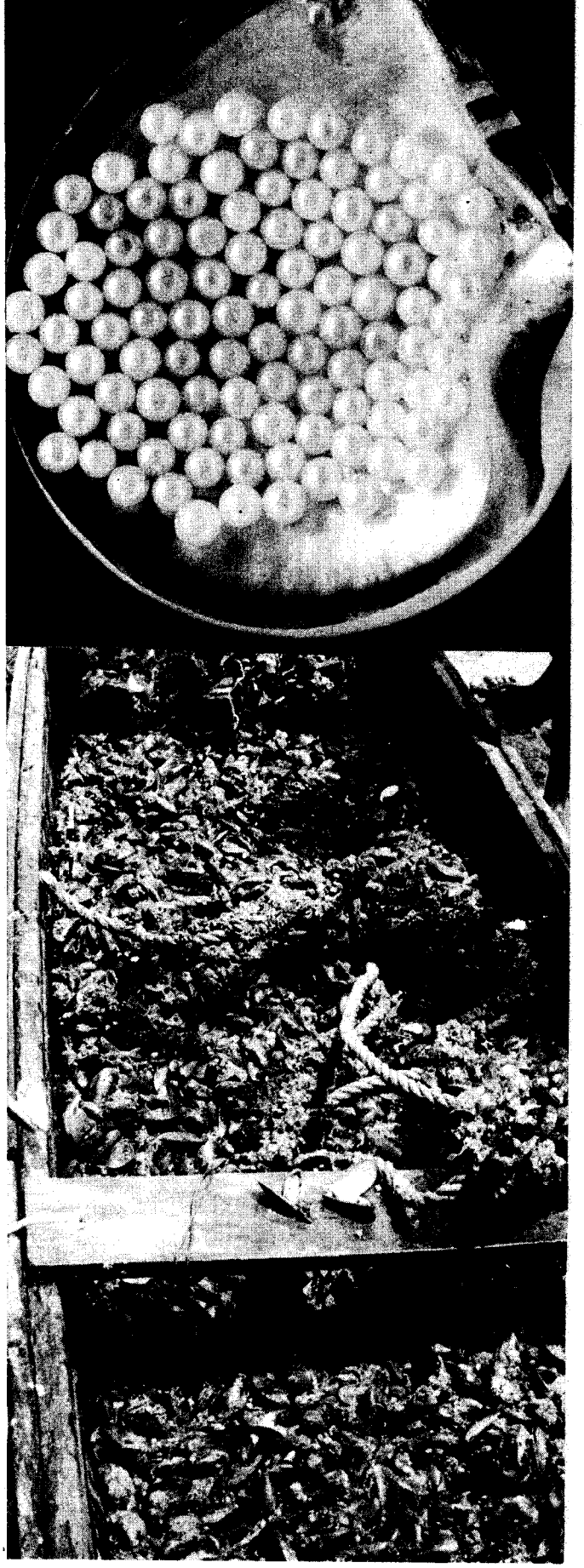
If India needs to take advantage of the enormous opportunities presented by the need for protein in diets and specifically the 55 million ton deficit in seafood expected by 2025, it is imperative that a well thoughtout and co-ordinated strategy to harness India's potential, be instituted immediately. Mariculture both in terms of coastal aquaculture as well as sea cage farming is essential and a matter of extreme priority. Apart from helping conservation of our seas and addressing important issues in nutrition, this acivity can dramatically change coastal economies and can make our lengthy coastline, the hub of a new economic front. In addition, extrapolating the fact that India is the second largest aquaculture producer in the world and the seafood industry is the third largest foreign currency earner, we have the opportunity to not only lead the world but generate considerable foreign currency reserves.

Status and prospects of bivalve mariculture in India

K. K. Appukuttan
Central Marine Fisheries Research Instute, Cochin-682 014.

ABSTRACT

Farming systems and hatchery techniques for mussels, edible oysters and pearl oysters were developed at the Central Marine Fisheries Research Institute by late 70's. In the present decade intense efforts were made to test the suitability of different ecosystems for bivalve mariculture. The attempts made to demonstrate the economic feasibility of mussel and oyster culture in Kerala had a positive impact leading to development of group farming activities in the coastal communities. Relative to this intensification of farming, preliminary attempts were made to explore the potential domestic market. The changing scenario from experimental farming to commercialisation of mussel and oyster culture in India is



presented. New strides have been made in large-scale production of pearl oyster spat and pearl culture. Initiation of pearl culture projects by private entrepreneurs has widened the scope for marine pearl production in India. A review of the status, opportunities and prospects for expansion of the bivalve mariculture along with the technical, economic and social problems are analyzed and discussed. Enhanced use of underutilized areas for future mariculture programmes are analyzed in the light of potential new markets. Thrust areas for future research and development activities are also highlighted.

Introduction

The occurrence of fast growing species of edible bivalves like mussels and oysters in the Indian coastal regions prompted marine researchers to evolve bivalve mariculture technologies. The pearl culture technology was also developed utilizing the pearl oysters from the Gulf of Mannar and Palk Bay. These technologies were tested in different regions. The chronological events in the development of pearl culture as an industry in India are given in Table 1. Consequent to the technology development, efforts were made to transfer these to the end users through implementation of pilot projects, short term and long term training programmes, publication of experimental results, and through organization of symposia, seminars and workshops. These efforts were successful and the main constraints, which impeded commercialisation of the technologies developed, were identified as lack of awareness about the technologies among the end users, financial constraints and low market demand. Hence the research programmes were reoriented and thrust was given to Transfer of Technology programmes with direct participation of the fishers. The accomplishments were encouraging leading to industrialization of pearl culture and development of mussel/oyster farming as a rural / community development programme in the southeastern and southwestern states of India. A brief account to the developmental activities of bivalve mariculture is presented.

Table.1. Chronological events in the development of bivalve mariculture in India

Pearl culture

1972	Initiated a project on pearl culture at Tuticorin Research Centre of CMFRI along the southeast coast
1973	Production of first cultured marine pearl in India
1981	First batch of pearl spat produced in hatchery
1985 - 87	Initiation of pearl culture programmes in different maritime states
1985 - 90	Sea ranching of pearl oyster larvae and spat to revive the natural stock
1991	India hosted the FAO/NACA* training programme on pearl culture at CMFRI –imparting training on pearl culture to trainees from other South, South East and East Asian countries
1993	Village level pearl production through direct involvement of small-scale fishers. Pearls worth US $ 2178 were produced
1994	Pearl produced along west coast through farming operations in the Arabian Sea
1996 - 99	Signing of memorandum of understanding with private entrepreneurs, increased survival rates in hatchery production, initiation of experiments for pearl production through tissue culture

Mussel farming

1973	Development of brown mussel culture technology at Vizhinjam
1974	Green mussel successfully cultured at Calicut
1977	Technology for edible oyster culture developed at Tuticorin
1976-82	Raft / longline culture of mussel tried at Madras,Karwar,Goa, Ratnagiri, A& N Islands, Vishakapatnam, Kakinada
1979	Pilot scale culture at Calicut
1984-85	Experimental brown and green mussel hatchery set up at Vizhinjam and Madras respectively
1997 -99	Production of green mussel spat at Calicut RC of CMFRI under DBT sponsored project
1995-2000	Intensification of transfer of technology programmes through

farmers participation, establishment of small rack units through group farming activities under DWCRA/ IRDP programmes, Commercial opensea farms set up by fishers along Kerala and Karnataka coast

Oyster farming

1977	Development of farming systems for oyster
1979	Lab to Land programme on oyster culture
1982	Succesful spat production in the hatchery, production of cultchless spat
1992 -95	Implementation of NABARD sponsored project for commercialisation of oyster culture
1993 - 95	Location testing programme at different maritime states
1996	Technology adoption by different fishers, setting up of oyster farms in the estuaries of Kerala
1999 -2000	Financial support extended by BFFDA for oyster culture in Kerala

Clam mariculture

1978	Farming technology for culture of *Anadara granosa*
1987	Hatchery technology for clam seed production developed
1993 - 96	Seed production and ranching of *Paphia malabarica* seed under MPEDA sponsored project
1994 - 99	Production and stock enhancement programmes of *Meritrix casta*
1998-2000	Trial expeiments to test the feasibilty of bivalve culture in shrimp ponds for maintaining water quality

* Network of Aquaculture Centres in Asia

Pearl mariculture

The need to develop pearl culture as a rural upliftment programme was recognized only in the early nineties. One of the successful programmes involving fishermen was carried out at Valinokkam, a small coastal village of Tamil Nadu in southeast coast of India. Twenty-five fishermen of the village

were selected and given training in various aspects of pearl culture. The initial reluctance noticed among the fishermen was overcome by proper motivation. They were educated about the importance and economic returns of the pearl culture. Active participation of the fishermen and their family members was observed from the fabrication of grow-out structure to pearl harvest. Part of the pearls produced was given to the fishermen as an incentive. The scope for large scale pearl production through village level community participatory programmes with proper technical and financial support from developmental organizations was clearly indicated by the 'Valinokkam Bay Programme' which yielded the following result.

Number of oysters implanted	9414
Total expenditure incurred	Rs.65982
Total pearls harvested	1849
Pearls distributed to fishermen	250
Revenue earned from sale of pearls	Rs.91476

Industrialization of pearl culture

Along the Tamil Nadu coast, M/s Tamil Nadu Fisheries Development Corporation Ltd (TNFDC) and M/s Southern Petrochemicals Industries Corporation Ltd (SPIC) took up a joint commercial project on pearl production in 1983 with technical know-how from CMFRI. This was a laudable pioneering effort by the government and the industry. The technical problems faced when the technology was commercialized were duly solved. The Department of Fisheries, Gujarat started a research and development programme along the Gujarat coast with the natural pearl oyster resource. Later, to enhance the depleted stock, pearl oyster spat were also supplied from the shellfish hatchery of CMFRI at Tuticorin. However, commercial ventures by industrial houses were restricted to the areas around the natural pearl oyster beds in India.

After a gap of nearly a decade, other firms also started pearl culture programmes. They are ITAP Ltd, Tuticorin; Orkay Company, Mandapam;

Master Pearls Ltd, Chirala and Pearl Beach Hatcheries, Visakhapatnam. Some of the pearl farms are located in the Krusadai Island while others are in the Palk Bay, Gulf of Mannar and in Andhra Pradesh. What started as an experiment in 1972, has supported the growth and development of an industry. While India has been a net importer of raw pearls during the early nineties, from 1996 onwards it has also been able to export cultured pearls, albeit in small quantities.

Development of mussel and oyster farming as seasonal group farming occupation

a) Oyster culture: Though oyster farming was successfully done in the farms of CMFRI's research centres, the actual momentum of technology adoption was felt only during the past five to six years. Considering the wide variation in the different water systems, a programme on location testing and demonstration of oyster farming with farmer's involvement was started in 1993 in Ashtamudi Lake. The livelihood of more than 3000 villagers of this area is linked directly or indirectly to this resource. First, a trial was done to test the growth and survival of oysters by suspending a few oyster rens from the platform of a Chinese dipnet in the Lake. Later, by the beginning of 1994, a demonstration farm was set-up by constructing a bamboo rack by involving fishermen. The first commercial oyster farming area was developed in Kerala in Ashtamudi Lake (Dalavapuram) during 1995-96. In August 1996, more than 100 tonnes of full-grown oysters were harvested. This motivated several farmers to arrogate edible oyster farming leading to the establishment of a number of commercial oyster farms in this large estuarine system.

The rens were harvested after a period of 5 to 6 months. The heat-shucked meat in the frozen form was sold in the internal market. Later, the Institute refined on the technology by introducing seasonal and perennial oyster farming depending on the estuary's physico-chemical characters. By the year 1997, oyster farming became increasingly popular, and a progressive farmer took up oyster farming on a large scale producing annually 10 to 15 tonnes of oyster. This hard working hexagenrian farmer received the ' Best Farmer ' award from the Government of Kerala for his integrated farming approach. Similarly, in other estuaries also the farming methods were

demonstrated. In Kerala, the BFFDA now gives financial assistance to farmers to set up oyster farms. This confirms the fact that the end users and the planners have recognized oyster culture as a viable project ideal for rural development and income generation.

In addition to creating awareness on the profitability of oyster farming, the demonstrations in the states of Karnataka, Tamil Nadu and Andhra Pradesh, gave valuable information on the spatfall, growth, and optimum period of harvest, etc. Though the level of technology adoption .varied from place to place, the overall impact was acceptance of the farming method as a part time avocation for additional income to rural fishers.

b) Mussel farming : In the past decade several demonstration farms were set up along the southwest coast of India mainly in Kerala and Karnataka. These two states have well-established traditional mussel fishery. The demonstration farms in the open sea and the estuarine systems were fabricated, stocked, managed and with full participation of the coastal fishers. The estuarine ecosystems are calm and shallow (< 4m). Accordingly, racks were constructed in different estuaries to suspend the seeded ropes. The first site was in Padanna (Kasargod region), which is famous for its offshore mussel fishery. About 100 seeded ropes were suspended during January 1996, which reached harvestable size within 5 months with a total weight of 12-15 kg/m length. A farmer who was impressed by the farming methods set up his own farm for mussel in a 200 m2 area and seeded 175 ropes during the same period. The meat yield was 30% of shell-on weight. This was the first instance of large-scale mussel farming in the estuaries of India. This demonstration impressed the local mussel picking families as well as the village level co-operative sector banks and what followed was a virtual revolution in mussel production from the region.

The scientists of the CMFRI in consultation with the district administration developed a master plan to transfer the technology to potential women beneficiaries. The Development of Women and Children in Rural Areas (DWCRA) was identified as the most suitable scheme intended for groups of women beneficiaries below the poverty line. The local governing bodies identified the beneficiaries with the help of village extension officers and district

administration. The selection criteria took into consideration (i) primary school as the minimum education level; (ii) age of the beneficiaries between 28-62 years, and (iii) fisheries / agriculture as the major occupation. After the selection of beneficiaries, a series of awareness camps on mussel farming were conducted by the Institute in each panchayat (village). Beneficiaries were given training in their own farms from seeding to harvesting. One-day workshops were organized in different villages involving bank officials, officers of the district administration and village extension workers.

Loans from the government developmental agencies like DWCRA, IRDP (Integrated Rural Development Programme), TRYSEM (Training of Rural Youth in Self Employment) and Farmers Co-operative Banks to newly formed village mussel farming groups (average 13 members in each group) resulted in starting of several mussel farms in this region. All technical help to the farmers was provided by the CMFRI.

The entire farming operation viz., starting from seed collection to marketing was done by the women themselves. They were able to pay back the loan within the stipulated period. In succeeding years the farming activities were intensified by the involvement of more groups. Now, mussel farming is a part time avocation of the coastal women of North Kerala. The local banks and district administration have taken a lead in providing financial assistance to these fishers. Mussel farms are usually set up by November-December and the crop is harvested before June (to avoid large-scale destruction due to monsoon). Though, it is only seasonal, women have recognised that it is something which they can do with minimum effort.

Open sea mussel farming by the longline was demonstrated at Andakaranazhi and at Byndur in Karnataka while mussel rafts were deployed at Narakkal in Kerala. Motivated by the CMFRI's demonstration at Byndur, a small scale gillnet fisherman launched his own mussel line during December 1996. With CMFRI's help he set his long-line with seeded ropes of 4m at a depth of 6 m. Floatation was ensured through small buoys and FRP drums. He looked after his line by examining them during his fishing trips in the sea. In total he harvested close to 400 kg of mussels from his single line and it

fetched him US $ 130. The profit margin was comparatively high because many of the materials he used were those already available with him.

Along Kerala coast, one group of fishers suspended the mussel ropes from racks constructed in the sea. This structure was able to withstand the turbulent sea during certain periods. The mussel growers were able to harvest about 2.4 tonnes from a small unit of 0.05 ha. From 1998 onwards opensea mussel farms (rafts and racks) have been launched in Central Kerala. The demonstration programmes reaffirmed the following facts

- High production rates and growth rate 15 kg per metre
- Possibility of two crops, Nov. – Feb; Mar- May
- High survival (>95 %) and growth of mussels in estuaries
- Low fouling in estuarine systems
- Possibility of Integrated bivalve farming of mussel and oyster
- Possibility of harvesting mussel in a phased manner for more profit.

In addition to the above mentioned facts bivalve mariculture programmes were able to make social changes such as

- Privatization of mussel farming
- Group farming activities by women under IRDP / DWCRA/ TRYSEM
- Employment opportunities for women during seeding and harvesting
- High consumer demand for farmed mussel

c) Clam mariculture: Experiments on culture of commercially important clams like *Anadara granosa,Meritrix casta* and *Villorita cyprinoides* have been done at different estuaries. Though the growth and survival rates were high, the technology transfer has not been intensified, mainly due to the low market demand. Moreover clams being bottom dwellers, the growth largely depends on the substratum of the ecosystem. In the recent years clam fishermen of Kerala have resorted to clam relaying or semiculture. The black clam *Villorita cyprinoides* and the yellow clam , *Paphia malabarica* are commercially fished from Vembanad and Ashtamudi Lakes of Kerala. When seed clams occur in the catch, the fishermen segregate these and stock them in the areas adjacent to their homestead. Later when they reach marketable size these are harvested and sold.

Bivalves being filter feeders have been used to control the water quality of the shrimp ponds in many Southeast Asian countries. Experiments to try the efficiency of this system has been initiated in Kerala, Karnataka, Goa and Gujarat using different species of bivalves like *Perna viridis*, *Villorita cyprinoides*, *Paphia malabarica* and *Crassostrea madrasensis*.

Hatchery development

Availability of seed is recognized as one of the major requirements for farming and aquaculture industry throughout the world. Seed production for commercially important pearls, mussels, oysters and clams were developed in CMFRI. The ' cultch less ' or the free spat of edible oyster produced in the hatchery can be used for the production of the highly priced individual oysters. For the present, the requirement of spat of edible oyster and mussel is met from the nature, although, the hatchery technology is perfected. However, with the growth of pearl culture industry, entrepreneurs have realized the importance of producing pearl oyster spats in areas where natural resources do not exist, by adopting the pearl oyster hatchery technology. In the last two years the survival rate of spat has been improved through repeated trials at the shellfish hatcheries of CMFRI at Tuticorin and Mandapam. The Institute has also supported the growing pearl culture industry by supplying spat.

Stock enhancement of pearl oysters and clams

Revival of pearl oyster beds from extinction and creation of new beds in the Gulf of Mannar and Palk Bay through ranching of the seed produced in the hatchery is one of the most significant achievements of CMFRI. The first programme on ranching was launched in 1985 and in the subsequent years, this was continued and more than one million spat were ranched during a period of 5 years. Subsequent dives made in the beds clearly indicated replenishment of the pearl beds as seen in the number of oysters collected in one diving hour. Similarly hatchery produced seed of *Paphia malabarica* in the Astamudi Lake of Kerala was done during 1992-93. This was significant as this clam supports the livelihood of more than thousand fishermen families living near this estuarine system which was under intense fishing pressure.

Market improvement

The establishment of mussel farms in Kerala State led to a dramatic increase in farmed mussel production (more than 500 tonnes in 1998). As a

direct result of this, new marketing channels were opened up and now there is wider acceptance by the public about mussels as quality seafood. This has partly solved the main constraint, which hampered the growth of bivalve mariculture in India. The edible bivalve market channel is relatively straightforward and fresh and frozen farmed mussels and oysters have a healthy and growing domestic demand in maritime regions of the country. There is now increasing appreciation of the fine texture and taste of mussel and oyster meat and these comparative new products look set to captivate the urban connoisseurs mainly through the developmental efforts of the Integrated Fisheries Project, Cochin. On the export front, in the case of mussels, Indian products have found a place in the markets of UAE, Germany and Republic of South Africa, and the list is growing. Although live oysters are an expensive gourmet food in Europe and America, they have not found such a niche in India. Markets are limited to few isolated pockets like the Parsi community in Mumbai, who have a specific preference to smoked oysters marketed in cans.

Problem areas

With the wider acceptance of bivalve mariculture it has been possible to scrutinize the technical constraints of culture systems and commercial constraints of production costs to maximize returns and minimize constraints. The developments made in the bivalve mariculture have been possible only with the interactions from other governmental agencies. However to make the development of bivalve mariculture complete the following technical points should be considered.

With the increased production of farmed edible bivalves more emphasis should be given to ensure that only high quality products reach the market. The European Community Directives, which are comparable with the FDA standards allow only those cultured in clean waters (A quality) can be marketed for direct consumption while those coming from other categories must be depurated. When the global demand for live shellfish is expanding it is essential that purification of farmed molluscan shellfish must be made an integral and mandatory part of Indian shellfish industry. In addition to these products such as oyster and mussel on half shell with different sauces, vacuum - packed /IQF bivalves, battered or breaded molluscs have to be developed.

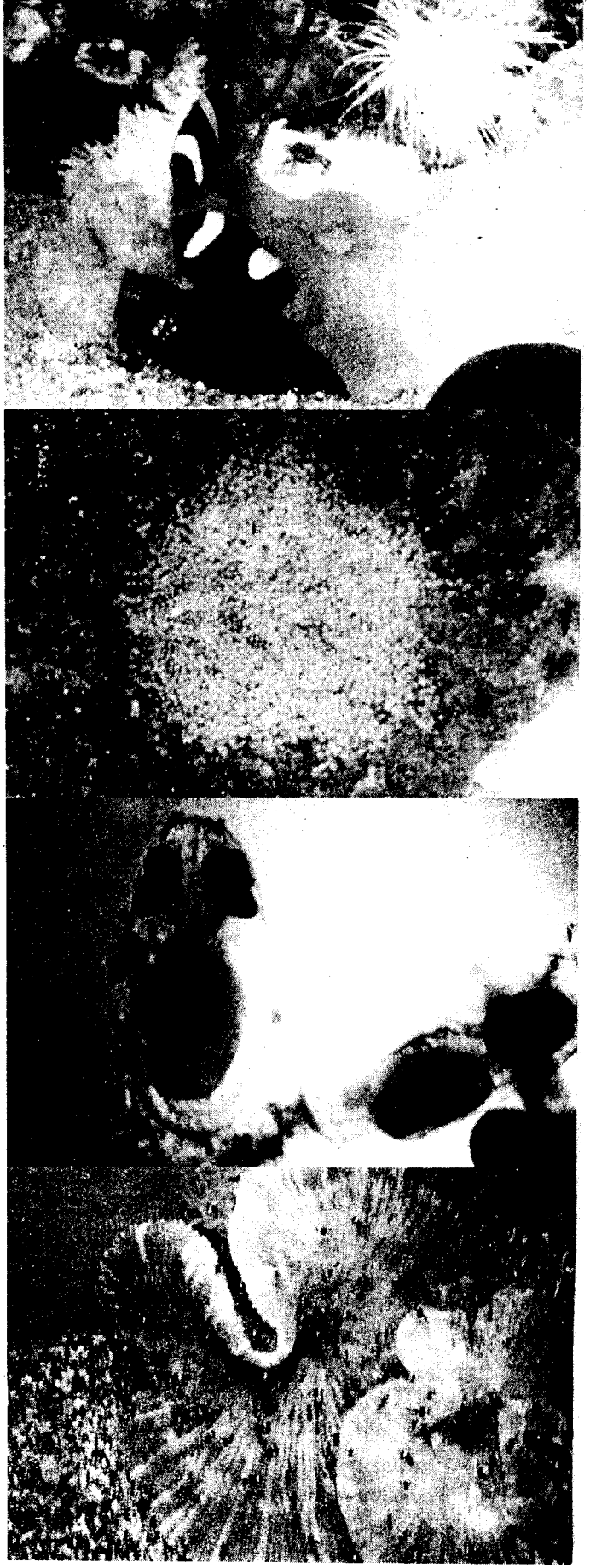
Harvest and post harvest process in bivalve mariculture is labour intensive. Mechanization of bivalve processing which started years back in the major molluscs growing areas have developed Multifunctional Lines which can process a variety of resources from the same site. The Indian bivalve industry should concentrate to mechanize the various processes like washing, declumping, grading, debyssing to make " ready-for-cooking' products with its flavour and appearance intact.

Issues, which cannot be neglected, are those related to use of water bodies and theft of crops. Conflicts between fishermen and bivalve growers have posed problems in certain areas. Similarly poaching of farmed material when the crop is ready for harvest has also been reported. These issues can be solved through generating awareness among the fishermen and also through increased participation of small-scale fisher.

New areas for development

Along with pearl culture the scope for developing other products from pearl industry can be explored. The mother of pearl shells which are used to make buttons and inlays in jewelry and furniture are sold @ Rs 3 lakhs per tonne. Dried adductor muscle which is considered as an aphrodisiac is sold @ Rs.11, 500 /kg. In India the expanding pearl culture industry can support the growth and development of such small - scale industries.

The most promising new opportunities for aquaculture are the products, which are in demand by the tropical marine aquarium trade. Among bivalves giant clams have the highest potential to be cultured for marine trade. The giant clam resource of A&N islands is currently harvested for the ornamental shell industry. Development of giant clam mariculture, which can produce small sized clams for aquariums, can lead to improvement of island economy.



Hatchery production of the clownfish *Amphiprion chrysogaster*

G. Gopakumar, Rani Mary George and S. Jasmine
Vizhinjam Research Centre of C.M.F.R.I., Vizhinjam - 695521

ABSTRACT

Tropical marine aquarium fishes have demand in the international market. Clownfishes or anemonefishes are most popular among marine aquarists due to their attractive colours and interesting display of behaviour with sea anemones. A technology for the hatchery production of the clownfish, Amphiprion chrysogaster *was developed for the first time in India. The broodstock maintained in the hatchery spawned frequently and methods were developed for hatching the eggs. The hatching period was 6-7 days. A simple biological detoxifying filtration system with effective circulation was designed and fabricated for larval rearing. The larvae were fed with the rotifer* Brachionus rotundiformis *at a*

concentration of 6-8 numbers per ml for the first four days. From the fifth day onwards they were fed with a mixture of B. rotundiformis *and freshly hatched* Artemia *nauplii. The larvae metamorphosed into juveniles in 12 to 15 days from the day of hatching. The juveniles were kept in the rearing tank for one week to one month by feeding them with* Moina micrura *and then transferred to grow out tanks with sea anemones. By upscaling the present technology, large scale hatchery production of clownfish young ones could be achieved.*

Introduction

The marine ornamental fish trade is rapidly expanding and tropical marine aquarium fishes are in great demand in the international market. About 15% of the world aquarium fish industry is constituted by marine aquarium fishes and more than 90% of these fishes are contributed from countries like Singapore, Hong Kong, Sri Lanka and Maldives. Eventhough India has a vast potential of marine ornamental fishes, an organised marine ornamental fish trade has not yet been developed in the country.

It is well known that the marine ornamental fishes are mostly associated with coral seas. The coral reefs provide a variety of ecological niche which are the abode of extremely rich and complicated animal communities consisting of a great diversity of species. By virtue of the shapes and bright colour patterns these fishes are attractive and many of them can be grouped as ornamental fishes. More than fifty reef fish families consisting of nearly 175 genera and about 400 species of ornamental fishes are distributed in the Indian seas. The major oceanic areas of coral fish distribution in Indian are the Lakshadweep Islands and the Andaman-Nicobar groups of Islands. The other areas of coral fish distribution are the coastal areas of fringing or patchy reefs of Gulf of Kutch to Bombay, areas of central west coast between Bombay to Goa, certain locations at south west coast (Vizhinjam to Cape Comorin), Visakhapatnam area, Gulf of Mannar and Palk Bay. The indiscriminate exploitation of these areas for the collection of ornamental fishes can cause severe damage to the delicate coral reef ecosystem. Hence it is evident that the exploitation of marine ornamental fishes from the wild should be done rationally purely on the basis of scientific management regime without inflicting any damage to the ecosystem.

The clownfishes belonging to the family Pomacentridae are among the most popular tropical marine ornamental fishes due to their generally small and hardy nature, attractive colours, high adaptability to life in captivity and the interesting disply of behaviour due to their association with sea anemones. The breeding of clownfishes has fascinated many marine aquarists

and some of the species were successfully bred and reared under captive conditions. The breeding and rearing of marine ornamental fishes in India is still in its infancy and successful technologies for the hatchery production of marine ornamental fishes are yet to be developed. In this context, the captive breeding and hatchery production of the clownfishes *Amphiprion chrysogaster* was attempted.

Materials and methods

The broodstock of *A. chrysogaster* was developed by collecting the fish along with the anemone belonging to the genus *Stoichactis* from Tuticorin/ Mandapam area. They were kept in 2.5 m x 0.6 m x 0.6 m glass aquarium tanks as well as in rectangular FRP tanks (2.2 m x 1.2 m x 1.2 m) along with sea anemones. The tanks were installed with biological-filters. In each tank 4-6 numbers of fishes were introduced. They were fed with boiled mussel meat two times daily. The eggs were removed carefully without exposing to air and were hatched in 100 litre FRP tanks containing filtered sea water from a biological filter. The eggs were continuously aerated by adjusting the position of the air stone so as to create the effect of fanning the eggs by the parents. Eggs were also hatched by keeping them in the parental tank itself till hatching. The newly hatched larvae were carefully removed by siphoning or transferring in small buckets. The larval rearing tanks (100 to 200 litres capacity) were provided with special type of filtration system. The water from an overhead tank with biological filter was re-circulated through the larval rearing tanks. The water circulation in the larval raring tanks were effected through fine pores put at the bottom of PVC pipes which were placed inside the rearing tanks. The filtration rate was adjusted around 100% per hour. The larvae were fed with the rotifer *Brachionus rotundiformis* at a concentration of 6-8 numbers for the first four days. From the fifth day onwards they were fed with a mixture of *B. rotundiformis* and freshly hatched *Artemia* nauplii. The young ones were fed with *Moina micrura.*

Results

Broodstock development

In all the broodstock tanks one pair grew ahead of others and became the spawning pair. The size of the mature fish ranged between 8-9 cm. Sexual dichromatizem was noted in the spawning pair. The snout of the female was dusky yellow whereas that of the male was bright yellow.

Spawning

The fish spawned several times in the broodstock tanks. The spawning pair drove out other fishes intruding into their territory. The spawning started with the cleaning of the substratum at which the eggs were to be

laid. Then the egg laying started which lasted for about an hour. The spawning took place invariably during 0900 to 1400hrs. The eggs were attached to small earthen pots, granite stones, on the sides of the broodstock tanks and even to the PVC pipes of the biological filter of the tank. The number of eggs at a single spawning ranged from 300 - 800. The interval between successive spawning of a pair varied between 10 days to 45 days. Both the parents continuously guarded the eggs and fanned the eggs with their fins and mouth.

The freshly laid fertilised egg was orange in colour and it started swelling within a few hours. The eggs were stalked, capsule shaped and the length ranged from 1.7 to 2.9 mm. A bright silvery spot inside the egg was obvious through the egg capsule. The unfertilised eggs were more orange in colour and they remained thin.

Hatching the eggs

The eggs started darkening from the second day and the developing larvae were clearly visible through the egg capsule from the third to fourth day. The larval hatching period was between 6 and 7 days. On the day of hatching the egg capsules became very thin and transparent. Glowing of the larval eyes was prominent. The larvae broke the capsules and came out. Darkness accelerated the hatching process. The mass hatching of the eggs occurred during night with peak during 1900 to 2200 hrs. In most cases 60 - 70% of the viable eggs hatched on the same night. But in a few cases half of the eggs hatched on the next day night. The hatching rate obtained was above 90% in the method of keeping the eggs in the parental tank itself till hatching, whereas the hatching rate was below 50% in the case of eggs incubated without parental care. The viability of the eggs was highly variable. The non-viable eggs became white from the third day of incubation.

Larval rearing

The length of the newly hatched larvae ranged from 2.5 to 3 mm (mouth gape varied from 200 to 250 μm). The larval survival after the critical period (after fifth day from the day of hatching) ranged from 50 - 60%. Contamination with unhatched *Artemia* cysts was detrimental to larval survival. When this factor was checked there was no further mortality. The larvae metamorphosed into juveniles in 12 to 15 days from the day of hatching. The average length of just metamorphosed young one was 8 mm. In the first batch 40 numbers of clownfish juveniles were produced at Vizhinjam by employing the same methodology.

Discussion

The exploitation of marine ornamental fish from the wild would lead to the overexploitation of the coral seas. The indiscriminate methods of harvest can damage the coral reef ecosystem, which provides the microhabitat requirement for the recruitment of the different species of coral reef fishes. Hence the only option to meet their demand is the hatchery production.

Several studies have been made on the biology of clownfishes (Moyer and Bell, 1978; Moyer and Nakazono, 1978; Moyer, 1980; Ochi, 1986; Hattori and Yanagisawa, 1991 a,b; Nelson *et al.*, 1996). They are benthic egg layers and protandrous hermaphrodites. Social hierarchies in anemonefish were also studied in detail (Moyer, 1976; Moyer and Nakazono, 1978; Hattori and Yanagisawa, 1991b). The largest individual in an anemone is usually a female with a smaller male and a variable number of juveniles. The anemonefishes show considerable aggression towards other individuals present in the anemone. Hirose (1995) studied the patterns of pair formation in *A. clarkii, A. frenatus and A. perideraion* on coral reefs of Okinawa, Japan which indicated that re-pairing occurred in these species. The results of the experiments on population restocking were found to be encouraging (Nelson *et al.*, 1996). Hattori and Yamamura (1995) described the coexistence of sub adult males and females as alternative tactics for breeding post acquisition in *A. clarkii.* The salient aspects of biology of *A. chrysogaster* agree with the general pattern noted in the other clownfish species studied.

The breeding and rearing of anemonefishes is promising due to the production of large eggs and larvae, frequent spawning in captivity and the hardy nature of the fish. The breeding and rearing of two species of clownfish viz, *A. clarkii and A. percula* was reported by Alva and Gomes (1989). Several fish hobbyists were able to breed different species of clownfishes. Malpass (1996) described the details of raising *A. percula.* Allen (1998) reported on the clownfish hatchery production by two companies connected with marine aquarium hobby. The species they reared were *A. percula, A. melanopus, A. perideraion, A. ocellaris, A. frenatus* and *Premnas biaculeatus.* There has been no report on the breeding and rearing of clownfishes from Indian waters.

The major technological aspects of clownfish rearing programme are the successful development of broodstock, methods of hatching the eggs, development of a biological detoxifying filtration system for larval rearing and appropriate larval feeding schedule. It is felt that by upscaling the present technology large scale hatchery production of clownfish young ones for export market could be achieved. This technology can be considered as a milestone towards the development of a marine ornamental fish trade in India, which

has immense potential for foreign exchange earning. Besides, this technology can also pave the way for the development of suitable techniques for the larval rearing of valuable marine food fishes in hatcheries.

References

Alva, V.R. and L.A.O. Gomes. 1989. Breeding marine aquarium animals: The anemonefish. *Naga*, **12**(3): 12-13.

Allen, Bob. 1998. Clowns in the desert. *Tropical Fish Hobbyist*, Jan 1998: 60-64.

Hattori, A. and Y. Yanagisawa. 1991a. Sex change of the anemonefish *Amphiprion clarkii* in a habitat of high host density: A removal study. *Japanese Journal of Ichthyology*, **41**: 1-8.

Hattori, A. and Y. Yanagisawa. 1991b. Life history pathways in relation to gonadal sex differentiation in the anemonefish, *Amphiprion clarkii* in temperate waters of Japan. *Environmental Biology of Fishes*, **31**: 139-155.

Hattori, A. and N. Yamamura. 1995. Co-existence of subadult males and females as alternative tactics of breeding post acquisition in a monogamous and protandrous anemonefish. *Evol. Ecol.*, **9**(3): 292-303.

Hirose, Y. 1995. Patterns of pair formation in protandrous anemonefish, *Amphiprion clarkii, A. frenatus* and *A. perideraion* on coral reefs of Okinawa, Japan. *Environ. Biol. Fish.*, **43**(2): 153-161.

Malpass, Don, Jr. 1996. Raising *Amphiprion percula*. *Tropical Fish Hobbyist*, August 1996: 55-61.

Moyer, J.T. 1976. Geographical variation and social dominance in Japanese populations of anemonefish *Amphiprion clarkii*. *Japanese Journal of Ichthyology*, **23:** 12-22.

Moyer, J.T. 1980. Influence of temperate waters on behaviour of the tropical anemonefish *Amphiprion clarkii* at Miyake-Jima, Japan. *Bull. Mar. Sci.*, **30:** 261-272.

Moyer, J.T. and J.T. Bell. 1976. Reproductive behaviour of the anemonefish *Amphiprion clarkii* at Miyake-Jima, Japan. *Japanese Journal of Ichthyology*, **23:** 23-32.

Moyer, J.T. and A. Nakazono. 1978. Protandrous hermaphroditism in six species of the anemonefish genus *Amphiprion* in Japan. *Ibid.*, **25:** 101-106.

Nelson, J.S., V.P.E. Phang and L.M. Chow. 1996. Survival and growth rates of the anemonefish, *Amphiprion ocellaris*: A transfer experiment. *J. Fish Biol.*, **48:** 1130-1138.

Ochi, H. 1986. Growth of the anemonefish *Amphiprion clarkii* in temperate waters with special reference to the influence of settling time on the growth of 0-year olds. *Mar. Biol.*, **92:** 223-229.

Survival and growth of seabass *Lates calcarifer* (Bloch) fry reared at different stocking densities

M. Kailasam, P. Kishore Chandra, M. Muralidhar, A.R. Thirunavukkarasu and Mathew Abraham

Central Institute of Brackishwater Aquaculture, Chennai - 600 034

ABSTRACT

Asian seabass, Lates calcarifer *larvae were reared under different stocking densities in the hatchery in order to understand the optimum density that can be maintained in rearing medium under controlled conditions. The growth and survival of the larvae reared in the hatchery condition vary with densities. The length increment was high (10.6 mm) in the lowest densities (5 Nos/l) when compared to that of highest densities (5.1 mm) in 30 Nos/litre showing significant variation. Higher survival rate (90%) was observed in lower densities (5 and 10 Nos/litre) the survival rate was decreased to 65% in the larvae reared in higher stocking densities of 25 and 30 Nos/litre. The survival and growth of seabass larvae were found to be density and duration dependent. Results indicated that a stocking density of 5 - 15 Nos/litre is optimum for better survival, growth and production of seabass fry in the hatchery.*

Introduction

The Asian seabass *Lates calcarifer* (Bhetki) is one of the important food fishes in India. It forms minor constituent in the perch fishery from nearshore waters normally caught by artisanal fishermen with hook and line. It is carnivorous in feeding habit, preying on small fish and

crustaceans. Owing to its fast growth rate and capacity to withstand wide environmental fluctuations and good market demands seabass is cultured extensively in cages and ponds and provides an important component in the aquaculture activities in SouthEast Asia (Mackinnon, 1985). In India though seabass aquaculture is practiced in some parts like West Bengal, Konkan area and Tamil Nadu in a traditional manner, culture could not be adopted on large scale due to non-availability of adequate quantity of seed. For the first time in India, the Central Institute of Brackishwater Aquaculture (CIBA), Chennai has successfully developed technology for the large scale production of seabass seed in hatchery (Thirunavukkarasu, 1997; Kailasam *et al.*, 1998).

Successful rearing of fish larvae in the hatchery depends on numerous critical factors. An understanding of the role of these factors on the fish larvae makes it possible to regulate them effectively under confined conditions which will help in better survival, growth and health of the larvae. These factors include initial food requirements, larval rearing densities and food densities in rearing larval fishes (Barlow *et al.*, 1996). Information on larviculture of seabass in relation to different stocking densities is meagre. Kailasam *et al.*, (1999) observed cannibalism as a crucial factor in the larval rearing of seabass. Direct relationship was observed with stocking densities and cannibalism in the larval rearing of seabass by Parazo *et al.* (1991). Seabass being carnivorous and cannibalistic, the stocking densities in rearing medium is an important factor to be considered. Stocking density is one of the factors, which would affect the survival of the larvae (Macintosh and De Silva, 1984). Knowledge in the growth and survival under different stocking densities would help in optimizing the stocking density in rearing medium which will help in reducing the cannibalism resulting in better survival, growth and health of the larvae. Therefore, the present investigation was taken up to assess the survival and growth of seabass fry at different densities under hatchery rearing conditions.

Materials and methods

Seabass larvae produced in the experimental finfish hatchery of Central Institute of Brackishwater Aquaculture (CIBA), Chennai

(Muttukkadu) were used for the experiment. Hatchery reared fourteen days old seabass larvae were used. Larvae in the size range of 6.5 ± 1.2 mm were collected and acclimatized to tank condition prior to experiment. Larvae were distributed to 3 L capacity plastic containers at a density of 5, 10, 15, 20, 25, and 30 nos / L. The experiment was carried out for a period of 16 days. Fry were fed with *Artemia* nauplii twice daily at 10 00 hrs and 17 00 hrs. Depending upon the density of the larvae, adequate quantity of *Artemia* nauplii @ 50 nauplii/larvae was provided in each tank to avoid under feeding. Water quality parameters such as temperature, salinity, dissolved oxygen; pH, ammonia and nitrite in the rearing medium were estimated regularly. Dead larvae were removed as soon as mortality was observed. Surviving larvae were counted at four days interval i.e., 4^{th}, 8^{th}, 12^{th} and 16^{th} days after starting the experiment. Final lengths of the larvae were measured at 16^{th} day and length increment was computed. All the experiments were conducted in duplicate and the mean value is considered for statistical analysis.

Data on percentage of survival from different densities was subjected to arc sign transformation (Abdussamad and Thampy, 1994) using the formulae, $\theta = \text{Sin}^{-1} \sqrt{X100}$, where θ is the arc sign value and X is the % values observed This transformation is needed as they are in percentage values, which do not follow normal distribution. Multiple correlation was carried out for larval growth, survival and time to find out their interaction relationship.

Results

Fig 1 illustrates the growth increment of *L. calcarifer* fry reared at different stocking densities upto 16 days of experimental duration. At the lowest density of 5 nos/L average growth increase was 10.6 mm i.e., from 6.5 ± 1.2 mm initial mean length to 17.2 ± 1.9 mm final mean length. Under stocking density of 10, 15, 20, 25 and 30 nos/L the larvae attained final mean length of 15.5 ± 1.9 mm, 14.1 ± 1.8 mm, 12.8 ± 1.1 mm, 12.2 ± 1.7 mm and 11.6 ± 1.6mm respectively. The overall average length increment was 9.0 mm, 7.6 mm, 6.3 mm, 5.7 mm and 5.mm

respectively in the densities of 10, 15, 20, 25 and 30 nos/L. Results of present study has indicated that the larval growth and survival rate were higher when they grow in lower stocking densities and in higher stocking densities, the larval growth and survival were comparatively lower (Table 1).

Table 1 . Growth of *L. calcarifer* fry reared at different stocking densities

Density Nos/ L	Initial Length (mm)	Final Length (mm)	Length Increment (mm)
5	6.5 ± 1.2	17.1^{a} ± 1.9	10.6
10	6.5 ± 1.2	15.5^{b} ± 1.9	9.0
15	6.5 ± 1.2	14.1^{c} ± 1.8	7.6
20	6.5 ± 1.2	12.8^{d} ± 1.1	6.3
25	6.5 ± 1.2	12.2^{de} ± 1.7	5.7
30	6.5 ± 1.2	11.6^{f} ± 1.6	5.1

Mean values with same alphabet as superscript are not significant (P > 0.05)

Survival of *L. calcarifer* fry reared at different stocking densities is shown in Fig 2. In lower densities (5 larvae/L and 10 larvae/L) higher survival rate (90%) was observed during the experimental duration. However, when larvae were reared at higher densities of 25 larvae/L and 30 larvae/L, the survival was only 65% in both the cases. The rate of survival was 85% and 70% in the densities of 15 nos/L and 20 nos/L respectively (Table 3).

The results of the analysis of variance for growth and survival are given in Tables 2 and 4 respectively. The growth of the fry in relation to different stocking densities was found to be significant at 5% level

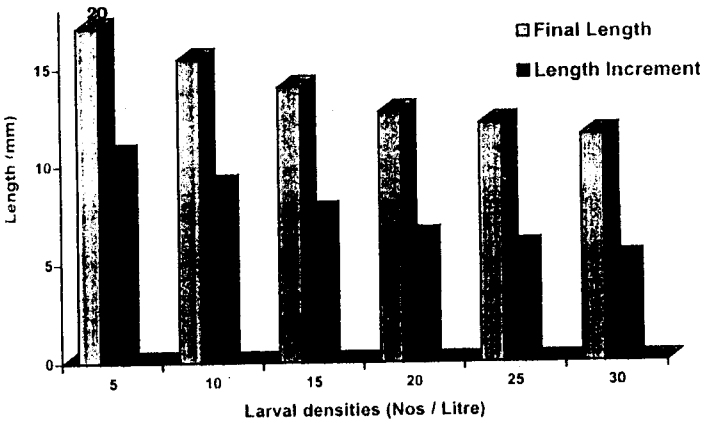


Fig. 1. Growth of *Lates calcarifer* fry reard at different stocking densities

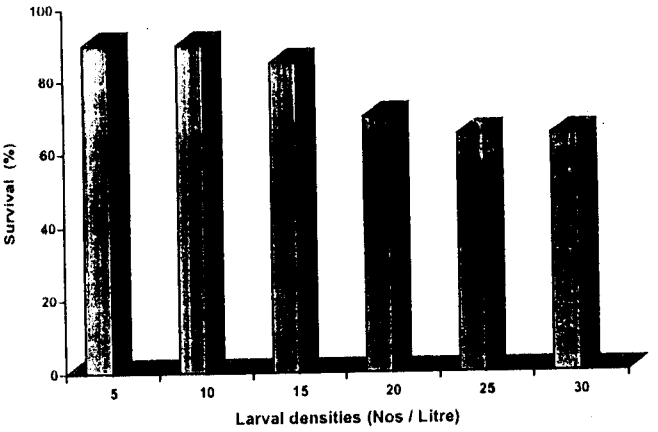


Fig.2. Survival of *Lates calcarifer* fry reared at different stocking densities

(Experiment.1). Length increment of the fry was higher (10.6 mm in 5Nos/L) in lower densities than that of higher densities (5.1 mm in 30 Nos/L). The stocking density and rearing days were significantly affecting the survival of the fry. The percentage of survival was found to be lower in higher densities and higher in lower densities.

Table 2. Analysis of variance for growth (length) in different density experiments

Source	Degrees of Freedom	Sum of Squares	F. Value
Density	5	66.66	
Error	12	2.08	77.01*
Total	17	68.73	

** Significant at 5% level.*

Among water quality parameters, basic parameters such as water temperature and salinity were at 29.0 ± 0.5°C and 29.0 ± 1.0 ppt respectively. pH value also did not vary much and it was 7.9 ± 0.30 in all the containers. Dissolved oxygen concentration was around 7.0 ± 0.05ppm during the experiment. However, highest ammonia value of 0.52 ± 0.12 ppm was estimated when the larvae were reared in higher densities of 30 nos/L and ammonia concentration was low at 0.32 ± 0.04 ppm at lower density 5 nos/L density. Nitrite too showed variations in the range between 0.08 ± 0.01 ppm (5 nos/L) and 0.16 ± 0.03 ppm (30 nos/L) showing significant increase in the level as the density of larvae increases in the rearing medium.

Table 3. Survival (%) of *Lates calcarifer* fry reared at different stocking densities*

Density Nos/L	Survival %			
	4th Day	8th Day	12th Day	16th Day
5	100[a]	100[a]	90[abc]	90[abc]
10	100[a]	100[a]	90[abc]	95[ab]
15	100[a]	95[ab]	95[ab]	85[abcd]
20	90[abc]	80[bcde]	75[cde]	75[cde]
25	100[a]	85[abcd]	70[dc]	65[e]
30	97.5[ab]	85[abcd]	75[cdc]	65[e]

· Mean values with same alphabet as superscript are not significant (P>0.05)

Table 4. Two factor analysis of variance for survival in different stocking densities experiments

Source	Degrees of Freedom	Sum of Squares	F. Value
Density(A)	5	2652.60	8.14*
Days(B)	3	2568.23	13.5*
A x B	15	966.15	0.99*
Error	24	1562.50	
Total	47	7749.48	

* *Significant at 5% level*

Discussion

Optimizing stocking density in the rearing of seabass larvae in the hatchery to maximize growth and survival would be a major benefit for seabass seed production in the hatchery. It would help in better management of the rearing medium and maintenance of water quality. The volume of water per fish is a significant factor in determining larval production in hatcheries. Stocking with optimum number in the container will help in the selection of optimum tank size for rearing fish larvae. It may vary with the species because of the space requirements of larval fish depends on the behaviour of fishes, food intake, metabolic rate etc., Swimming behaviour and inherent capability to adopt to the space available are also important (Kinne, 1977).

In the present study, survival was low when the larvae were reared in high stocking densities of 25 and 30/L. Higher stocking density has been observed to influence survival and growth probably affecting the social behaviour of fry. This has been demonstrated in several species including Mozambique tilapia *Oreochromis mossambicus* (Macintosh and Silva, 1984), large mouth bass *Micropterus salmonoides* (Fleming and Juhansen, 1984) and grouper *Epinephelus suillus* (Duray, 1995). It is inferred that at high stocking densities, there is reduction in social dominance and antagonistic behaviour among individuals and this led to decrease in growth. In this

study also *Lates calcarifer* fry showed decreased growth in high stocking densities when compared to that of lower stocking densities though feed is equally provided in the rearing medium. Water quality maintenance is yet another important factor to be considered in larval rearing. Deteriorating water quality is a concern at high stocking densities because of high nitrogenous wastes like ammonia and nitrite are added to the tank. In the high stocking densities, large quantity of feed also have to be added in the rearing tanks, which would also increase the organic load and thereby deterioting the quality of the rearing medium. But at the same time, very less stocking density lead to large requirements of rearing space which will cause more capital and operational costs. Increasing stocking density results stress in rainbow trout leading to enhance energy requirements causing reduced growth as reported by Leather land and Cho (1985). Bridges and Kling (2000) have also reported poor survival of Atlantic cod *Gadus morhau* larvae when reared at high stocking density as result of deteriorating water quality. Increased space reduces the chance of larval damage, since larvae have more freedom to swim continuously without contacting tank walls and the favourable water quality makes larger rearing tanks better for fish larvae (Estudillo *et al.*, 1998).

Feeding activities of fish are governed by a number of biotic and abiotic factors. Water quality, temperature, light regime, shelter and stocking density are known to be important (Brett, 1971). These factors and their interactions determine scope for growth (Hogendoorn, 1983). Cannibalism has been observed in numerous fish species including cod, *Gadus morhua* (Oiestad *et al.*, 1985) and *Dicentrachus labrax* (Katavic *et al.*, 1989). Some of the main factors in cannibalism and antagonistic behaviour are size variation, availability of prey and population density (Hecht and Pionaar, 1993). In the *Lates calcarifer*, Kailasam *et al.*, (1999) observed the size variation as a major factor. When small and large fry are reared together, the larger ones tend to prey upon the smaller fry even in the presence of adequate feed in the rearing medium.

The influence of population density on the cannibalism behaviour has

to be ascertained in the seabass larvae though it has been observed in other species. The present investigation though revealed that lower densities give better survival and growth rate in the laboratory experiment, larval rearing is done on larger tanks in CIBA, Muttukkadu for ascertaining the optimum stocking density with economic considerations. This would help in designing an efficient hatchery production system.

Acknowledgement

The authors are thankful to Dr.G.R.M. Rao, Director, Central Institute of Brackishwater Aquaculture , Chennai for his encouragement.

References

Abdussamad E.M., and D.M. Thampy, 1994. Cannibalism in the tiger prawn *Penaeus monodon* (Fabricus) in nursery rearing phase. *J. Aqua. Trop.* **9** : 67 – 75.

Barlow C., K. Williams and M. Rimmer 1996. Seabass culture in Australia. *Infofish International* **2** : 26 – 33.

Brett, J.K., 1971. Satiation time, appetite and maximum food intake of sockeye salmon *Chorhynchus nerka. J. Fish. Res. Board Can.* **28** : 409 - 415.

Bridges B.B. and L.J. Kling, 2000. Larval culture of Atlantic Cod (*Gadus morhua*) at high stocking densities. *Aquaculture*, **181** : 61 – 69.

Duray M.N., C.B., Estudillo and L.G. Alpasan 1995. Optimum stocking density and tank size for larval rearing of Grouper *(Epinephelus suillus)*. Fourth *Asian Fish.Forum*, October 16 – 20, 1995, Beijing, China.

Estudillo C. B, M.N, Duray, E.T, Marasigan, 1998. Growth and survival of milk fish (*Chanos chanos*), Seabass *(Lates calcarifer)* and Rabbit fish (*Siganus guttatus*) larvae reared in the same density in different sized tanks.. *The Israeli Journal of Aquaculture* Badmideg, **50** (1): 20 – 24.

Fleming, I.A., P.H. Johansen, 1984. Density and agonistic behavior in young-of-the year Largemouth bass (*Micropterus salmoides*) *Canadian Journal of Zoology*, **62** : 1454 – 1455.

Hecht, T., A.G. Pienaar, 1993. A review of cannibalism and its implications in fish larviculture. *Journal of world Aquaculture Society*, **24** : 246 – 261.

Hogendoorn, H., 1983. Growth and production of the African catfish *Clarias gariepinus* (C and V) : II Bioenergitic relations of body weight and feeding level. *Aquaculture* **35** : 1 – 17.

Kailasaam, M. A.R. Thirunavukkarasu, P. Kishore Chandra, P. Shiranee, and K.V. Rajendran, 1998. Induction of maturity and spontaneous spawning of captive broodstock of Bhetki *Lates calcarifer* (Bloch) through hormonal manipulation. Paper presented in *"National workshop on Recent advances in Hormonal Physiology of fish and shellfish Reproduction"*. CIFA, Bhubaneshwar, 16-18 April 1998, PP-30.

Kailasam, M, A.R. Thirunavukkarasu, Mathew Abraham, P.Kishore Chandra and R. Subburaj, 1999. Influence of size variation and feeding on cannibalism of Asian Seabass *Lates calcarifer* (Bloch) during hatchery rearing phase. (In Press)

Katavic, I., J. Judgujakovic, B. Glamuzina, 1989. Cannibalism as a factor affecting the survival of intensively cultured Seabass (*Dicentrachus labrax)* fingerlings. *Aquaculture,* **77** : 135 - 143.

Kinne, O., 1977. Pisces, Rearing of larvae. pp. 968 - 1004. In : O Kinne (ed.). *Marine Ecology* Vol. 3. Wiley, N.Y.

Leather land, J.R and Cho., 1985. Effect of rearing density on thyroid and interrenal gland activity and plasma hapatic metabolic levels in rainbow trout, *Salmo gairdneri* Richardson.*J.Fish.Biol.*, **27**:583-592.

Macintosh, D.J., S.S. Desilva 1984. The influence of stocking density and food ration on fry survival and growth in *Oreochromis mossambicus* and *O. niloticus* female X *O. aureus* male hybrids reared in a closed circulated system. *Aquaculture,* **41** : 345 - 358.

Mackinnon, M.R., 1985. Barramundi breeding and culture in Thailand. Queensland Dept of Primary Industries study tour report. 1 - 21, June, 1982. Sohgkla, Thailand.

Oiestad, V., P.G. Kvensth, A. Folkvord, 1985. Mass production of Atlantic Cod juveniles *Gadus morhua* in a Norwegian salt water pond. *Transactions of the American Fisheries Society,* **114** : 135 - 143.

Parazo M.M., D.M. Reyes Jr. and E.M. Avila, 1991. Hatchery rearing of Seabass *Lates calcarifer* (Bloch). *Philip. Sci.*, **28** : 65 - 76.

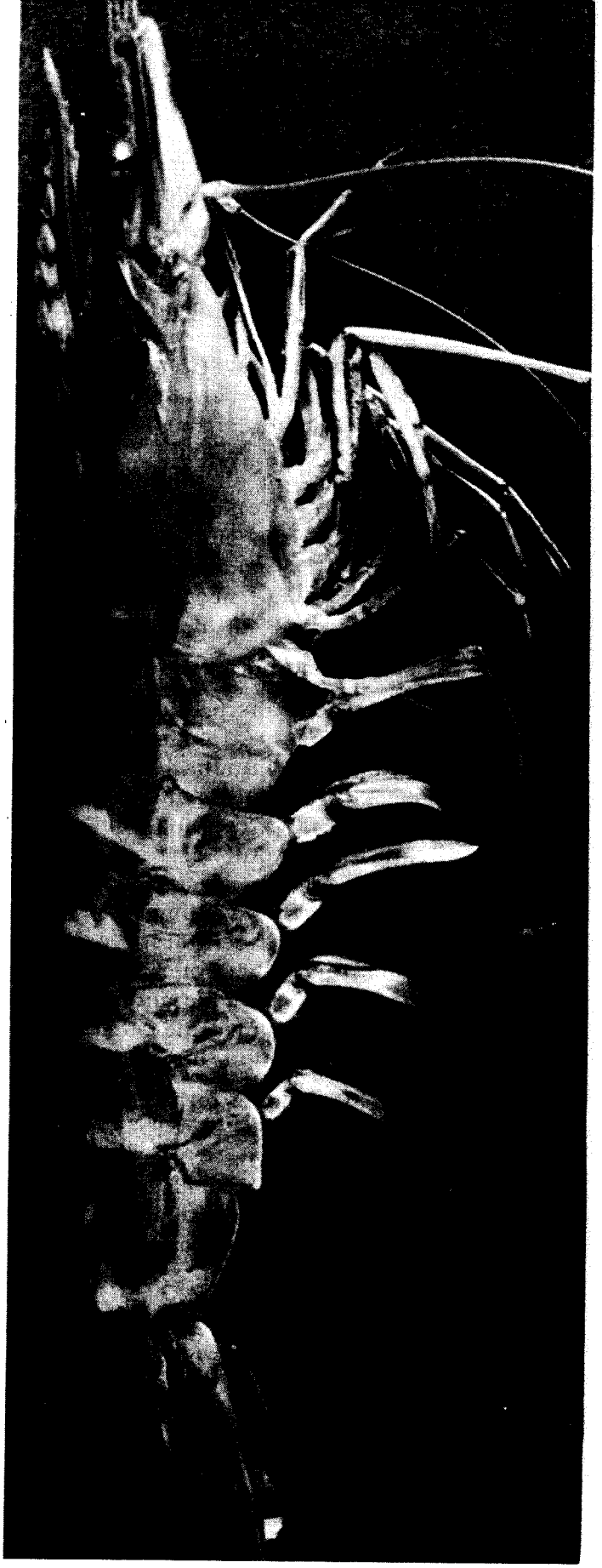
Thirunavukkarasu ,A.R. (1997) Marine fin fish seed production . Paper presented in *"Indo-Egyptian Workshop"*, CMFRI, Kochi, Dec. 8-9, 1997.

First experience of commercial farming of *Penaeus monodon* (Fabricius) along the Okhamandal coast, Gujarat

N. S. Subrahmanyam, G. P. S. Rao and V. J. Somaiya
Tata Chemicals Ltd., Mithapur-361345.

ABSTRACT

First experiences of commercial shrimp farming of Penaeus monodon *(Fabricius) in the Okhamandal coast Saurashtra, Gujarat, India are reported. The effects of the prolonged winter temperature on the survival and growth of the shrimp and the overall, impact on the economic viability of the farming process are discussed.*



Introduction

Coastal shrimp farming, which gained momentum in India in the nineties have immense potential to generate self employment and improve the living conditions of the rural population. It has been reported that Gujarat has over 0. 376 million hectares of marshy land along its coast , potentially suitable for aquaculture (Dixitulu and Papa Rao, 1994), though the brackishwater area is limited (Sarvaiya, 1992) . In continuation of earlier attempts (Gopalakrishnan, *et al.*, 1990. Gopalakrishnan and Thaker, 1995) to explore the possibilities and prospects of shrimp farming in the economically backward and agriculturally unfit coastal land areas of Okhamandal, along the Saurashtra coast of Arabian Sea, attempts to raise commercial shrimp crops were made in the Fisheries Demonstration Farm of M/s. Tata Chemicals Ltd, at Mithapur, during 1997-1998. The results of this study are reported in this paper.

Materials and methods

The Fisheries Demonstration Farm is located at 22 ° 25' N and 68° 0' E facing the Arabian Sea . The farm has a water spread area (including canals and bundhs) of 9.39 hectares and a production area of 5.75 hectares having 9 ponds of 0. 5 hectare and 5 ponds of 0.25 hectare, and a wastewater receiving pond of 0.5 hectare. Each pond has separate inlets and similar outlets, placed in opposite direction. The farm receives the full strength seawater from the Arabian Sea, pumped through a canal and settled and sedimented in a masonry tank before use in the farm. Paddle wheel aerators @ 4 nos. per hectare are provided. The farm is constructed with the main purpose of demonstration and training in economically viable and ecologically sustainable aquaculture practices to the fishermen and others to encourage them to pursue it as a full time occupation, in this area.

Three crops, of different culture periods , were raised during September 1997 to August 1998. The semi- arid type coastal land received a rainfall of 509 mm during the year under study, with maximum rains in July-September. The ambient and pond water temperature data was collected on a fortnightly interval (Fig. 1). The water quality parameters of the source seawater and the pond water were analysed during each crop.

Pond preparation

The ponds were limed with CaO @ 300 kg/ ha. and tilled manually, in the beginning of the first crop and thereafter, before subsequent

crops, the pond bottom was only dried till it cracked and then tilled. The ponds were filled with seawater, filtered through 20 and 40 mesh micron filter cloth bags, and then fertilised with Urea and Superphosphate at

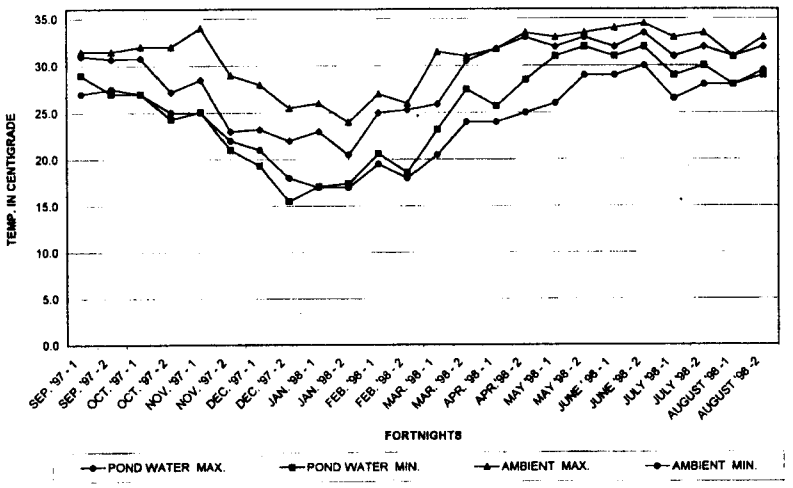


Fig. 1. Ambient vs pond water temperature

required quantities, to develop the algal bloom, prior to stocking.

Seed selection and stocking

Penaeus monodon postlarvae-20 produced in the hatchery of M/s. Tata Chemicals, at Mithapur, were stocked, after testing quality using the following methods:

i. morphological examination to check deformities, external infections etc.,

ii. temperature stress under 15-20 °C,

iii. salinity stress in 15-18 ppt salinity,

iv. chemical stress in 100 ppm formalin and

v. environmental stress in hapa fixed in the pond water.

In each test, 100 nos of seed were used, in 20 ltrs. of medium for 120 minutes , without aeration . The hapa used for the seed test, was 1m x 1 m x 1 m. The active and surviving seed after 48 hrs were removed and a cumulative average survival percentage was worked out to arrive at the quality of the seed stocked. The selected seed was acclimatised before releasing into the ponds.

Seawater management

Due to the high porosity of the soil, weekly water replenishment of the level ranging between 0.03 to 0.07 meter was done. The source water and pond water quality parameters (Table 1) were maintained using Dolomite and Zeolite @ 100kg. / ha as and when required.

Feed management

Feed management was done using the information provided by the feed manufacturer, and the pond conditions and the observations on the requirements of the shrimp based on the pond sampling. The feed had the mean values of components as below (information provided by manufacturer) :

Crude protein : 38-42%, Crude fat : 5-7%; Crude fibre : 3%; Moisture : 11%; Ash : 13%;

The FCR was calculated for each crop using the total feed presented against the total harvested shrimp biomass.

Table 1. Data of source water and pond water quality parameters

S.No.	Parameter	Crop 1	Crop 2	Crop 3	Source
1	Temperature (oc)	15.5 - 28.5	15.5 - 33.5	28.0 - 33.5	21.9 - 28.2
2	Salinity (o/oo)	35.0 - 36.0	35.0 - 36.0	28.0 - 33.6	35.0 - 36.0
3	Dissolved Oxygen (mg / l)	7.5 - 8.5 4.8 - 8.3	4.6 - 8.8	4.6 - 7.8	
4	pH	7.9 - 9.0 8.1 - 9.0	8.1 - 9.0	8.1 - 8.2	
5	Sechi depth (mts.)	0.45 - 0.75	0.35 - 0.65	0.45- 0.75	NA
6	Ammonia Nitrogen (NH3-N) mg/l	ND	ND	0.01	NA
7	Nitrite Nitrogen (N02-N) mg/l	ND	ND	0.02	NA
8	Hydrogen Sulphide (H2S) mg/l	ND	ND	0.0001	NA

ND : not detected
NA : not analysed
Note : in crop 1&2 parameters 6-8 were analysed using e.merck test kits
In crop 3 the above parameters were analysed using
HACH DR 2010 Spectrophotometer

Shrimp growth and health monitoring

After 30 days of culture (DOC) , regular sampling was conducted to monitor the growth characteristics of the shrimp in the pond, to obtain the information on the biomass to regulate the feeding schedule, and to evaluate the quality of the health of the shrimp (Fig. 2).

Prevention of any bacterial / viral diseases in the shrimp stock was done through treatments using iodine compound (10% active iodine) and Benzyl Chloride (BKC 50 % active) @ 4 lt. / ha as and when found necessary, especially during the winter crop. Harvesting of the crop was done after reducing the water level and removing the shrimp partly through the drain sluice gates and partly manual methods of cast netting and hand- picking.

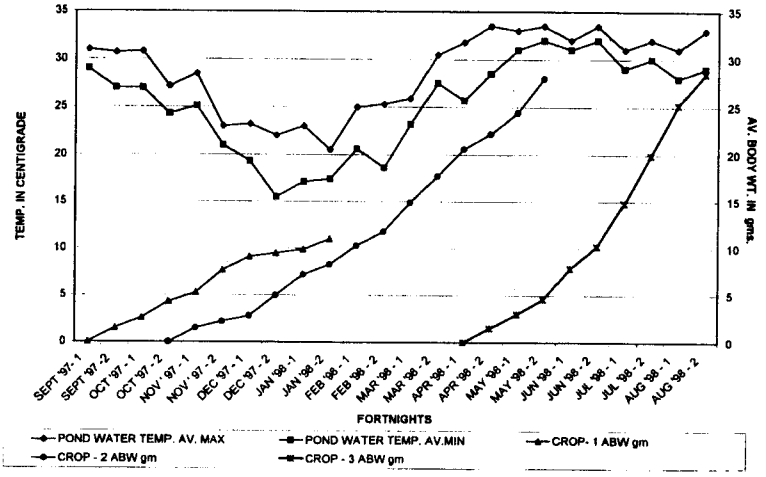


Fig. 2. Water temperture vs shrimp growth trends

Results and discussion

The survival rate of the shrimp ranged from 78 to 83.7 % . It has been established beyond doubt that *Penaeus monodon* can be successfully cultured in the temperature range of 15. 5 to 33. 5 ° C of pond water conditions found during the year (Tables 1& 2). However, in the crop I, which recorded a survival of 78 % in 128 DOC , shrimp registered a growth rate of only 0. 086 g/ day as for the most part of crop, temperature remained in the range of 24.3 ° to 15. 5 °C (86 DOC) when the growth rate decimated to 0. 073 g. / day . The feeding activity of the shrimp was very poor . The feed in the check tray was mostly

unconsumed. The gut was found not full when observed during the sampling. The moulting cycle was disrupted by the lowered metabolism. The crop was in fact terminated at 128 DOC only because of these observations. The harvest size was only 11 g. Similar poor metabolism and rate of growth had been reported in *Penaeus indicus* culture (Gopalakrishnan and Thaker, 1995), recording 0. 105 g/ day under temperature conditions below 25 °C. This proves that minimum ambient temperature for viable shrimp growth shall be above 26 ° C .

Table 2. Details of crop management

Sr.No.	Parameter	Crop 1	Crop 2	Crop 3
1	Water Spread Area [ha]	3.5	1.0	1.0
2	Pond Size [ha]	0.5	0.5	0.5
3	Water Depth [mt]	1.0 +/- 0.2	1.0 +/- 0.2	1.0+/-0.2
4	Crop Period	Sept.'97-Jan.'98	Nov.'97-May'98	May'98-Aug.'98
5	Stocking Density [Nos. / Sq.mt.]	8.0	9.0	9.0
6	Water Exchange [Nos. / Crop]	2	6	2
7	Days of Culture [Doc]	128	212	128
8	Survival [%]	78	79.4	83.7
9	Final Mean Harvest Size [gm.]	11.0	28.0	28.0
10	Shrimp Yield [kg./ha.]	686.0	2000.0	2109.0
11	F C R	3.0	3.60	1.53
12	Feed Used [kg / ha]	2058.0	7200.0	3226.7
13	Water Replenishment Per Week [mt.]	0.05 +/- 0.02	0.05 +/- 0.02	0.05 +/- 0.02
14	Sale price [Rs. / kg]	65.00	235.00	235.00

During the crop II, which had begun during the winter, and ended

in summer, as an extended crop, during the first 86 DOC the temperature was 24. 3 °C to 15. 5 ° C and the growth was similarly 0.083 g /day. It was interesting to note that the shrimp which exhibited all the characteristics of lowered metabolism during this period, showed remarkable improved growth rate of 0. 165 g / day in the next 126 DOC in a temperature range of 23. 0 °C upto 28. 0°C, and achieved final harvest size of 28 grams and very good quality, at the end of total DOC of 212 days.

Compared to these observations of the crop I and II , the shrimp in the crop III, under summer conditions of temperature range of 28 °C to 32 °C , recorded a growth rate of 0. 222 g / day and achieved the size of 28 grams in 128 days. It is therefore seen that the shrimp continue to survive under retarded growth in the temperature less than 23. 0 °C to 15.0 ° C, but with the commencement of favourable temperature a spurt of growth is evident. Similar retardation of growth in low temperature conditions have also been reported by Canese *et al.* (1993)

The data presented above also indicate that a winter crop is totally unviable, whereas the extended winter growth can lead to marginal profits . The economic evaluation of the crops presented in Table 3 shows that the summer crop can generate a gross gain of Rs.2,69,878 / ha. in 128 DOC, in this region. However, when shrimp farming is considered as a source of generating full time occupation and income round the year, it is necessary to consider the farming also during the remaing period of the year. The Table 4 shows a suitable pattern of the cropping, based on the observations presented here, whereby a summer crop of 120 - 140 DOC and an extended winter crop of 160 - 180 DOC , together produce 3700 kg. / ha / annum of the optimum marketable sizes of 22 - 28 g shrimp to generate an overall gross gain of Rs. 3, 04, 350 / ha ensuring round the year employment to the farmer. Critical and careful feed management in the pond to bring down the FCR from 3.6 to 2. 0- 2. 5, during the extended winter crop, and adoption of a build – operate - own method of farming, wherein the maintenance labour expenditure can be avoided, by the members of the family of the farmer severally and jointly sharing the farm operations, will further improve the gross gain from the farming. Besides, an improved marketing strategy, whereby, a cluster of farms can simultaneously harvest and offer the product together or independently at the same time , direct to the exporter (who would need a sufficient container capacity load for the specific type/ count export for a specific price), to realise better remunerative prices also should be considered. Thus commercial

shrimp farming, using *Penaeus monodon*, is economically viable and ecologically sustainable in the Okhamandal coast, and offers potential as a powerful tool to improve the living standards of the coastal fisher-folk and other downtrodden communities, which can lead them to self sufficiency and economic independence.

Table 3. Showing deails of operating expenditure of three differrent crops

Sr.No.	Item details	Crop 1	Crop 2	Crop 3
1	Period	15.09.97-20.01.98	27.10.97-27.05.98	06.04.97-27.08.98
2	Days of culture	128	212	128
3	seed stocking density (nos. / ha)	80 000	90 000	90 000
4	food conversion ratio [f c r]	3.0	3.6	1.53
5	shrimp yield [kg. / ha]	686.0	2000	2110
6	harvesting size [gm.]	11.0	28	28
7	value realised [rs.]	44 590.00	4 70 000.00	4 95 615 . 00
8	operating expenses			
	i. seed	40 000.00	45 000 . 00	45 000
	ii. feed	92 610.00	3 24 000 . 00	1 45 201
	iii. labour	15 000 . 00	25 000 . 00	15 000 . 00
	iv. harvesting charges	2744.00	8 000 . 00	8 436 . 00
	v. others	12 100 . 00	12 100 . 00	12 100 . 00
	total	1 62 454 . 00	4 14 100 . 00	2 25 737 . 00
9	gross gain (4-7) [Rs.]	1 17 864 . 00	55 900 . 00	2 69 878 . 00

Remarks :

1. The supervisory charges not included, 2. The seed produced in the hatchery of tcl used so ex-hatchery cost considered, 3. Harvested shrimp sold to local buyers who supplies to freezing plants, as credit payment by freezing plants was not acceptable, 4. The expenditure on staff for supervision is not indicated

Table 4. Showing the proposed annual cropping pattern for shrimp farming in Okhamandal coast , Gujarat

Sr.No	Item details	Summer crop	Eded winter crop
1	Period	May to September	October to March
2	Days of culture	120 - 140	160 - 180
3	Seed stocking density (nos. / ha)	90000	90000
4	Food conversion ratio [f c r]	1.5	3.0
5	Shrimp yield [Kg. / Ha]	2100.0	1600.0
6	Harvesting size [gm.]	28.0	22.0
7	Value realised [Rs.]	493500.00	296000.00
8	Operating expenses		
	Seed	45000.00	45000.00
	Feed	141750.00	216000.00
	Harvesting Charges	8400.00	5000.00
	Others	12000.00	12000.00
	Total	207150.00	278000.00
9	Gross gain (4-7) [Rs.]	286350.00	18000.00

Acknowledgements

The authors are extremely thankful to the management of M/s. Tata Chemicals Ltd., for the facilities received and for the permission to present this paper in the Symposium . Thanks are due to Sri. P. Gopalakrishnan, Superintendent - Fisheries Department, Tata Chemicals, Mithapur for critically going through the manuscript and offering valuable suggestions for improvement.

References

Camese, S., A. Ponticelli ,M., L. Corbari And L.Polidori 1993. Semi- intensive farming of *Penaeus monodon* (Fabricius) in Italy and first experiences of controlled reproduction in captivity. Production, Environment and Quality. Bordeaux Aquaculture 1992. G.Baranbe and P. Kestemont (Eds). *European*

Aquaculture Society Spl. Publ.,18 , Ghent, Belgium.

Dixitulu, V.H. and G.Papa Rao, 1994. *Handbook on Fisheries. Global Fishing Chimes* Pvt. Visakhapatnam. pp. 46

Gopalakrishnan , P.,V. Krishna Raju and Surendra R. Thaker. 1990. Some Observations on the growth and survival rate of the Jumbo prawns *Penaeus monodon* Fabricius and *Penaeus indicus* H.M.Milne – Edwards in a solar saltworks along the Okhamandal coast of the Gulf of Kutch. *Fish. Technol.* 27, 2: 120-122.and

Surendra R. Thaker .1995. Prospects of farming the Indian white shrimp *Penaeus indicus* H. Milne- Edwards in Gujarat, India. *Fish. Technol.* 32, 2 : 80-84

Sarvaiya , R.T.1992. Prospect for Coastal aquaculture along the Jamnagar coast in Gujarat. *Fishing Chimes*, June. Pp. 41-43.

Rao, G.R.M. 1997. Coastal Aquaculture in India. Workshop on Environmental Impact assessment of Aquaculture Enterprises. Chennai. Dec. 10 Organized by Rajiv Gandhi Centre For Aquaculture, Mayiladuthurai . (under publication).

Multi crop farming system as fish aggregating device to enhance marine production

G. P. Kumaraswamy Achary, Joseph Andrews and K. T. Thomas

Vizhinjam Research Centre of CMFRI, Vizhinjam.

ABSTRACT

The results of the studies during 1995 to 2000 March using different types of cages, spat collectors and other gadgets for farming pearl oysters and mussels at Vizhinjam showed that large aggregations of edible fishes, ornamental fishes, cephalopods and other marine organisms frequently appear in the farming areas in search of substratum, shelter, food and breeding purpose. It is also observed that this system acts as a sanctuary conserving these resources by providing them breeding habitat.

The organisms collected by regular operation of different types of cages are presented in this paper describing their behaviour showing how this multi crop farming system simultaneously acts to improve resources generation and conservation.

Introduction

The Central Marine Fisheries Research Institute has initiated marine farming in India in 1972 by starting mussel farming at Vizhinjam. As the marine resource exploitation from the coastal sector has reached the threshhold level, there is an urgent need to enhance production through improved sea farming and sea ranching activities. India's marine production swings between 2.3 to 2.8 million tonnes during the past few years. Realising this there has been considerable improvement and research prioritization in marine farming activities especially in the fields of mussels, pearl oyster, edible oyster, prawn, crab, lobsters, algae etc.

The research results of the project "Development of low cost technology system for sea farming of pearls and mussels", started in 1995 at Vizhinjam, were highly encouraging (Achary *et. al.*, 1998). The studies also showed that the multi crop farming system developed using new designs of cages in the sea bottom was also acting as fish aggregating device just like the concrete modules and other structures used for developing artificial reefs in India as well as in some of the developed countries. Vik (1982) and Grove and Sonu (1983) have reviewed the Japanese artificial reef technology showing the possibilities of enhancement of marine production through the introduction of modules. It was also observed that the cages and the nylon frills used for farming were giving better results than the cement modules and the former used for multi crop farming system can be raised to the surface periodically for stocking candidate species suitable to the farming area and the operations can be regulated as a "farming cum fishing activity" using series of two-in-one trap cages. James and Lazarus (1996), Lazarus (1996), Parameswaran Pillai (1996), Raja (1996), Rajamani (1996) and Sanjiva Raj (1996) have elaborately given the details of the

artificial reefs operated in some of the localised regions in India and other countries showing the functional merits and demerits of the artificial reef system and the associated fisheries. As these reefs are permanently submerged and is launched in deeper areas it is practically difficult to monitor the biosystem from the surface. The multi crop farming system presented here shows that while the farming operation is done using a main crop like pearl oyster, mussels, lobsters, crabs, edible fishes, etc. ornamental fishes, crinoids, echinoids, bryozoans, sponges, gorgonids etc. also can be raised according to the depth and local conditions of the farming area for extracting medicines. In addition the system attract the larvae and adults of the above organisms just like the fish aggregating device (FAD) and function as a sanctuary. In short the multi crop farming system functions as a "mobile artificial reef" for farming different desirable animals and can be lifted to the surface for stocking, farming and harvest as well as simultaneously manipulating the ecosystem for induced regulation of the faunal and floral complexity.

The farming system for developing fish aggregating device

The farming system is developed as a multicrop sea bottom activity by introducing *high density stocking cages* fabricated with additional lateral pedestals to avoid the cages touching the silty bottom. A 64 cm^3 cage with four shelves could accommodate 4000 mussels or pearl oysters with normal growth. Cages with radial pedestals which are known as *satellite cages* are also designed to protect the cages and the stocked animals in any adverse circumstances. Nylon frills of 50 cm length are suspended from the lateral pedestals for attracting mussel and pearl oyster spats. As these cages are launched in the sea with the stocked animals, large aggregations of fishes and other animals are noticed around the cages. For catching the fishes, the high density cages are converted into two-in-one cages with trap mouth for fishing cum-farming of oysters using the top and bottom shelves (Achary *et. al.*, 1998). A series of all these cages could act as 'Modules' of the artificial reefs forming a new biosystem in the sea with high rate of biomass production (Fig.1).

Raft system with cages, breeding hapas and spat collector frills maintained in the Vizhinjam bay is also found to act as fish attracting

Fig. 1 (Above) : High density stocking cages with individual lid for each shelf.
Fig. 2 (Below) : Fishes caught using two-in-one trap cages (Collection from a single cage)

device while operated as a multicrop system. However since the protected bays are very much limited, along the Indian coast, sea bottom farming by using these cages are more desirable to compensate the rough sea conditions.

Fresh settlement of different sedentary and free living animals like pearl oysters, mussels, ascidians, barnacles, bryozoans, sponges, holothurians, crinoids, echinoids etc. were noticed within three months after launching the cages. Penaeid and caridian prawns, crabs, lobsters, edible fishes and ornamental fishes, cephalopods etc. were the other groups attracted by this newly developed biosystem. It is also observed during the period of study that by introducing few numbers of a particular group like pearl oyster or mussel it will be possible to raise a sizable population of these animals within two to three years and an associate faunistic complex as shown above can also be built up during this period to have a multicrop self sustaining system, which will later function as a fish aggregating device (Fig. 2).

Fishes and other marine organisms collected from multicrop farming system

During the period of studies 93 species of fishes belonging to 46 genera and 25 families were collected using the fishing-cum-farming two-in-one trap cages. In addition lobsters, crabs, cephalopods and holothurians were also regularly collected.

Fishes of the family Serranidae, Lethrinidae, Lutianidae, Mullidae, Siganidae, Pomacentridae and Acanthuridae were the common food fishes entrapped. A single trap cage could collect 0.5 to 2 kg of fishes based on the frequency of operation and the availability of fishes. Most of the fishes caught using the trap cages were belonging to the ornamental fishes which are of high export value.

Panulirus homarus and *Panulirus polyphagus* were commonly collected from the farm area. Lobsters of 100 to 180 mm were frequently trapped during November to June which could be used for subsequent farming and fattening. *Scylla oceanica* and *Scylla serrata* measuring 145 to 540 mm were collected from the interspace of cages during July and November, immediately after rains.

Loligo duvaucelli, Sepioteuthis lessoniana, Sepia pharaonis and *Sepiella inermis* use to congregate in the multicrop farming area for depositing their egg masses on the spat collectors and on the cages during October to January period. Fishermen catch them using hooks and lines, at a catch rate of 2 to 5 kg.

Holothurians of the species *Thelenota ananas* weighing 1.5 to 2 kg were occasionally caught from the trap cages.

Settlement of *Ulva* spp, *Caulerpa* spp, *Gracilaria* spp and other green and red algae in the shallow water multicrop farming system indicates the possibilities of farming these algae also along with other animals.

List of edible and ornamental fishes caught

1. Family: **Acanthuridae**

Acanthurus lineatus (Linnaeus), *A. triostegus* (Linnaeus), *A. nigricans* (Linnaeus), *A. edlongatus* (Lacepede).

2. Family: **Apogonidae**

Rhabdamia cypselurus (Weber), *Pristiapogon synderi* (Jordan and Evermann), *Ostrorhynchus nubilus* (Garman), *O.endekataenia* (Bleeker), *O. quadrifasciatus* (Cuvier), *Apogon sangiensis* (Bleeker).

3. Family: **Blennidae**

Istiblennius sp

4. Family: **Callyodontidae**

Callyodon sordidus (Forskal), *C. bataviensis* (Bleeker), *C.sexvittatus* (Ruppell), *C. scaber* (Valenciennes), *C. jordani* (Jenkins).

5. Family: **Canthigasteridae**

Canthigaster amboinensis (Bleeker), *C. cinctus* (Richardson), *C. margaritatus* (Ruppell).

6. Family: **Chaetodontidae**

Chaetodon aurige Forskal, *C. collare* Bloch, *C. melannotus* (Bloch and Schneider), *C. bennetti* Cuvier, *C.vagabundus* Linnaeus, *Heniochus acuminatus* (Linnaeus), *H. monoceros* Cuvier, *Megaprotodon strigangulus* (Gmelin)

7. Family: **Diodontidae**

Diodon hystrix (Linnaeus), *Lophodiodo calori* (Bianconi)

8. Family: **Gaterinidae**

Gaterin nigrus (Cuvier)

9. Family: **Hemirhamphidae**

Hemirhampus georgii (Valanciennes)

10. Family: **Holocentridae**

Holocentrus sammara (Forskal), *H. spinifer* (Forskal), *H. diadema* Lacepede, *Myripristis murdjan* (Forskal).

11. Family: **Labridae**

Labroides dimidiatus (Valenciennes), *Anampses caeruliopunctatus* Ruppell, *Thalassoma janseni* (Bleeker), *Iniisteus pavo* (Valenciennes).

12. Family: **Lethrinidae**

Lethrinus harak (Forskal), *L. mahsena* (Forskal).

13. Family: **Lutianidae**

Lutjanus kasmira (Forskal), *L. russelli* (Bleeker)

14. Family: **Monacanthidae**

Paramonacanthus choirocephalus (Bleeker)

15. Family: **Mullidae**

Upeneus tragula Richardson, *U. vittatus* (Forskal), *Parupenius indicus* (Shaw), *P. barberinus* (Lacepede)

16. Family: **Muraenidae**

Echidna delicatula (Kaup), *E. zebra* (Shaw), *E. nebulosa* (Ahl), *E. polyzona* (Richardson), *Gymnothorax pictus* (Ahl), *G. pseudothyrsoidea* (Bleeker), *G. ruppelli* (Meclelland).

17. Family: **Ostraciontidae**

Ostracion tuberculatus Linnaeus, *O. melagris* Shaw, *Rhynchostracion nasus* (Bloch), *Lactoria cornuta* Linnaeus.

18. Family: **Pomacentridae**

Abudefduf bengalensis (Bloch), *Pomacentrus opercularis* (Gunther), *P. pavo* (Bloch), *P. melanopterus* Bleeker , *P. nigricans* (Lacepede), *Dascyllus trimaculatus* (Ruppell), *Amphiprion crysogaster* Cuvier, *Chromis caerulea*(Cuvier), *C.chrysura* (Bliss), *C.nigrurus* Smith.

19. Family: **Priacanthidae**

Priacanthus cruentatus (Lacepede)

20. Family: **Scorpaenidae**

Pterois volitans (Linnaeus), *P. antennata* Bloch, *P. radiata* Cuvier, *Scorpaenodes guamensis* (Quboy and Gaimard), *S. parvipinnis* (Garret).

21. Family: **Serranidae**

Epinephelus tauvina (Forskal), *E. hexagonatus* (Block and Sceneider), *E.merra* Block, *E. fasciatus* (Forskal) , *E. flavocaeruleus* Lacepede, *E. melanostigma* Schultz, *Cephalopolis boenack* (Bloch), *C. miniata* (Forskal), *C. pachycentron* (Valeneiennes), *Grammistes sexlineatus* (Thunberg).

22. Family: **Siganidae**

Siganus stellatus Forskal , *S. canaliculatus* (Park)

23. Family: **Sillaginidae**

Sillago sp

24. Family: **Tetrodontidae**

Tetrodon hispidus Linnaeus, *T. meleagris* Lacepede, *T. stellatus* Bloch and Schneider, *T. nigropunctatus* Bloch and Schneider

25. Family: **Zanclidae**

Zanclus cornutus

Farming of side crop and sea ranching in natural system

Many animals collected from the farm area can be cultivated further as side crops for fettening, brood stock development, sea ranching etc. in the natural system and for extraction of medicines from some of the rare organisms.

Edible and ornamental fishes

The juvenile fishes which get entrapped in cages can be stocked in

separate cages for further fattening and brood stock development. Some of the fishes belonging to the family Serranidae, Chaetodontidae and Pomacentridae are of high commercial value as brood stock and as export commodity. A regular system can be maintained in the farm area for protecting and further fattening of these fishes.

Lobsters and crabs

The experimental results at Vizhinjam indicated that by keeping separate lid for each shelf of the cage, berried lobsters and crabs can be farmed for releasing their larvae into the natural system. This will also help to grow them into commercial size while replenishing the area with the larvae released by them. As there is high competition for the export of lobsters and crabs in the live condition there is decrease in the spawning population. A large scale attempt on the fattening of the under sized lobsters and crabs to the first maturity condition in the natural system using the cages will compensate this situation.

Ascidians, bryozoans, sponges, gorgonids and other marine organisms

The system also attracts and colonizes a wide variety of organisms like sponges, anthozoans, tunicates, gorgonids, annelids, bivalves, brachiopods, echinoderms and algae. Many of them have biochemical compounds with anti viral, anti tumor and anti cancer properties. Thomas and Rani (1987) have discribed the biochemical diversity in sponges and gorgonids listing a series of compounds of medicinal value from these animals.

Marine algae

Just like stocking various animals in the high density stocking cages, different species of algae like *Ulva* spp, *Caulerpa* spp, *Gelidiella acerosa*, *Gracilaria* spp and *Hypnea muciformis* can be stocked in different shelves of the cages placed in shallow tidal areas and the out growths through the large meshes of the netting can be harvested periodically. The antibacterial and antifungal activities of marine algae have been

assessed by and projected the scope for making medicines from these algae by Padmakumar and Ayyakkannu (1997).

Discussion and conclusion

The results of the study presented here show that the multi-crop farming system using the cages function as an efficient fish aggregating device. It is also of interest that in addition to the reef dwelling fishes, other pelagic fishes also congregate in the farm area as reported by James and Lazarus (1996). As the high density stocking cages and the two-in-one cages along with the spat collectors (Nylon frills) give sufficient protection to fishes and other organisms, the biomass production in unit area is also increased considerably compared with other habitats. One of the disadvantage of the 'modules" used for the construction of *conventional artificial reefs* in India and other developed countries is that the modules are permanently submerged in the sea and are usually in greater depths and consequently the monitoring of the system and harvesting becomes difficult after the establishment of the reef. The cages and other gadgets used for the multicrop farming system is also functioning just like the cement or other types of modules used for developing the conventional reefs and these cages (modules) can be lifted to the surface as and when required just like a "mobile artificial reef" and any desired species can be stocked and farmed at any depth of the sea and can be periodically harvested. Another important advantage is that this type of farming encourages *sea ranching in the natural system* as described elsewhere in this paper and can function as sanctuary for the conservation of desired species. Under the light of this study it is recommended that the multi-crop sea bottom farming system can be taken up at an International collaboration of the maritime countries so that a joint venture of the participating countries will help to increase the biomass of the marine eco system on a global basis and will thereby enhance the marine resource potential. However on a national basis, all the maritime states of India can be co-ordinated for a joint programme for establishing multi-

crop farming system along the Indian coast and this will help to increase the marine production and also will serve as fish aggregating devise to conserve the marine resource.

Acknowledgments

The authors are deeply indebted to Dr. V. N. Pillai, Former Director, Dr. K. K. Appukuttan, Head of Molluscan Fisheries Division, CMFRI, Cochin and to Mr. K. Prabhakaran Nair, Officer-in-charge, VRC of C. M. F. R. I. Vizhinjam for all the helps in this study. The senior author is very much grateful to Dr. S. Z. Qasim, Dr. R. Velappan Nair, Dr. E. G. Silas, Dr. P.S.B.R. James and Dr. M. Devaraj, the former Directors of the Institute during whose period he could initiate the mariculture programmes in India at Vizhinjam and could further develop into a multi-crop farming system with all the supports received from them.

References

Achary, G.P.K. Joseph Andrews and K.T. Thomas, 1998. Shallow Sea bottom farming: Multicrop System developed at Vizhinjam *Mar. Fish Infor. Serv. T&E Ser* No.156: 16-21.

Grove, R. S. and C. J. Sonu, 1983. Review of Japanese fishing reef technology *South California Edition Comp*, 83-RD - 137: 116 pp.

James P.S.B.R. and S. Lazarus, 1996. Artificial reef for artisanal fisheries enhancement - an attempt off Trivandrum coast *Bull. Cent. Mar. Fish Res. Inst.* **48** - pp. 18-23.

Lazarus, S. 1996. Artificial fish habitat in traditional fisheries of south west coast of India. *Bull. Cent. Mar. Fish. Res. Inst.* **48**: 37-41.

Padmakumar, K and K. Ayyakkannu, 1997. Seasonal variation of antibacterial and antifungal activities of the extracts of marine algae from southern costs of India. *Botanica marina* **40**: 501 - 515.

Parameswaran Pillai P. 1996. Artificial reef research in Minicoy. *Bull. Cent. Mar. Fish. Res.* **48**:11 & 12

Philipose K. K. 1996. Artificial reef habitat enhancement and increasing fisheries potential Bull. *Cent. Mar. Fish. Res. Inst.* **48**: 4-7.

Raja G. 1996. Fish aggregating device and artificial reefs. *Bull. Cent. Mar. Fish. Res Inst* **48**: 8-10

Rajamani, M. 1996. Artificial reef and its role in marine fisheries development *ibid* 13-17.

Sanjeevaraj, P. J. 1996. Artificial reefs for a sustainable costal ecosystem, in India involving fisher folk participation. *Bull. Cent. Mar. Fish. Res. Inst.* **48**: 1-3.

Thomas, P.A. 1996. An Appraisal of the biological and biochemical diversity in sponges. *Mar. Fish. Infor. Serv. T&E* Ser.No. **142**. 1-5.

Thomas P. A. and Rany Mary George, 1987. Gorgonid resources of India *Mar. Fish. Infor. Serv. T&E Ser.*, No. **74** p.1-13.

Vik, S. F. 1982. Japanese artificial reef technology *Aquabio Inc.*, Florida 380-pp.

Studies on the remote setting of two commercially important Indian bivalves

U. Unnikrishnan, V. Kripa, K.S. Mohamed and T. S. Velayudhan
Central Marine Fisheries Research Institute, Kochi-682014

ABSTRACT

The concept of remote setting which involves transporting bivalve larvae, under moist condition and at 5-10°C lower than the atmospheric temperture, to distant areas for settlement was tried for the first time on two species of Indian bivalves. About 68% of the 'pediveliger' larvae of Indian backwater oyster, Crassostrea madrasensis *which were transported at 27±1 °C for 18 hours metamorphosed to settle as spat. The post-set survival rates were 66.2% to 73.4% in 30 ppt and 71.3% to 87.5% in 15 ppt salinity. Higher settlement rate and post-set survival was observed in larvae transported under low temperature than in atmospheric temperature. Transportation of late 'umbo' larvae of Indian pearl oyster* Pinctada fucata *gave high survival rate, but failed to metamorphose as spat.*

Introduction

During the initial stages of commercialisation of edible oyster culture, farmers were dependent exclusively on wild seed. With the expansion of oyster culture the seed requirement could not be met fully from the wild seed stock, which had seasonal and regional limitation. To cop up with demand, technology had developed for hatchery production seeds as early as 1890s. This has also paved the way for the expansion of oyster culture into new cultivable areas where no natural stocks were available. As the setting up of hatcheries and transportation of broodstock to these new areas were not feasible, attempts were made to transport their larvae and juvenile. Initially the set larvae (spat) on cultch were transported from hatchery to culture site. But this procedure which necessitated a large consignment space was uneconomical. Similarly the maintenance of larvae till the spat stage in the hatchery lead to the increase in seed price. Another alternative was to transport the larvae at swimming stages - from "D" to "pediveliger" - in seawater. However this resulted in larval mortalities due to deteioration of water quality. It was at this stage remote setting of spat was tried. Remote setting is the method by which eyed or pediveliger larvae are transported without water, in moist condition to distant places where they are made to set on the cultch material. By this method culturists can store and transport millions of larvae, which require a storage space of a matchbox.

Remote setting, which was first tested in the 1960s got recognition for its importance only in the mid 1970s. The concept of remote setting was first described by Jones and Jones in early '80s and Chew (1984) in his review on recent advances in the cultivation of molluscs, described remote setting as an "exciting technique" successfully employed by several oystermen in the U.S. Significant results were obtained in larval transport and distant setting of Pacific oyster *Crassostrea gigas* (Henderson, 1982), *Crassostrea virginica* (Gibbons, 1988) hard clam, *Mercenaria mercenaria* and the bay scallop, *Argopecten irradians* (Rhodes and Manzi, 1988). Pilot scale experiments with industrial collaboration were also done at the planting site to test the feasibility of supplementing wild seed with hatchery reared remote set spats (Castagna *et al.*, 1989).

To ensure good growth and survival of remote set spats, studies were focused on defining criteria for sighting natural nursery area (Roland *et al.*, 1989). Different aspects of remote setting, like, density (Baud *et al.*, 1991), cultch material (Bitaud and Herve 1991), cost of remote set spat (Holliday *et al.*, 1991, Thomas *et al.*, 1991; Thomas and Burnell, 1992) and economic assessment of setting on natural and artificial cultch (Langan *et al.*, 1997) were studied. In recent years demonstration programmes were scaled up to promote the technology of remote setting among oyster farmers (Meritt, 1998). In India commercial farming of the dominant species *Crassostrea madrasensis* using wild spat started from 1996 in the estuarine systems mainly along the southwest coast. However there are no commercial bivalve hatcheries, and the seed requirement for demonstration purposes and pearl culture are met from the shellfish hatchery of CMFRI. In edible oyster culture, the farmers collect spat by setting rens during the spat fall. The present work is an attempt to study the feasibilty of remote setting in two commercially important bivalves of India, the edible oyster *Crassostrea madrasensis* and the pearl oyster *Pinctada fucata.*

Materials and methods

The pediveliger larvae of *Crassostrea madrasensis* (Preston) and the umbo larvae of *Pinctada fucata* (Gould) produced in the shellfish hatchery at Tuticorin Research Centre of C.M.F.R.I were transported by road to Kochi, about 315 km away from Tuticorin. Transportation was done during the late hours to reduce stress and mortality due to the summer heat. The larvae were filtered through an 80 μ sieve, transferred into a 10 *l* beaker and the number estimated. The seawater with larvae was gently stirred to ensure homogenous distribution and then divided into 10 equal parts of 1 *l* each. For the experiment on remote setting, 1 *l* of larval suspension was filtered through the filter cone made of 20 × 20 cm size fine polyester cloth. After folding once, it was kept over a piece of cotton wetted with seawater. This was placed within a wide mouthed transparent plastic bottle of 250 *ml* capacity which was kept open throughout the transportation period. Eight such moist larval packages were made. Of the remaining larvae in 2 *l* of seawater, 1 *l* each were

transferred into the oxygenated seawater in the double layered polythene bags which were placed in transparent buckets and covered. These packets with water kept under atmospheric temperature were treated as "Control" for the experiment .

Out of the eight moist packets for remote setting, four were transported under cool condition within an ice box at 27±1 °C. To maintain uniform temperature and avoid direct contact with the ice pieces, the chamber within the ice box was partitioned into a lower ice chamber and an upper storage/larval chamber by 2 cm thick perforated thermocol. The larval packets were kept above the thermocol. The remaining four moist larval packets for remote setting were kept within a bucket and transported under atmospheric temperature. At Kochi, the edible oyster larvae, transported under moist condition at atmospheric temperature (A) and at low temperature (B) were stocked in 15 and 30 ppt salinities (A-15 ppt, A-30 ppt, B-15 ppt and B-30 ppt) in 15 *l* polythene lined troughs while pearl oyster larvae were maintainted at 25 and 30 ppt (A-25 ppt, A-30 ppt, B-25 ppt and B-30 ppt), with a stocking rate of 4 larvae per *ml*. Water exchange was done daily till the settlement was completed. Controls of both the experiments were maintained at 30 ppt salinity. All the experiments were duplicated.

Standard larval rearing and nursery techniques for *Crassostrea madrasensis* (Nayar *et al.*, 1983; Utting and Spencer, 1991) and *Pinctada fucata* (Alagarswami *et al.*, 1983) were followed during the course of experiment. Up to their settlement the larvae were fed with pure cultures of *Isochrysis galbana* at a rate of 12000 cells per larva per day. During the later stages they were fed with a mixture of *Isochrysis* spp., *Nanochloropsis* spp. and *Chaetoceros* spp. in the equal proportions, at the same feeding rate as mentioned above. The same procedure was followed for Pearl oyster larvae till the closure of experiment.

Experiment 1. Activity of larvae a after transportation

The activity of larvae was observed at hourly intervals during the first 10 hours after transportation. During the subsequent days the activity was monitored once in the morning prior to water exchange and

feeding till the completion of settlement. The activity of larvae was categorised into four, active, partially active, dead or empty and spat, based on the following characteristics:-

1. Active larvae: Actively swimming or crawling with well developed velum (eyed stage) or foot (pediveliger stage).
2. Partially active: Though the valves were closed the movements of respiratory apparatus could be seen through the transparent shell valves.Valves were rarely opened and therefore their movements, either by swimming or by crawling were much less.
3. Dead or empty shells: During the initial hours after transportation the dead shells were identified as those with no gill or body movements. Later, while observing the daily activity, most of the dead shells were found to be empty.
4. Spat: These were more flattened in appearance with prominent, irregular growth processes and valve margins. They were sedentary, but were active in feeding. Though the shell valves were more or less translucent, gill and other body movements were visible.

For the activity studies, 4 sub-samples of 1 *ml* each were taken from different points, pooled into an embryo cup and observed through microscope for counting. The sampling was done thrice to minimize the error.

Experiment 2. Settlement rate of larvae transported

Number of edible oyster spat settled per cultch per day was noted to find out settlement rate and also to find out the time required to complete the settlement. 1.2 mm thick P.V.C. pieces of 10 cm length and 6 cm breadth were used as cultch. A hole was drilled at the centre of each cultch to facilitate ren making. The cultch were washed repeatedly in fresh water to remove any noxious substances present on it. They were then aged by keeping them immersed in filtered seawater for at least two to three days. Two pieces of cultch were kept in each trough, which were replaced daily till the completion of settlement. Number of

spat attached in each cultch, both inside and outside, were noted to determine the settlement rate. The cultch with spat were then hung on a nylon rope to form a ren which was tagged for identification. The rens were hung into 75 *l* F.R.P. tanks and reared in seawater of required salinity. Water exchange and feeding were done as per the standard procedure (Nayar *et al.*, 1983).

Experiment 3. Growth and survival rate

Once the settlement was over, the total number of spat in each trough were recorded seperately. The initial growth and survival rate were calculated from this data. There after, every 5th day growth and survival were noted.

Results

Remote setting of edible oyster larvae

The pediveliger larvae of edible oyster which were transported in the moist condition were healthy and showed velar movements when stocked in seawater. Details regarding the percentage of active larvae transported under low and atmospheric temperature is given in Table-1. The percentage of active larvae were low, ranging from 24 to 36 % during the 1st hour after stocking in seawater, while 62 % of the larvae in the control showed active velar movements.

Table 1. Percentage of active larvae of edible oyster in 15-30 ppt salinities during the first 10 hrs after transportation.

Hours	1	2	3	4	7	8	9	10
Control	62	41	42	43	35	28	27	28
A-15	32	14	15	18	62	45	45	48
A-30	32	17	18	20	43	43	45	35
B-15	24	33	43	47	60	66	65	77
B-30	36	44	47	52	66	68	66	67

After the first hour, the percentage of active larvae decreased and reached the lowest value of 28 % in the control. A reverse trend was observed in the larvae transported without water. Their activity gradually increased during the later hours, reaching 35 to 77 %, at the end of the 10th hour. This was higher than the percentage of active larvae in the control. Among the larvae transported under moist condition, those kept in low temperature showed higher activity (67 to 77 %) than the larvae transported under atmospheric temperature (35 to 48 %).

The larvae which were 19 days old at the time of transportation showed settlement on the next day of transportation, ie, on the 20th day. It was seen that the settlement rate in all treatments were very high during the 1st day after transportation. The settlement pattern during the first 10 days is graphically represented in Fig. 1. A-30 had the highest settlement rate, which was followed by B-15, control, B-30 and A-15 respectively. During the subsequent days the settlement rate in all treatments showed a decreasing trend except in A-15 in which high settlement was observed on 7th day.

Variation in percentage survival was shown by the edible oyster larvae after the remote setting. On the first day after transportation 100 % survival was observed in all treatments. In both the treatments A and B high survival rate was found in 30 ppt salinity. Among the larvae transported under moist condition, B-30 showed maximum survival (68

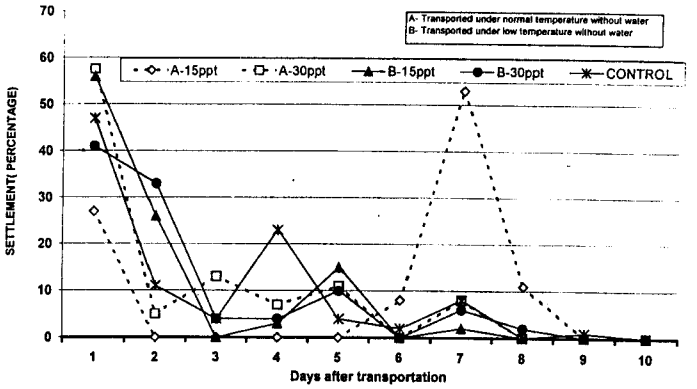


Fig.1. Settlement pattern of *C.madrasensis* larvae

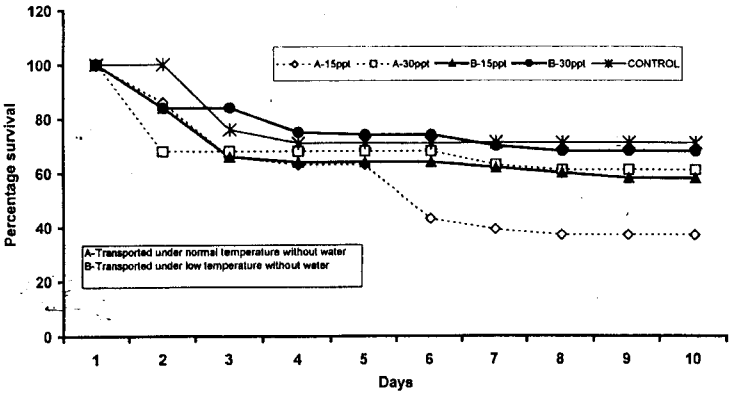


Fig.2. Percentage survival of edible oyster during the first 10 days after transportation

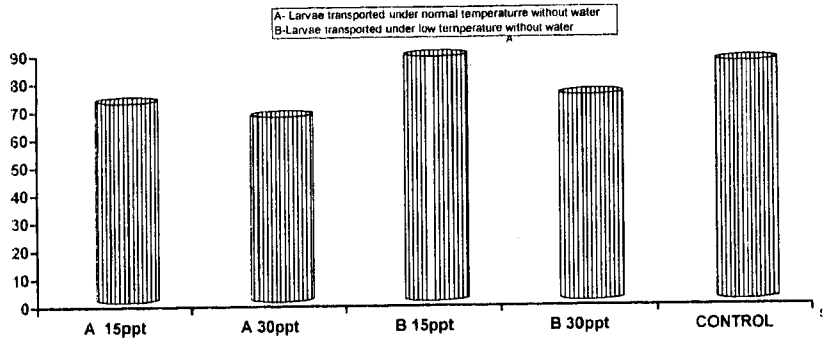


Fig.3. Percentage survival of remote set spat of *C. madrasensis* on the 25th day of rearing

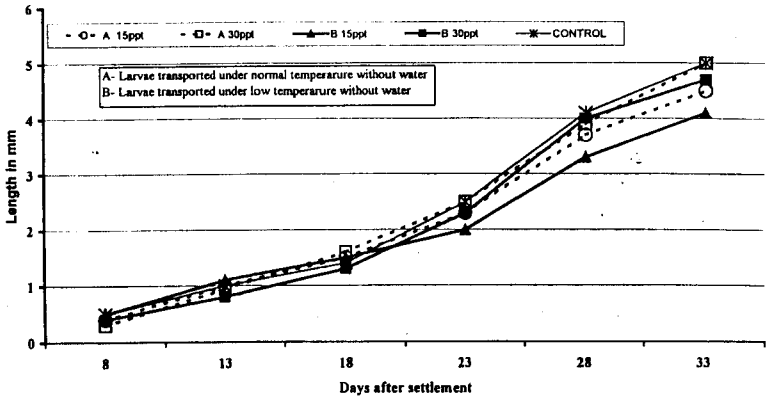


Fig. 4. Growth pattern of remote set spat of *C.madrasensis*

%) but was slightlty lower than that of control (70 %) on 10th day. Details regarding the survival pattern is depicted in Fig. 2. The treatments at 15 ppt showed lower survival rate. Lowest survival (37 %) was observed in larvae transported at atmospheric temperature and reared in 15 ppt.

The spat which had settled were reared in the respective salinities. On 25th day after settlement high survival rate ranging from 66.36 % to 87.59 % were observed in different treatments. The highest survival, 87.59 % was found in B-15, which was higher than the survival in "Control" (85.27 %). The lowest survival, 66.23 % was noted in A-30 (Fig.3).

From the growth rate of the first 33 days after settlement, it is evident that the spat maintained in A-30 treatment showed a growth rate almost equal to that of "Control". Slightly lesser growth rate was shown by B-30 and A-15 while B-15 was the lowest. (Fig. 4).

Remote setting of pearl oyster larvae

The pearl oyster larvae transported under moist condition, under the atmospheric temperature (A) and low temperature (B) were stocked in salinities 25 and 30 ppt after about 18 hours of transportation. The larvae showed more or less similar activity during the first few hours. The percentage of active larvae in different treatments is given in Fig. 5. During the later hours it is seen that greater activity was shown by the larvae transported at cold temperature. The peak activity in both the cases were found at 7th hour. Further, the activity was found to be lowered between 40 to 60 % in the 9th hour. The "Control" treatment showed more or less 100 % activity throughout the period of observation.

Hundred percentage survival was seen during the first 4 days after transportation. The survival of the pearl oyster larvae in different salinities is given in Fig. 6. On 6th day there was a steep decrease in survival in all treatments. Complete mortality was observed in A-25, B-25 and B-30 on 10th day, while 77 % and 40 % survival were observed in "Control" and A-30 respectively.

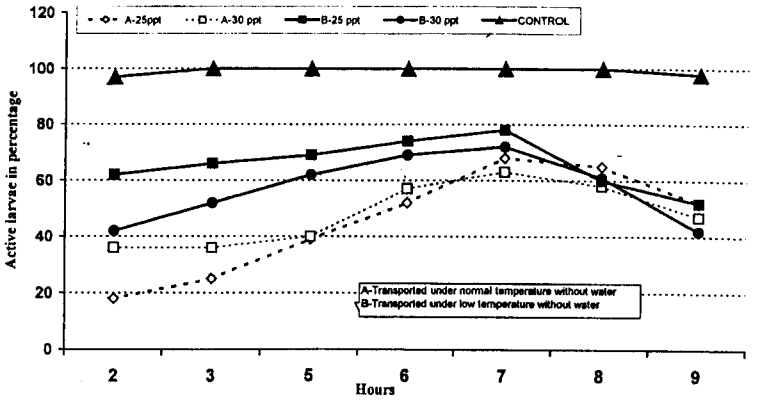


Fig.5. Percentage of active larvae of pearl oyster after transportation

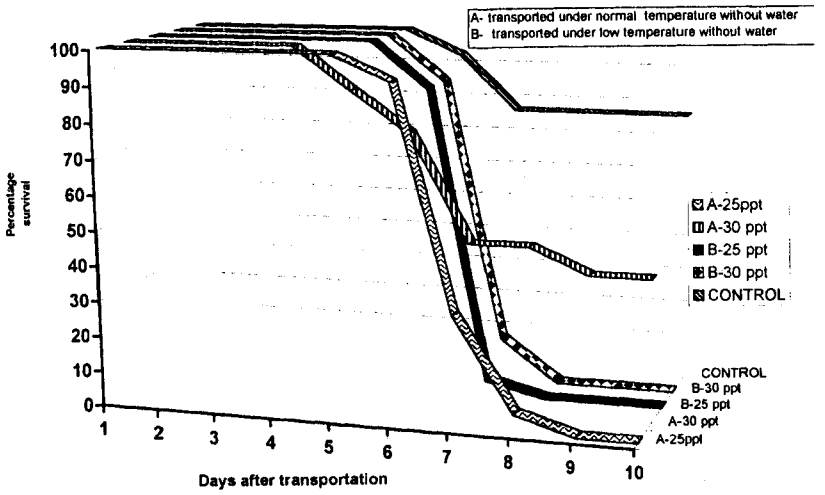


Fig. 6. Percentage survival of *P.fucata* larvae after transportation

Discussion

Remote setting, a recently developed technique to get hatchery-produced setting size pediveliger larvae shipped to near or distant locations for final settlement as seed on cultch materials has been tried successfully in many species of oyster (Chew, 1991). In the present study the concept of remote setting which was tested in the Indian edible oyster *C. madrasensis* gave encouraging results. The larvae transported under moist condition at two storage temperatures of 32±1 °C and 27±1 °C for

18 hours had showed 100 % survival after 24 hours.The settlement rate was 61 to 68 %, which was comparable to the settlement rates observed by Holliday *et al.* (1991) for *C. gigas*. Higher duration of storage up to 6 days in moist condition at 5 °C in a moist wrapping did not produce any depreciation in health, setting or juvenile growth abilities for *C. gigas* (Henderson, 1982).

Commercial settlement rates of 20-30 % were reported for larvae of Pacific oyster during the '80s (Henderson, 1982; Roland *et al*, 1989). For the same species Holliday *et al.* (1991) have reported 68 % set rate after a storage period of 98 hours at 6 °C. The high settlement rate was attributed to the fact that the larvae were fed live algae during the settlement. Low commercial settlement rates were noted when the larvae were unfed or fed on stored algal paste (Roland *et al.*, 1989).

Panggabean *et al.* (1989) investigated the feasibility of straight-hinge larval storage under a variety of test conditions and their subsequent performance. They found that 48 hours storage is feasible and larvae stored at 5 °C exhibited greater survival than those held at room temperature. In the present study also slightly higher settlement rate was observed in larvae transported under low temperatue than those held under room temperature. Holliday *et al.*(1991) observed that percentage of Sydney rock oyster, *Saccostrea commercialis* which set following a cold storage at 11 °C for up to 98 hours was excellent, with 77-85 % survival. These settlement rates are higher than those observed for *C. gigas* and *C. madrasensis*.

The post-set survival rates were high in 15 and 30 ppt salinities indicating that the larvae can be made to set and grow even in low salinities. The survival rates in 15 ppt salinity were as high as 87.50 % which hints the potential for remote setting in the estuarine regions of India. The growth of remote set spat is also comparable to that of larvae transported in water even after 33 days of culture period. This clearly indicates that remote setting does not harm the health and survival of *C. madrasensis* larvae and spat. Roland *et al.*(1989) observed that the proportion of larval setting was affected by circulation rate, temperature, salinity, cultch type and feeding rate. In the present study high settlement was observed when the circulation of water was increased by aeration.

The pearl oyster larvae transported under moist condition showed 100 % survival for 4 days upon exposure to proper rearing conditions.

However their settlement rate was negligible. The pearl oyster larvae were in the "umbo" stage and had not developed into "pediveliger" stage which may be one of the reason for low settlement rate. Survival rate after transportation has been found to depend on age or size of larvae in *C. gigas*.

Holliday *et al.* (1991) studied the use of shell length and eye spot diameter as criteria to decide when the oyster larvae will be ready for the remote settlement. They observed that faster growing Pacific oyster larvae had a significantly larger shell length. They have also indicated the appropriate measurements of shell length of Pacific oyster larvae and Sydney rock oyster larvae which will give high settlement rate. In the present study study the pearl oyster larvae had small shell length. Better results would have been achieved if larger pediveliger were used.

A direct correlation was observed between shipping duration and mortality of seed of hard clam, *Mercenaria mercenaria* and between the seed size and mortality of the seed of bay scallop, *Argopecten irradians* (Rhodes and Manzi, 1988). Greater mortalities occurred in the smaller sized bivalves shipped over the longest period of time. In the present study the duration was limited to 18 hours and this gave good survival rates. However in small size larvae of pearl oyster low settlement rates were noted.

The encouraging settlement rates and survival obtained for *Crassostrea madrasensis* larvae and spat indicate that the remote setting technique can be of considerable potential for this species. The oyster larvae ready to set can be transported to farm sites in distant areas from the hatchery location by means of the simple technique of remote setting and with relatively inexpensive and commercially available materials. The concept of "remote setting" which has revolutionised the oyster industry in temperate countries, can help in the development of oyster farming in India. Remote setting can be recognised as the key to solve the chronic need for the reliable and economical source of oyster seed. With remote setting oyster growers can purchase eyed oyster larvae from a hatchery and set them on the cultch in their own settlement tanks, where they can be fed with cultured algae until they are ready for planting. These techniques can be used in small or large scale undertakings.

Acknowledgements

Authors are thankful to Shri. Sampson Manickam, O.I.C., F.M.C., C.M.F.R.I. for having permitted to avail laboratory facilities during the experiments. Thanks are also due to Dr. P. Muthiah and Shri. S. Dharmaraj, Senior Scientists, T.R.C. of C.M.F.R.I. for providing larvae for the study. Authors are greatly obliged to Dr. K.K. Appukuttan, Head, M.F.D., C.M.F.R.I. for the encouragements and suggestions during the study. The first author is grateful to I.C.A.R. for providing him with the Junior Research Fellowship during the course of study.

References

Alagarswami K., S. Dharmaraj, T.S. Velayudhan, A. Chellam, A.C.C. Victor and A.D. Gandhi, 1983. Larval rearing and production of spat of pearl oyster *Pinctada fucata* (Gould). *Aquaculture* **34** : 287-301.

Baud P., C. Joll and A. Bodoy, 1991. Improvement on remote setting and nursery rearing of the Pacific oyster, *Crassostrea gigas* on French plastic pipes. *Proc. I.C.E.S. Council Meeting Papers*, I.C.E.S., Copenhagan (Denmark), 12 pp.

Bitaud G. and P. Herve, 1991. Remote collection on micro-tiles. *Aqua Rev.*, No. **38** : 13-5.

Castagna. M., M.C. Gibbons and K. Kurkowski, 1988. Remote setting and post-set strategies for growing *Crassostrea virginica* in Virginia. *Journal of Shellfish Research*, **7** (3): 571-572.

Chew K.K., 1984. Recent advances in the cultivation of molluscs in the Pacific United States and Canada. *Aquaculture*, **39** : 69-81.

Chew K.K., 1991. Hatchery development and remote setting of the Pacific oyster larvae. *Aquaculture Magazine*, **17** (4): 72-75.

Gibbons M.C., 1988. Development of nursery techniques remote set larvae of *Crassostrea virginica* in Virginia. *Journal of Shellfish Research*, **7** (1): 160.

Henderson B.A., 1982. Practical methods of handling and remote setting eyed- pediveliger larvae of the Pacific oyster, *Crassosrea gigas* (Thurnberg). *Journal of Shellfish Research*, **2** (1): 119-120

Holliday J.E., G.L. Allan and J. Frances, 1991. Cold storage effects on setting of larvae of the Sydney rock-oyster, *Saccostrea commercialis* and the Pacific oyster *Crassostrea gigas*. *Aquaculture*, **92**: 179-185.

Langan R., D. Gress, I. Walker, P. Flanigan, J. Sheeny, J. Drake and K. La Valley, 1997. Remote setting of the eastern oyster (*Crassostrea virginica*) on natural and artificial cultch. *Journal of Shellfish Research*, Vol. **16**(1): 289-290.

Jones G. and B. Jones, 1982. Methods for setting hatchery produced oyster larvae. *Min.of Environment*, Prov. of British Columbia, 61 pp.

Jones G. and B. Jones, 1988. Advances in the remote setting of oyster larvae. *Min. of Agri. and Fish.*, Victoria, B.C. (Canada). 88 pp.

Meritt D., 1998. Oysters and hatcheries, *Aquaculture Magazine*, **25** (2): 22-23.

Muthiah P., 1987. Techniques of collection of oyster spat for farming, *C.M.F.R.I. Bulletin*, No. 38: 48-51.

Panggabean L., P.R.Waterstrat, S.L.Downing and J.L. Beattie, 1989. Storage and transportation of straight-hinge oyster larvae. *Journal of Shellfish Research*, **8** (1): 323-324.

Nayar K.N., M.E. Rajapandian, A.D. Gandhi and C.P.Gopinathan, 1987. Larval rearing and production of spat of the oyster *Crassostrea madrasensis* (Preston) in an experimental hatchery. *Indian J. Fish.*, **31**: 233-243.

Rhodes E.W. and J.J Manzi, 1988. Interstate shipment of juvenile bivalves : Effects of shipping duration method survival. *Journal of Shellfish Research*. **7** (1): 130-131.

Roland W.G., T.A. Broadley and I.R. Sutherland, 1989. Solving problems with remote setting Pacific oyster larvae in British Columbia. *Journal of Shellfish Research*. (8), p. 324.

Rouse D., 1993. Growth of micro-cultched and remote set oysters in coastal waters of Alabama. *Journal of Shellfish Research*, **7** (1), p. 134.

Supan J.E. and C.A. Wilson, 1994. The technical feasibility of commercial hatchery and remote setting operations in Louisiana. *Journal of Shellfish Research*, **3** (1),p. 286.

Thomas J., G.M. Burnell, N. De Pauw and J. Joyce, 1991. A biological and economic appraisal of the remote setting of Pacific oyster larvae in Ireland. *Aquaculture And The Environment* [Spec. Publ. Eur. Aquacult. Soc.] No.14, pp. 310-311.

Thomas J. and G.M. Burnell, 1992. Commercial trials to assess the growth and survival of remote set Pacific oyster (*Crassostrea gigas*) larvae in shallow nursery ponds. *Journal of Shellfish Research*, **11** (1), p. 207.

Observations on fattening, growth and sex reversal of the greasy grouper *Epinephelus tauvina* (Forskal)

A. Raju, D. Kandasamy, I. Rajendran, I. Jagadis and N. Ramamurthy

Mandapam Regional Centre of Central Marine Fisheries Research Institute, Madapam Camp.

ABSTRACT

This paper deals with the experiments conducted on the development of broodstock, rearing of fingerlings and sex conversion of the greasy grouper, Epinephelus tauvina *under controlled conditions. Groupers collected from the wild and harvested from the culture ponds were raised to broodstock in 10 x 5 x 2.3 m (net water capacity of 100 tons) R.C.C. tanks by feeding with freshly killed sardines, cod liver oil and vitamin tablets. Experimental*

rearing of fingerlings was carried out in 30 ton tank. In broodstock rearing, the growth rate varied from 10.1mm/106.8g to 13.1mm/178.3g for the wild and 16.6mm/116.8g to 21.1mm/271.5g for the pond harvested stock with a feed conversion ratio of 4.6-7.7 and 3.2-3.5 respectively. Maximum growth increments of 27.2mm/404g and 25.5mm/305g have been realised for the wild and pond harvested stock respectively. In general, pond harvested groupers showed higher growth with less conversion ratio. The fingerlings registered a growth rate of 24mm/42g with a conversion ratio of 10. Preliminary experiments on sex reversal using androgen hormone (Testoviron Depot containing Testosterone Enanthate) revealed the possibility of converting female to male by the indication of regression in ovary size and disappearance of oocytes.

Introduction

Although there are several reports on the breeding and larviculture of groupers, controlled breeding and hatchery production of fingerlings for large scale commercial farming is scarce due to several technical and hatchery constraints and poor larval survival (Leis, 1987; Kohno *et al.*, 1988; Tookwinas, 1990; Al-Thobaiti and James, 1996). Among several species of groupers, *Epinephelus coioides* (previously reported as *E. tauvina*) was the first species to be cultured in the Middle East and the natural spawning of this species in captivity was reported by Hussain and Higuchi (1980) in Kuwait. The artificial spawning and larval rearing of *E. tauvina* in Singapore and *E. salmonoides* in Philippines have been reported by Chen *et al.*, (1977) and Kunvankji *et al.*, (1986) respectively. In Japan, Tawada (1989) described on the breeding and larval rearing of the camouflage groupers, *E. polyphekadion* as *E. microdon* with poor larval survival. Debas *et al.*, (1989) reported the fully mature *E. polyphekadion* caught all year round in polynesian waters and focused in the identification of the life cycle of reproduction and sex reversal phenomena of this hermophrodite species. Successful year round natural spawning of *E. polyphekadion* under captive conditions and hatchery rearing of larvae has been reported in the hypersaline waters along the Red Sea coast of Saudi Arabia by James *et al.*, (1997).

Most of the grow out culture systems for groupers depend on the seeds collected from the wild (Quinitio and Toledo, 1991). The availability of grouper fingerlings from the wild for farming are very erratic and inconsistent due to seasonal and environmental conditions. A more reliable source is to produce the seed in hatchery under controlled conditions. An important aspect in controlled breeding is broodstock raising and fattening which enters on promoting sexual maturation and enhancing broodstock quality to ensure better quality of eggs and sperm. This paper reports the results of experimental fattening and growth of the grouper *Epinephelus tauvina* conducted from June to December 1998 in captive conditions and sex reversal using androgen hormone.

Material and methods

Grouper, *E. tauvina* used for this study were collected from the wild by traps, gillnets and stake net ('patti') and were transported to the experimental site by jeep. They were segregated into three size groups such as below 1 kg, 1-2 kg and above 2kg and stocked separately in 10 x 5 x 2.3m R.C.C. tanks F, H and G respectively (net water capacity 100 tons) at the rate of 1 kg biomass/cubic metre. Besides, cultured groupers, harvested from the ponds were transported from Tuticorin and used for the investigation. These stocks were screened out as below 0.5 kg, 0.5-1 kg and above 1 kg and stocked separately in another R.C.C. tanks (L,K and J) of same size and at the same density. Fingerlings of *E. tauvina*, collected from the wild along Tuticorin coasts were transported and stocked in 30 ton capacity R.C.C. tank. The stocks in 100 ton tanks were fed with freshly killed sardines (*Sardinella* spp) at the rate of 2-6% of the body weight of the biomass. Apart from this, sardines injected with Seven Seas cod liver oil and orally implanted vitamin E tablets were provided for the stock weighing above 2 kg. The fingerlings were fed with chopad sardines at the rate of 5-10% of the body weight.

Tanks were cleaned twice in a week and 100% water exchange was done with water drawn directly from the sea through pumping in

addition to 50% daily change. One third portion of tank on the top was covered by mats made out of coconut leaves to provide shade for the fishes. Considering the behaviour of the fish, hide outs were provided with asbestos sheets and fibre glass tanks at the bottom. The injured/diseased fish were given antibiotic dip treatments using malachite green, methylene blue, chloramphenical and oxytetracycline. Hydrological parameters such as water temperature, salinity, dissolved oxygen and pH were monitored regularly. Stocks were sampled at an interval of thirty days and the measurements of length and body weight of broodstock and fingerlings were done to ascertain the growth rate. For the experiments on sex reversal, the androgen hormone, a derivative of testosterone was used as inducing agent. Fish are regularly monitored for gonadal condition by ovarian biopsy.

Results

Growth: The growth progress and increments in length and weight for the broodstock of each size group and fingerlings reared in R.C.C. tanks are presented in Tables. 1-4

Table 1. Rearing of wild grouper, *Epinephelus tauvina* during June - December 1998 in R.C.C. tanks

Days	Tank F (Below 1.0 kg)		Tank H (1.0-2.0 kg)		Tank G (above 2.0 kg)	
	Average length (mm)	Average weight (gm)	Average length (mm)	Average weight (gm)	Average length (mm)	Average weight (gm)
Initial	378.2	742	472.3	1515	560.0	2600
30	382.0	763	480.0	1681	562.1	2765
60	400.4	948	495.4	1785	568.8	2817
90	427.1	1100	496.3	1833	586.0	2942
120	429.3	1180	522.6	2237	588.6	3200
150	438.3	1341	535.0	2400	600.4	3495
180	442.6	1383	550.9	2480	620.4	3670

Table 2.Rearing of pond harvested stock of grouper, *Epinephelus tauvina* during August December 1998 in R.C.C. tanks

Days	Tank L (Below 0.5 kg)		Tank K (0.5 -1.0 kg)		Tank J (Above 1.0 kg)	
	Average length (mm)	Average weight (gm)	Average length (mm)	Average weight (gm)	Average length (mm)	Average weight (gm)
Initial	298.8	324	389.7	755	481.8	1534
30	306.6	395	402.8	876	502.8	1800
60	338.2	584	422.8	1128	521.9	2105
90	363.7	726	442.4	1289	540.3	2394
120	383.2	791	456.1	1485	551.7	2622

Table 4. Rearing of fingerlings of grouper, *Epinephelus tauvina* during August 1997 - May 1998 in R.C.C. tanks

Days	Average length (mm)	Average weight (g)	Growth increments Length (mm)	weight (g)
Initial	69.8	4.5	-	-
30	126.0	42.4	56.2	38.1
60	152.0	62.2	26.0	19.8
90	210.6	117.7	58.6	55.5
120	211.2	175.1	0.6	57.4
150	247.9	204.5	30.7	29.4
180	257.5	224.1	15.6	19.6
210	271.4	252.7	13.9	28.6
240	280.5	327.0	9.1	74.3
270	284.6	377.0	4.1	50.0

Table 3. Growth increments of broodstock of *Epinephelus tauvina* in R.C.C. tanks

Days	Wild stock						Pond reared stock					
	Below 1.0 kg		1.0 -2.0 kg		Above 2.0 kg		Below 0.5 kg		0.5-1.0 kg		Above 1.0 kg	
	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)
30	3.8	21.8	7.7	166	2.1	165	7.8	71	13.1	121	21	266
60	18.4	184.8	15.4	104	6.7	52	31.6	189	20.0	252	19.1	305
90	26.7	161.9	0.9	48	17.2	125	25.5	142	19.6	161	18.4	289
120	2.2	70.0	26.3	404	2.8	258	19.5	65	13.7	197	11.4	228
150	9.0	161.0	12.4	163	11.6	295	-	-	-	-	-	-
180	4.3	42.0	15.9	80	20.0	175	-	-	-	-	-	-

Wildstock: The stocks weighing below 1 kg in Tank F have grown to an average size of 400.4 mm (948g) in 60 days, 429 mm (1180g) in 120 days and 442.6 mm (1383g) in 180 days. The growth was observed to be better at 60,90 and 150 days period. The growth increments varied from 2.2-26.7 mm in length and 21.8-184.8g in weight. The overall monthly average growth rate was 10.7 mm and 106.9g in length and body weight respectively. The feed conversion ratio was 7.7 : 1.

In Tank H (1-2 kg), the fishes grew to 495.4 mm (1785g), 522.6 mm (2237g) and 550.6 mm (2480g) in 60, 120 and 180 days respectively. Encouraging growth was observed at 30 and 120 days period. The growth increments ranged from 0.9 mm to 26.3 mm and 480-404g in length and weight respectively. The average growth increment per month was 13.1 mm in length and 160.8 g in weight. The feed conversion ratio was 4.8 : 1.

The fishes in Tank G weighing above 2 kg showed the growth progress of 568.8 mm (3200g) in 120 days and 620.4 mm (3670g) in 180 days. The growth was fast upto 150 days. The growth increments varied between 2.1 and 20 mm in length and 52 and 225g in weight with an average growth rate of 101 mm and 178.3g in length and body weight respectively. The feed conversion ratio was found to be 4.6 : 1.

Pond harvested stock: In Tank L (below 0.5 kg) the fish were found to grow to an average size of 338.5 mm (584g) in 60 days and 383.2 mm (791g) in 120 days. Better growth was observed upto 90 days. The growth increment ranged from 7.8-31.6 mm in length and 65-189g in body weight. The monthly average growth rate was 21.1 mm and 116.8g in length and body weight respectively. The feed conversion ratio was 3.2 : 1.

The stock weighing between 0.5 and 1 kg in Tank K progressed to 422.8mm (1128 g) and 456.1mm (1485g) in 60 and 120 days respectively. The growth of fish was encouraging throughout the rearing period. The growth increments ranged from 13.1-20.0 mm and 121-252g in length and weight respectively. The average growth increment was 16.6 mm and 182.5g. The feed conversion ratio was 3.3 : 1.

In Tank J (above 1 kg), the fishes have attained an average size of 521.9 mm (2105g) in 60 days and 551.7 mm (2622g) in 120 days. The growth of fish was found to be encouraging throughout the rearing period. The monthly growth increments varied from 11.4-21 mm in length and 228-305g in weight. The average growth increment was 17.5mm and 271.5g in length and weight respectively. The feed conversion ratio was 3.5 : 1.

Fingerlings: The fingerlings progressed to 152.0 mm (62.2g) in 60 days, 211.2 mm (175.1g) in 120 days, 257.5 mm (224.1g) in 180 days, 280.5 mm (327g) in 240 days and 284.6 mm (377g) in 270 days. Encouraging growth was observed at 30, 90 and 150 days. In the rest of the period, increase in body weight was pronounced rather than length. The growth increments varied from 0.6-58.6 mm in length and 19.6-74.3g in weight. The average increment was found to be 24.0 mm and 42.0g. The feed conversion ratio was 10:1.

Sex reversal : Six female fishes measuring 60-68 cm in length and 3.4-5.4 kg in body weight were given intramuscular injection with methyl testosterone (Testoviron Depot containing Testosterone Enanthate) at the dose of 2 mg/kg body weight. The injection was given twice in a week. The fish were found healthy and surviving with the injections for 3 months. The intake of food was poor and as a result the body weight decreased. After receiving a total dose of 40-60 mg, 4 fishes were observed to be disease affected and found sluggish but did not survive. On examination of the body cavity of these fishes, it was observed that the ovary has reduced in size and oocytes disappeared.

Remarks

In broodstock rearing, a maximum weight increment of 404g for the wild and 305 g for the pond harvested stock has been observed for the fishes weighing above 1.0 kg. The growth of fish weighing below 1.0 kg was found to be good in pond harvested stock. It has also been revealed from the study that pond harvested groupers raised in R.C.C. tanks showed

better growth with less conversion ratio when compared to wild groupers. The reason may be attributed due to their domestication in the pond environment from the fingerling stage itself.

Anon (1986) stated that grouper fingerlings of 75-100 mm and 120-150 mm in size are stocked at the rate of 100-150 and 44 per sq.m. in hapa and nursery nets respectively for culture. In the present study, 49-87 mm size fingerlings of *E. tauvina* were stocked in R.C.C. tank at the rate of 1 per sq.m. Panbanpaew and Sakara (1990) reported that fingerlings of *E. malabaricus* have grown to 90-100 mm after 60 days in concrete tanks from the initial size of 43 mm and 141 mm (49.9g) in net cages 75 days from the size of 57 mm (3.1g). In the present observation, fingerling of *E. tauvina* registered the monthly growth rate of 24.0 mm (42.0g) by growing to 284.6 mm (377g) in 270 days.

Most of the grouper species are protogynous hermophrodite, meaning that they are females during early stage of their life cycle and become males during later phase (Shapiro, 1987). Abdullah *et al.* (1983) stated that sex transmission in *E. tauvina* occurs in fish measuring 55-75 cm in length under natural condition. Tan-Fermin *et al.* (1994) showed that 2 year old *E. coioides* weighing 1.2 kg can be sex reversed when 0.5-5.0 mg/kg body weight Methyl Testosterones (MT) injection at fortnight intervals for 5-6 months. Marte *et al.*(1995) reported that bi-monthly implantation if MT (4 mg/kg body weight) or MT (4 mg/kg body weight) + Luteinizing hormone-Releasing hormone (20 μg/kg body weight) were more effective than bi-weekly injection of MT (1 mg/kg body weight). In the present investigation, androgen hormone injection was given twice in a week at the dose of 2 mg/kg body weight. The androgen hormone used was a local derivative of testosterone (Testoviron Depot containing Testosterone Enanthate). The reason for slow development of sex reversion to male may be due to the quality of the product used. There is possibility of quick sex conversion of male from female with original MT. However, the reduction in ovary size and disappearance of oocytes confirm the possibility of sex reversion from female to male.

Acknowledgements

The authors are grateful to Dr. M. Devaraj, Former Director, CMFRI for suggesting this problem and for his encouragements. They also express their sincere thanks to Dr. V. Sriramachandra Murty, Head, Demersal Fisheries Division for his valuable guidance and suggestions during the course of this study.

References

Abdullah, M.A.S., S. Akaten, K.M. Al-Abdul-Elah, and B.K. Teng, 1983. *Annu. Res. Rep. KISR.* : 55-57.

Al-Thobaity, S. and C.M. James, 1996. Developments in grouper culture in Saudi Arabia. *INFOFISH International* 1/96 : 22-29.

Anon. 1986. Manual on floating netcage fish farming in Singapore. Fisheries Handbook No.1. *Prim. Prod. Dept. Republic of Singapore* : 17 pp.

Chen, F.Y., M. Chow, T.M. Chao, and R. Lim, 1977. Artificial spawning and larval rearing the grouper, *Epinephelus tauvina* (Forskal) in Singapore. *Singapore Journal of Primary Industry* **5** : 1-21.

Debas, L., A. Fostier, J. Fuchs, M. Weppe G. Nedlec, A. Benett, C. Cauty, B. Jalabert, and Aquacop, 1989. The sexuality of cultured hermaphrodite fish species: analysis of morphological and endocrinological features in a protogynous hermaphrodite, *Epinephelus microdon,* as a basis for further research to control reproduction in the grouper. In : *Advances in Tropical aquaculture* (Ed. J. Barret). pp. 543-557. AQUACOP, IFREMER. Actes de Colloque 9. Tahiti, French Polynesia.

Hussain, N. A. and M. Higuchi, 1930. Larval rearing and development of the brown-spotted grouper, *Epinephelus tauvina* (Forskal), *Aquaculture, **19** :* 339-350.

James, C.M., S.A. Al-Thobaiti, B.M. Rasem and M.H. Carlos, 1997. Breeding and larval rearing of the camouflage grouper *Epinephelus polyphekadion*

(Bleeker) in the hypersaline waters of the Red Sea coast of Saudi Arabia. *Aquaculture Research*, **28** : 671-681.

Kohno, H., M. Duray, and J. Juario, 1988. State of grouper (lapu-lapu) culture in the Philippines. *SEAFDEC Asian Aquaculture*, **10** : 4-9.

Kungvankij, P., L.B. Tiro, B.P. Pudadera and I.O. Potestas, 1986. Induced spawning and larval rearing of grouper (*Epinephelus salmoides* Maxwell), p.663-666. In J.L. Maclean, LB. Dizon and L.V. Hosillos (Eds). *The First Asian Fisheries Forum.* Asian Fisheries Society, Manila, Philippines.

Leis, J.M. 1987. Review of the early life history of tropical groupers (Serranidae) and snappers (Lutjanidae) In: *Tropical Snapper and Grouper: Biology and Fisheries Management* (Ed. J.J. Polovina & S. Ralston). pp. 189-237. Westview Press, Boulder, Colorado USA.

Marte, C.L., G. Quinitio, and N. Caberoy, 1995. Spontaneous spawning of sex inversed groupers *Epinephelus coioides* administered 17 -alpha methyl-testosterone implants. A paper presented at the *Fourth Asian Fisheries Forum.* 16-20 October 1995, Beijing, PROC.

Panbanpaew, S. and W. Sakaras, 1990. Experiment on sea grouper *Epinephelus malabaricus.* Technical paper No.38/1990. Rayong Brackishwater Fisheries Station, Dept. of Fisheries, 17 pp.

Quinitio, G.F. and J.D.Toledo, 1991. Mariculture techniques for *Epinephelus* spp. in the Philippines, p. 94-106. In : R.D. Guerrero III and M.P. Garcia, Jr. (Eds.) *Advances in Finfish and Shellfish Mariculture* : Proceedings of the First Phillippine-French Technical Workshop on Advances in Finfish and Shellfish Mariculture. 24-26 October 1990, Los Banos. Laguna, Philippines. Philippine Council for Aquatic and Marine Research Development; French Embassy in the Philippines.

Shapiro, D.Y., 1987. Reproduction in groupers, p.295-327. In : J.J. Polovina and S. Ralston (Eds). *Tropical Snappers and Groupers : Biology and Fisheries Management,* Westview Press. Inc., Boulder, Colorado.

Tan-Fermin, J.D., L.M.B. Garcia, and A.R. Jr. Castillo, 1994. Induction of sex inversion in juvenile grouper, *Epinephelus suillus* (Valenciennes) by injections of 17A-methyl testosterone. *Japan J. Ichthyol.* ***40 :*** 413-420.

Tawada, S., 1989. Spawning of broodstock of the grouper *Epinephelus microdon* (Bleeker). Suisanzoshoku III. 105-108.

Tookwinas, S. 1990. Review of knowledge on grouper aquaculture in Southeast Asia. In: *Advances in Tropical Aquaculture* (Ed. J. Barret). pp. 429-435. AQUACOP IFREMER, Tahit, French Polynesia.

Probiotics in aquaculture

M.Vijayakumaran

Madras Research Centre of Central Marine Fisheries Research Institute, Chennai - 600 006

ABSTRACT

Use of probiotics, the beneficial digestive bacteria, are well documented in human and animal nutrition. Bacteria belonging to the genera Lactobacillus, Enteroccocus, Pediococcus *and* Bacillus, *microscopic fungi and saccharomyces yeast have been widely used as probiotics. In aquaculture, the usage of probiotics is mainly confined to hatcheries of shrimps, bivalves and fishes. However, scientific studies are scanty on the use of probiotics in grow out systems of fish/shrimps and even its benefits are debated. Contrary to the traditional use of probiotics as feed additives, it is used in aquaculture and production systems to modify the microbial population of the environment ultimately leading to better growth and survival of the targetted species. Even non-pathogenic strains of pathogenic bacteria are being used as probiotics in shrimp culture. Use of various brands of commercial probiotics have become a regular farming practice in shrimp culture in India, particularly after the major viral disease outbreak in 1995. The paper reviews the use of probiotics in 'coastal aquaculture systems'.*

Introduction

Rapid stride in coastal aquaculture received a temporary setback due to widespread disease outbreaks in shrimp farms in early 90's. The panic reaction from the farmers resulted in large scale use of chemical and biological preparations to prevent and control disease. Indescriminate use of antibiotics, chemicals and pesticides coupled with unplanned,

mushrooming growth of shrimp farms in developing countries created social and environmental problems drawing attention from scientists, environmentalists and legislators. Consequently the emphasis of coastal aquaculture, especially shrimp culture, has shifted from maximising production to sustainable production with minimum damage to environment. The search for safe and permanent solution has led aquascientists to the use of beneficial microorganisms or 'probiotics' for environmental clean up and maintenance of water quality, in addition to their role in nutrition as well as in exclusion of endogenous pathogens. This article reviews the use of probiotics in aquaculture, with special reference to shrimp culture.

What are probiotics

The term probiotics was used originally to describe the organisms and substances that contribute to intestinal microbial balance. Such substances prevented colonization of gut by pathogenic organisms and promoted utilisation of feed. Use of probiotics is well documented in human and animal nutrition. Bacteria belonging to the genera *Lactobacillus, Enterococcus, Pediococcus, Bacillus*, microscopic fungi and Saccharomyces yeast are the main probiotics used in animal nutrition. Contrary to its traditional use as food additives, probiotics are used in aquaculture to mainly modify and manipulate the microbial population of the environment and to reduce or eliminate selected pathogenic species of microorganisms leading to better growth and survival (Jory, 1998). According to Morrarty (1966) microbial food webs are an integral part of aquaculture ponds and have direct impact on productivity.

Probiotics in live feed and fish culture

Probiotics are widely used in livefeed culture of rotifer, *Artemia* and copepods used as feed for larval fish in many hatcheries. Garriques and Arevalo (1995) described the use of spray dried probiotic bacterial preparation to increase production of rotifer and to limit bacterial proliferation in rotifer during enrichment with fish oil. Its use also reduced the proportion of vibrios in the bacterial flora of rotifer. Same authors reported that digestions of micro algae was enhanced in *Artemia*, when it is grown with bacteria (Flexibacter) as food supplement. The growth of Japanese flounder fry was enhanced when it was fed with rotifer fed on Lactic Acid Bacteria (LAB). The total count of bacteria reduced

from 99,000 to 54,000 in LAB treated rotifer after enrichment with cuttlefish oil (Gatesoupe *et al.*, 1989). Similar reduction in gram negative bacteria (*Virbio spp.*) was reported into Copepod *Aearlea tousa* by treatment with *Bacillus* spp. isolated from common stort (*Centropomus undecemalis*) larvae (Fernandez *et al.*, 1995). Differential growth rate was obtained when the treated copepod was fed to 7-14 day old tomato clown fish (*Amphiprion frenalus*). The same *Bacillus* spp. increased production and reduced gram-negative vibrios in rotifers (Gianelli *et al.*, 1995) and reduced total bacteria (*Staphylococcus*, *Monococcus* and *Vibrio* spp.) in *Artemia* (Gensler *et al.*, 1995). High survival and weight gain and enhanced resistance to pathogenic vibrios were reported in turbot (*Scophthalmus maximus*) larvae when fed with probiotic enriched rotifer and bacteria (Garriques and Arevalo, 1995).

Gut flora, which play prominant part in animal nutrition, do not have a major role in digestion in cold water fish but do play a significant role in intestinal fermentation in warm water fishes (Gatessoupe *et al.*, 1989). Besides enzymes, the gut bacteria in fishes may bring in nutrients like vitamin B12. Use of antibiotics have only temporary effects in gut microflora since plasmid mediated resistance will be transferred to pathogens. Lactic acid bacteria (LAB) like *Streptococcus*, *Lactobacillus* and *Carnobacterium* belong to the normal microflora of the gastrointestinal tract in healthy fish but the population of LAB in digestive tract is affected by nutritional and environmental factors like polyunsaturated fatty acids, chromic oxide, stress, salinity etc. LAB isolates from fish gut can act as probiotics and prevent colonization of gut by pathogenic bacteria (Ringo and Gatesoupe, 1998). Sujita and Shibuya (1996) were successful in isolating intenstinal bacteria, from seven species of fish. that have antibacterial activities against pathogenic bacteria.

A case of 'good vibrio' (which form yellow colonies in TCBS agar) acting as probiotic in fish was reported by Austin *et al.* (1995). A strain of *Vibrio alginolyticus* inhibited growth of fish pathogens like *Vibrio ordalli*,, *V.angullarum*, *Aeromonas salmonicida* and *Yersinia ruckeri*. When the probiotic was added to Atlantic salmon culture, a reduction in mortality was recorded when challenged with *A. salmonicida*. Smith and Davey (1993) isolated a fluorescent strain of pseudomonid bacteria that could competitively inhibit the growth of fish pathogen *A. salmonicida* in culture media, probably due to competition for iron.

Probiotics used in shellfish larval rearing

Growth and survival of prawn and crab larvae could be enhanced by use of selected bacterial strains that had vibriostatic activity (Maeda and Nogami, 1989). A similar result was obtained in the crab (*Portunus trituberculatus*) larvae by a bacterial strain isolated from crustacean culture pond (Nogami and Maeda, 1992). This bacteria strain, in addition to competitive exclusion of pathogens, also acted as a nutrient source.

Vibrios are among the natural microflora of shrimps (Lightner, 1993) and some strains like *Vibrio harveyi*, *Vibrio parahaemolyticus* etc. are opportunistic pathogens. As in the case of fish a strain of *Vibrio alginolyticus* is used as probiotic agent for larval rearing of *Penaeus vannamei* in Ecuador. Use of this strain increased survival of *P. vannamei* post-larvae by competitive exclusion of pathogenic virbios and reduced antibiotic prophylaxis in intensive larval culture systems (Griffith 1995). Hong *et al.* (1998), quoting Ecuadorian scientists, reported relationship between zoea syndrome and presence of *V. harveyi* as type 222 in *P. vannamei*. The zoea syndrome was effectively controlled by using a strain of *V. alginolyticus* as probiotic. Use of *V.alginolyticus* has reduced hatchery down time from 7 days per month to less than 21 days annually. The production of *P. vannamei* larvae was increased by 35% and antibiotic use was decreased by 94% between 1991 and 1994. Farm trial also showed that survival, production, feed conversion and growth rates were not negatively affected by hatchery use of probiotics (GomezGil, 1995).

Use of *Lactobacillus* spp. against pathogenic vibrios and white spot disease was reported in *Penaeus monodon*. Inhibitory activity of two *Lactobacillus* spp. against *Vibrio* spp. *Escherischia coli*, *Staphylococcus* and *Bacillus subtiles* was demonstrated in laboratory culture. Possibility of using two non-pathogenic vibrio strains against pathogenic viruses, is indicated in the results obtained in fish (Hong *et al.*, 1998).

Use of photosynthetic bacteria (*Rhodomonas* spp.) as probiotic improved water quality, reduced shell fouling in larvae, reduced metamorphosis time by one day and improved post larval production in *Penaeus chinensis*. It also acted as auxiliary food (Hong *et al.*, 1998).

Bacterial strain isolated from natural intestinal muciflora of *P.chinensis* increased desease resistance, low salinity tolerance and weight gain in its larvae. The probiotic bacteria produced digestive

enzymes which helped to improve digestion of shrimp larvae (Hong *et al.*, 1998).

Probiotics, serving as food supplement that promotes larval growth have been reported in *Penaeus japonicus* and the crab *Portunus trituberculatus* (Maeda and Nogami *et al.*, 1992) in *P.trituberculatus* larvae (Maeda & Liao, 1994) and in *P.monodon* (Sunilkumar, 1996). In India, most of the shrimp hatcheries use commercial probiotics with promising results in reducing vibrio population in culture water and decreasing disease outbreak, leading to higher survival of *P. monodon* larvae. Another hatchery practice followed in India, as in many South East Asian countries, is providing inoculam, (to the tune of 10% of total volume) from the most successful tanks (Jayagopal, Personal communication).

Probiotic bacteria are being used in molluscan hatcheries also for competitive exclusion of pathogens and as food supplement. Enhanced growth rate of Pacific oyster, *Crassosteria gigas* with probiotic bacteria at a rate of 0.1 million cell per ml, improved survival and growth (Douillet and Langdon, 1994). The probiotic provides essential nutrients or improve oyster digestion by supplying digestive enzymes to the larvae and remove metabolic wastes of the larvae.

Use of probiotics in grow-out

Use of a commercial probiotic at a rate of 6 kg/ha every 7 days for a period of 147-154 days in 0.19 ha ponds growing *P. monodon* was found to reduce ammonia levels, increase survival rates and increase production per hactre (Tobbu *et al.*, 1997). Addition of photosynthetic bacteria in food or culture water was found to improve quality of water by eliminating ammonia nitrogen, hydrogen sulphide, organic acids and other harmful materials rapidly and enhance growth rate of the prawn *P. chinensis* (Hong *et al.*, 1998).

Moriarty (1966) and Suhendra *et al.* (1997) have reported farm trials with probiotic *Bacillus* spp. in Indonesian shrimp forms. The use of probiotic considerably reduced luminiscent vibrio, *V. harveyi*, in water column and sediments, reduced outbreaks due to vibrios and viruses, reduced organic mater accumulation and improved water quality, shrimp size and production. However, strict maintenance of water quality and sludge removal were required to get maximum benefit from probiotic use.

Selective addition of nutrients and expansion of habitats and culture addition may cause shifts in microbial community that can result in faster carbon - nitrogen cycling and removal, eventually reducing ammonia and sludge accumulation in shrimp ponds (Browdy and Bratwold, 1997).

Detritus Management System (DMS) bacteria are used in many shrimp farms in Indonesia to improve water quality leading to enhanced production. DMS bacteria act by accelerating the natural process in the pond, reduce detritus by converting it into CO_2 and reduce the total organic content. It makes more oxygen available to fish/shrimp by reducing the oxygen requirements of heterotrophs. It also extracts phosphorus from water and controls the phytoplankton production by competing with it for phosphorus. It also serve as food source when it forms a coating over reduced detritus. DMS consists of bacteria that reproduce slowly but are highly efficient at reducing the amount of detritus in the pond. Hence they are to be added at regular intervals and at sufficient quantity to get best result.

Chandrika (1999) isolated pigmented *Bacillus* from paddy-cum-prawn fields and prennial prawn fields in Narakkal, Kerala that inhibit growth of pathogenic bacteria like vibrios, aeromonas and *E.coli*. Gram-negative slime forming myxobacteria were also inhibited by two of the isolated *Bacillus* spp. These strains have the potential to be used as probiotic through feed or in DMS.

Commercial probiotics are regularly being used in Indian shrimp farms since 1990. The use of probiotics is reported to give stable phytoplankton bloom, pH alkalinity and improve dissolved oxygen. The ammonia and nitrite levels and vibrio counts were also reduced in probiotic treated ponds. Application of 10 kg of probiotic at the cost of Rs.2500 per kg for a stocking rate of $10/m^2$ are being practiced in few corporate shrimp farms in India for *P. monodon* culture. Fermented juice of tea seed cake, groundnut oil cake, yeast, jaggery and toddy are also being used by some shrimp farmers in India.

Composition of probiotics used in aquaculture

Fifteen species of *Bacillus* constitute the generally recommended microorganisms used as probiotics in pond aquaculture and these form

the main component of many commercial probiotics. *Bacillus* are motile, produce endospores to tide over unfavourable conditions, produce antibiotics and enzymes. They grow at high temperature and are easily isolated and cultured in synthetic media using sugars, alcohols and organic acids as carbon sources and ammonia as sole nitrogen source. *Bacillus* are the main component of DMS bacteria. *Bacillus*, *Lactobacillus*, *Nitrobactor*, *Nitrosomonas*, *Pseudomonas*, *Cellulomonas*, *Rhodopseudomonas*, *Enterobacter*, *Enterococcus*, *Pseudococcus*, *Staphylococcus* etc. are the bacterial flora reported to be present in many commercial probiotics (Boyd 1995, Guillot, 1998). Among the vibros, *V. alginolyticus* strain has been successfully used as probiotic in the shrimp hatcheries in Ecuador.

Application of probiotics

Probiotics are marketed in liquid and powder forms. The hatcheries generally use liquid forms which are directly added to hatchery tanks. The liquid can be applied any time of the day in indoor hatchery tanks, while it should be applied either in the morning or in the evening in outdoor tanks. Good aeration in hatchery tank is required to get best results.

The dry probiotics that come in packets have to be brewed at farm site before application. Each kit of dry probiotic contains a packet of dry powder and a packet of enzyme catalist. Brewing has to be done in clean disinfected water after emptying the packets and blending thoroughly. Usually, it is brewed at 27-32°C for 16 to 18 hours with continuous aeration. The finished products must be used within 72 hours. Maximum aeration is required in semi-intensive culture ponds. If aeration is less, the application of probiotic has to be spread to two consecutive days, applying 50% of the dose each time.

Anaerobic probiotics, specially meant for extensive culture systems are also being marketed by few companies recently.

Action of probiotics

The probiotics act in aquaculture systems in the following manner.

1. Competitive exclusion of pathogenic bacteria.
2. Enhancement of digestion through production of exoenzymes.
3. By moderating and promoting direct uptake of dissolved organic materials.

4. By inhibiting growth of pathogenic bacteria through production of antibiotics.

Other possible mechanisms of action of probiotics are :

a. Controlling phytoplankton and blue-green algal bloom.
b. Preventing off-flavour.
c. Reducing toxic metabolites like nitrate, nitrites and ammonia and phosphates.
d. Increasing dissolved oxygen concentration by efficient removal of detritus and thereby heterotrophs.
e. Maximising carbon mineralisation as CO_2 to minimise sludge accumulation.
f. Maximising primary productivity.
g. Maintaining diverse and stable pond community.
h. Eliminating cost of cleaning ponds after harvest.
i. Reducing cost of disease treatment and crop management.

(Jory 1998, Moriarly 1996, Hong *et al.*, 1998).

Microbial activity in a pond is linked to water quality and disease outbreak. Heterotrophic bacteria use oxygen and produce toxic metabolites and since the diffusion of oxygen is minimum at pond bottom, the aerobic bacteria oxidize nitrate producing toxic metabolites and create an oxygen debt leading to an increase in reduced sediments and anoxic conditions. Shrimps exposed to reduced organic matter has less resistance to pathogens. *Bacillus* spp. produce wide range of exoenzymes which reduce available organic matter for other bacteria and reduce organic matter accumulation at the bottom. The species composition of a microbial community in a pond is determined by chance as well as by favourable physiological factors that helps it to grow and dominate. Chance favours the organisms that happens to be at the right place at the right time. Addition of probiotics is thus giving chance a helping hand (Moriarty, 1996).

Bacteria selected for bioremediation must be selected for specific funcitons and added at high enough density and under the right environmental conditions. According to Boyd (1995), in shrimp ponds, the major factors affecting bacterial activity is dissolved oxygen supply which is usually abundant. If activity of described bacteria is low it is

due to poor environmental conditions for bacterial activity and application of bacterial amendments or enzymes may not enhance bacterial activity and improve water quality. He could not get the desired beneficial activities by addition of probiotics in culture ponds.

Future of probiotics in aquaculture

Probiotics are widely used in USA. Japan, European countries, Indonesia, Thailand and India in aquaculture. It has been projected as a promising practice to improve the efficiency of aquaculture systems in general and this technology tends to have the potential to be a significant factor in sustainable shrimp culture. Experience of Boyd (1995), however, has demonstrated that simple addition of probiotics will only increase production cost and may not yield the desired results and the right environment has to be provided (Moriarty, 1996) for that. Many vendors of commercial probiotics are not aware of the physiological and ecological requirements of their bacterial products and do not pass the information to the clients (Boyd, 1995). Utmost care should be taken in selection of appropriate probiotic combinations and all necessary environmental safeguards should be provided to get optimum benefits from probiotic use. Exaggerated claims of probiotic combinations are to be scientifically evaluated in different culture conditions. Unfortunately, very few studies have been published on actual field trials. It is desirable to discourage use of imported probiotics, till its beneficiary effects in local conditions are properly evaluated. Another potential social problem in countries like India will be the handling of probiotics at farmsite. The farm hands, mostly, unskilled, should be properly trained in handling microbial preparation while brewing at farm site to avoid accidental infections to them.

References

Austin, B., L.F.Stuckey, P.A.W.Bertson, I. Effenti and D.R.Griffith 1995. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida, Vibrio angullarum* and *Vibrio ordalii, Journal of Fish Disease*, **18** : 93-96.

Boyd, C.E. 1995. Chemistry and efficacy of amendments used to treat water and soil quality imbalances in shrimp ponds. In C.L. Browdy and J.S.Hopskin (Eds). *"Swimming through troubled water"* proceedings of the special session on shrimp farming, Aquaculture 95, World Aquaculture, San Diego, California, 2-6 February 1995. pp.183-199.

Browdy and Bratwold, 1997. Pond microbial communities : Significance, assessment and Management. *Proceedings, Fourth Ecuadorian Aquaculture Congress, Guayaquil* Ecuador.

Chandrika, V. 1999. Incidence of antagonistic *Bacillus* spp. An ecofriendly aquatic probiotic from aquaculture ponds. *The Fourth Indian, Fisheries Forum Proceedings* 24-28 November, 1996. Kochi, p.147-150, published in 1999.

Douillet, P.A. and C.J.Langdon 1994. Use of a probiotic for the culture of larvae of the pacific oyster (*Crassostria gigas* Tunberg). *Aquaculture*, **119** : 25-40.

Ferrandez, E.M., S.B.Kennedy, J.D.Gianelli, A.L.Gensler and J.W.Tucker Jr. 1997. Reduction of gram-negative bacteria in copepods (*Acartia tonsa*) by treatment with *Bacillus* sp. isolated from common snook (*Centropomus undecimalis*) larvae. *World Aquaculture 97, Book of Abstracts*, p.154.

Garriques, D. and G.Arevalo 1995. An evaluation of the production and use of live bacterial isolate to manipualte the microbial flora in the commercial production of *Penaeus vannamei* postlarvae in Ecuador. In : C.L.Browdy and J.S.Hopkin, (Eds.) *"Swimming through troubled water"*. Proceedings of the special session on shrimp farming, Aquaculture 195. World Aquaculture Society, Baton Rouge, Lousiana, USA. p.53-59.

Gatesoupe, F.J., T.Arakawa and T.Watanalee 1989. The effect of bacterial additives on the production rate and dietary value of rotifers as feed for Japanese flounder, *Paralichithys olivaceus, Aquaculture*, **83** : 39-44.

Gensler, A.L., S.B.Kennedy, E.M.Fernandez, J.D.Gianelli and J.W.Tucker Jr. 1997. Reduction of bacteria in *Artemia* by treatment with *Bacillus* sp. isolated from common snook (*Centropomus undecemalis*) larvae. *World Aquaculture 97, Book of Abstracts*, p.172.

Gianelli, J.D., S.B.Kennedy, E.M.Fernandez, A.L. Gensler, J.W. Tucker Jr. 1997. Increased production of rotifers treated with *Bacillus* sp. isolated from common snook (*Centropomous undecemalis*) larvae. *World Aquaculture*, 97, *Book of Abstracts*, p.131.

GomezGil - RS, B. 1995. The use of bacteria as probionts in shrimp larviculture. In : *Larvi '95 - Fish and Shellfish Larviculture Symposium*. P.Lavens, E.Jaspers and I.Roelants (Eds.) European Aquaculture Society, Special Publication No.24, Gent. Belgium 1995, p.479.

Griffith, D.R.W. 1995. Microbiology and the role of probiotics in Ecuadorian shrimp hatcheries. In *Larvi '95 - Fish and Shellfish Larviculture Symposium.*

P. Lavens, E.Jaspers and I.Roelants (Eds). European Aquaculture Society, Special Publication No.24, Gent. Belgium, 1995, p.478.

Guillot, J.F. 1998. Probiotics in Animal nutrition. *Cabries Aquacultures* 7(1) : 49-54.

Hong, W.S., L.Jun, J.W.Shang, X.H.Shu 1999. Application of Probiotics in Aquaculture, *Ocean University of Qindao*, China.

Jory, D.E. 1998. Use of probiotics in penaeid shrimp growout. *Aquaculture magazine* January/February 1998, p.62-67.

Lightner, D.V. 1993. Diseases of cultured penaeid shrimp. In *CRC Handbook of Mariculture* (2nd ed) Vol.1 *Crustacean Aquaculture*, J.P. McVey (Ed.) CRC Press, Boca Raton, pp.393-486.

Maeda, M. and K. Nogami, 1989. Some aspects of the Biocontrolling method in aquaculture. Current topics in marine biotechnology. *Japan. Soc. Mar. Biotechnol.*, Tokyo, 395-397.

Maeda, M., K.Nogami and N.Ishibash, 1992. Utility of microbial food assemblages for culturing crab. *Portunus trituberculatus, Aquaculture*, **21** : 31-38.

Maeda, M. and I.C.Liao 1994. Microbial processes in aquaculture environment and their importance for increasing crustacean production. *JARQ* **28(4)** : 283-288.

Moriarty, D.J.W. 1996. Microbial biotechnology, a key ingredient for suistainable aquaculture. *INFO Fish International*, **4** : 29-33.

Nogami, K. and M.Maeda 1992. Bacteria is biocontrol agents for rearing larvae of the crab. *Portunus trituberculatus. Canadian Journal of Fisheries and Aquatic Sciences* **4(9)** : 2373-2376.

Ringo, E. and F.J.Gatesoupe 1998. Lactic acid bacteria in fish : a review. *Aquaculture* **160** : 177-203.

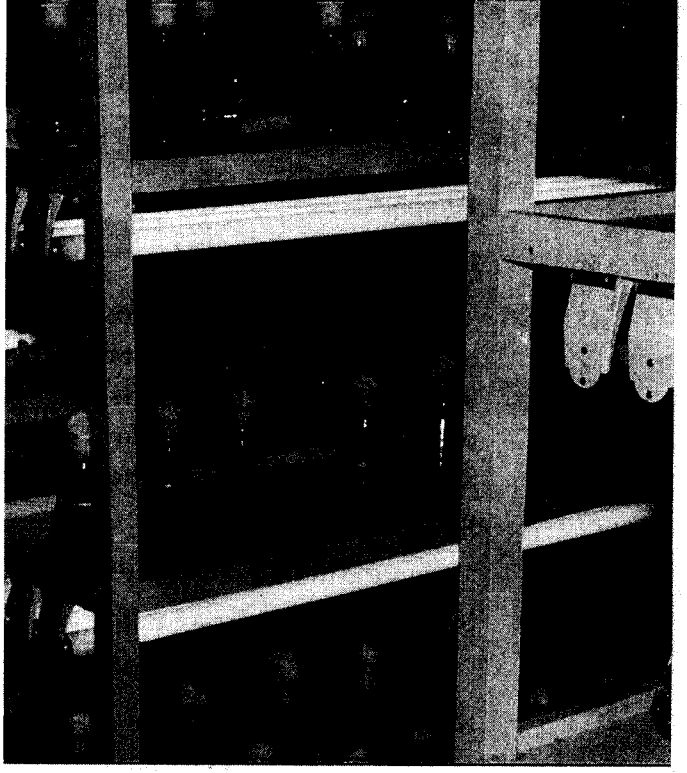
Smith, D, and S.Davey, 1993. Evidence of competitive exclusion of *Aeromonas salmonieida* from fish with stress - inducible furunculosis by a fluorescent pseudomonad. *Journal of Fish Disease*, **16** : 521-524.

Sugita, H. and K.Shibuya 1996. Antibacterial abilities of intestinal bacteria in freshwater cultured fish. *Aquaculture*, **145**(1/4) : 195-203.

Suhendra, T., J.Handoko, D.Octaviano, R.S.Porubean and P.A.Douillet 1997. Managemnet with bacterial probiotics for virbio and virus control in an Indonesean prawn farm. pp.201-202. In : D.E.alston, B.W.Green and H.C.Clefford III (Editors) Proceedings, IV Central American Aquaculture Symposium : sustainable culture of shrimp and *Tilapia*.

Sunilkumar, M.K. 1996. Heterotrophic marine bacteria as supplementary feed for larval *Penaeus monodon*, *NAGA the ICLARM Quarter* : 23-26.

Tabbu, M.Y., R.Q. Gacutan, and R.T. Dal 1997. The beneficial effects of a probiotic product on selected chemical and growth parameters in *Penaeus monodon* pond waters. *World Aquaculture 97, Book of Abstracts*, p.454.



Prospects of biotechnology in seaweed mariculture in India

P. Kaladharan and
C.P. Gopinathan
Central Marine Fisheries Research Institute, Kochi - 682014

ABSTRACT

Red seaweeds are the major source of economically important colloids, agar and carrageenan. Agar industry in India shall become commercially attractive only when the yield of agar from the raw material is enhanced. Inherently most Indian agarophytes contain 10-20% agar only. Though species of Gelidiella *and* Gelidium *contain 35-50% agar and the quality of agar in terms of gel strength is also superior, their biomass production is very less, occurrence is seasonal and their exploitation is difficult. Hence an attempt is underway to obtain a hybrid strain of red seaweed for large scale mariculture between slow growing but high agar containing seaweed and fast growing, poor agar yielders. This article enumerates the prospective areas of seaweed biotechnology and its immediate relevance to seaweed mariculture and the related industry in India.*

Introduction

Polysaccharides produced by seaweeds form the basis of an economically important and expanding industry such as agars, agaroses, algins and carrageenans, which are used as ingredients in food, pharmaceuticals and many other industrial and consumer products. Seaweed biotechnology has got to its credit the following prospective areas:

1. Production of genetically modified strains of commercial seaweeds.
2. Acclimatization, culture and propagation of exotic varieties of seaweeds under controlled conditions.
3. Repositories of seaweeds whose occurrence is seasonal or rare and are facing the threat of extinction due to indiscriminate exploitation.
4. Bioremediation of toxic pollutants in sea and land based mariculture systems and
5. Extraction, isolation and manipulation of bioactive peptides, polysaccharides, metabolites and drugs from marine micro and macro algae that are maintained in *vitro* conditions.

Of these aforelisted prospective areas, production of genetically modified seaweed strains has the direct and immediate relevance to the seaweed mariculture and their industrial utilization, as the Indian agar yielding seaweed species yield only around 10-20% agar and possess low gel strength (100-150g/cm^2; Anon, 1995). Hence, agar industries in India can't function economically viable. To make the industry commercially attractive, a raw material that can promise better yield and superior quality is the only prerequsite. Though species of *Gelidiella* and *Gelidium* contain 35-53% agar and the quality of agar in terms of gel strength is also superior (350-400 g/cm^2), their biomass production is very less (Wheeler *et al.*, 1981) as well as seasonal and as they are firmly attached to the rocky substratum, their exploitation is difficult. Hence an attempt has been made to obtain a hybrid strain of red sea weed for large scale mariculture, between crustose thalloid but high colloid containing

seaweed and fast growing foliose type poor-yielders through genetic modification of seed stock (Kaladharan, 1998).

Protoplasts as seed stock

For all current methods of macroalgae culture upto 25% of the cultivated material is required as the seed. This reduces productivity and makes the cultivation efforts labour intensive, where the seed stock is attached to the culture rafts by hand. Instead protoplats could be used as seed stock for seaweed mariculture (Renn, 1977). Significant progress in obtaining viable protoplasts has been reported for *Porphyra linearis* (Chen *et al.*, 1994), other species of *Porphyra* (Polne-Fuller and Gibor, 1986), *Gracilaria tikvahiae* (Cheney, 1984), *Gracilaria verrucosa* (Belleanger *et al.*, 1990), *Chondurus crispus* (Le gall *et al.*, 1990), *Kappaphycus alvarezi* (Zablackis *et al.*, 1993), *Gelidium sp* (Gusev *et al.*, 1987), *Pterocladia capillocea* (Liu *et al.*, 1990) besides many other species of green and brown seaweeds (Butler *et al.*, 1990). Techniques and conditions of protoplast isolation from seaweeds, their characteristics in *in vitro*, their culture and fusion were reported earlier (Kaladharan, 1999).

Genetic modification of seaweeds

Methods for the introduction of foreign genes into other organisms include protoplast fusion; introduction of the desired genes by appropriate vectors, including insertion of transformed plasmids; infection with transformed plasmids; infection with transformed viruses or other specific pathogens; and direct insertion of the gene by electroporation or ballistics. Protoplast fusion in seaweeds can best be achieved either by electrofusion through cell fusion system (Mizukami *et al.*, 1995) or chemically induced by Poly ethylene glycol (PEG). Somatic cells from the meristematic thallus of gametophytes (n) are isolated and viable protoplasts are isolated through enzyme treatment. These protoplasts are allowed to fuse each other and karyogamy is induced. From the diploid callus through morphogenic induction tetraporophyte (2n) can be developed (Fig. 1).

Development of interspecific hybrids of *Gracilaria*

a) *Gracilaria edulis vs G. verrucosa*

G. verrucosa grows in quiet warm shallow bays and in brackish waters throughout the year. Thus fusion of protoplasts from gametophytic thalli

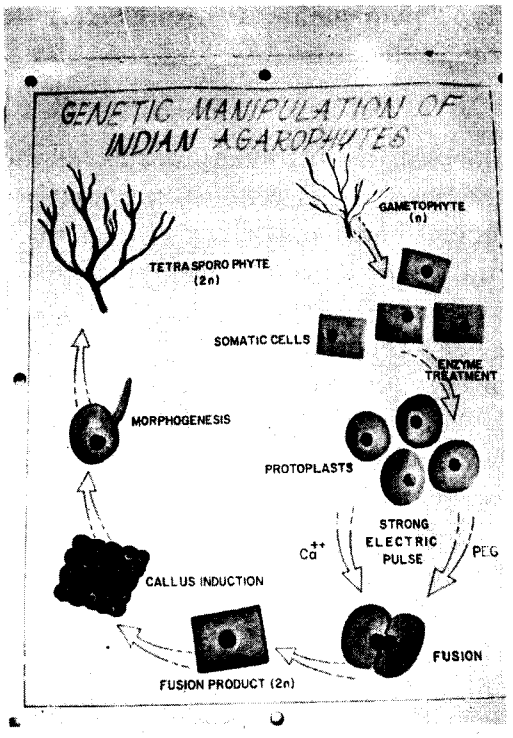


Fig. 1 : Schematic representation of hybrid strain of tetrasporophyte of *Gracilaria edulis* production through protoplast fusion

of *G. edulis* and *G. verrucosa* can result in a fusion product that can be regenerated to a new strain with improved agar and gel strength. This may be an ideal strain for brackish water habitats, as brackish water areas are the most suitable for large scale mariculture of seaweeds, integrated with bivalves and shrimps.

b) *Gracilaria corticata vs G. edulis*

Distribution of *G. edulis* is restricted to Tuticorin, Mandapam, Lakshadweep and Andaman-Nicobar Islands in India. Whereas, *G.corticata var. corticata* is widely distributed all along the Indian coasts especially the west coast (Anon. 1995). Hence the hybrid strain obtained through para sexual fusion would be a promising clone for large scale mariculture along the coasts of Kerala and Karnataka.

c) *G. edulis vs Gracilaria NBr 10*

Nova Biosciences of the Philippines has developed a fast growing subtropical strain termed *Gracilaria NBr-10* and successfully propagated in the laboratory and transferred for expansion of culture at Nova's Biological Research Station located at the Science and Technology Park of the University of Phillipines, Iloilo. Besides being a fast growing strain, *Gracilaria NBr-10* has a high agar content (18%) and a gel strength of 680-950 g/cm^2 (Anon, 1996). A somatic hybrid between *G. edulis* and NBr-10 would be suitable for tropical climate and can be cultivated in

ponds, raceways and other land based culture systems to get a highly priced product having higher gel strength.

Intergeneric hybrid between *Gelidiella acerosa* and *Gracilaria edulis*

Species of *Gelidiella* and *Gelidium* are known to contain high content of agar (45-53%) with higher gel strength (350-425 g/cm^2). An intergeneric hybrid produced either through fusion of protoplasts or through insertion of gene responsible for agar content into *G. edulis* either through vectors or mechanical means would make the seaweed mariculture and agar industry commercially attractive.

Gene mapping, sequencing and transformation

A prerequisite for genetic engineering is an adequate understanding of genome structure, sequence and gene expression. Although the homology is not complete, probes from land plants, unicellular algae and other micro organisms have been used with success to locate specific genes. Considerable work has been done using unicellular and green algae and Cyanobacteria, but reports of mapping and sequencing red marine microalgal genomes are scarce. The chloroplasts ribosomal-protein-encoding genes of the agarophyte, *Gracilaria tenuispitata* have been located, cloned and characterized (Kao *et al.*, 1990). A 1365 bp region around this gene was also sequenced; the gene order was found to be identical to that detected in the chloroplast DNA of liverwort, tobacco and maize. The plastid gene for the rp 122 protein in *Gracilaria tenuispitata* has also been isolated and sequenced (Kao and Wu, 1990).

The polymerase chain reaction (PCR) has been used to amplify *Gracilaria pacifica* nuclear and plastid ribosomal genes from algal herbarium specimens and spores (Goff and Moon, 1993). The glyceraldehyde 3 phosphate dehydrogenase gene system of the red alga *Chondrus crispus* has been investigated (Liaud *et al.*, 1993). Promoter structures, intron/exon organization, genomic complexity, differential expression of genes and transcript level of the genes in the gametophytes and the protoplasts were determined, paving the way for genetic transformation in *C. crispus*

Five of the twenty on red algal genera studied by Goff and Coleman, (1990) were found to contain circular double stranded DNA plasmids. Some

of these were isolated and one 3.0 kbp plasmid from *Gracilariopsis lemaneiformis* was sequenced to reveal two potential open reading frames. In this species, plasmids are present in a high copy number/cell and may provide useful vectors for algal transformations. The DNA sequence and structural organization of the GC2 plasmid from the agarophyte, *Gracilaria chilensis* has been determined (Vilemur, 1990). This 3827 bp circular plasmid has one major open reading frame that generates a transcript and could encode a 411 aminoacid polypeptide. Henry and Meints (1994) suggests that recombinant viruses, particularly the large ds DNA viruses that are known to infect eukaryotic algae can be used as transformation vectors for marine microalgae.

Conclusion

The polysaccharides from seaweeds are the basis of well established growing industries. Many consumer products rely on their unique properties and would not exist without their availability. With increased research emphasis and wide applications possibly even new polysaccharides from new seaweed strains might emerge. Genetic understanding and techniques for the introduction of foreign genes are evolving. On the whole the prospect of biotechnology are bright to seaweeds, their mariculture and polysaccharides they produce.

References

Anon. 1995a. *Economically Important Seaweeds.* Special publication No. **62**, CMFRI 1-35

Anon. 1995 b. *Infofish International*, 6 : 43-44.

Bellanger, F.m N,C. Verdus, V. Henocq and D. Christiaen1990. Determination of the composition of the fibrillar part of *Gracilaria verrucosa* cell wall in order to prepare protoplasts. *Hydrobiol.*, 204/205: 527-531.

Butler, D.M., L.V. Evans and B. Kloareg 1990. Isolation of protoplasts from marine microalgae. In : *Introduction to Applied Phycology* Ed: I. Akatsuka, SPB Academic Publishing, The Hague 647-668.

Chen, L.C-M, J. Craigie and Z.K. Xie 1994. Protoplast production from *Porphyra linearis* using a simplified agarase procedure capable of commercial application. *J. Appl. Phycol.*, **6**: 35-39.

Cheney, D.P. 1984. Genetic modification in seaweeds: applications to commercial utilization and cultivation. In *Biotechnology in the Marine Sciences* Eds: R. Colwell, E.Praiser and A. Sinsky, Wiley Intersciences, New York, 161-175.

Goff, L.J. and A.W. Coleman 1990. Red algal plasmids. *Curr. Genet.* **18**: 557-565.

Goff, L.J. and D.A. Moon 1993. PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores. *J. Phycol.*, **29**: 381-384.

Gusev, M.V., A.H. Tambiev, N.N. Shelyastina and R.R. Aslanyan 1987. Callus formation in seven species of agarophyte marine alga. *Mar. Biol.*, **95**: 593-597.

Henry, E.C. and R.H. Meints 1994. Recombinant viruses as transformation vectors of marine algae. *J. Appl. Phycol.*, **6**: 247-253.

Kaladharan, P. 1998. Protoplasts - A powerful tool in genetic manipulation of commercial seaweeds. In *Proceedings of the First National Seminar on Trends in Marine Biotechnology*, Ed. S.Lazarus and S.G. Prakash Vincent, 83-88.

Kao, J. and M. WU 1990. The sequence of the plastid encoded rp 122 protein in marine macroalgae *Gracilaria tennuistipitata. Nuclic Acids Res.*, **18**: 3067

Kao, J., M. WU and Y.M. Chiang 1990. Cloning and characterization of chloroplast ribosomal protein-encoding genes rp 116 and rp 3 of the macro marine algae *Gracilaria tennuistipitata. Gene* **90**: 221-226.

Le Gall, Y., J.P. Braud and B. Kloareg 1990. Protoplast production in *Chondrus crispus genotypes* (Gigartinales, Rhodophyta) *Plant Cell Rep.*, **8**: 582-585.

Liaud, M.F., C. Valentin, U. Brandt, F.Y. Bouget, B. Kloareg and R. Cerff 1993. The Gadph gene system of the red alga *Chondrus crispus:* Promoter structures, intron/exon organization, genomic complexity and differential expression of genes. *Plant Mol. Biol.*, **23**: 981-994.

Liu, X-W., C. Rochas and B. Kloareg 1990. Callus culture of *Pterocla capillacea* (Rhodophyta) and analysis of cellwall polysaccharides. *J. Appl. Phycol.*, **2**: 297-303.

Mizukami, Y., M. Okauchi, H. Kito, S. Ishimoto, T. Ishida and M. Fuseya 1995. Culture and development of electrically fused protoplasts from red marine

alga *Porphyra yezoensis and Porphyra suborbiculata. Aquaculture*, **132**: 361-367.

Polne-Fuller, M. and A. Gibor 1986. Calluses, cells and protoplasts in studies towards genetic improvement of seaweeds. *Aquaculture*, **57**: 117-123.

Renn, D. 1997. Biotechnology and red seaweed polysaccharide industry: Status, needs and prospects. *Tibtech*, Elsevier Science, **15**: 9-14.

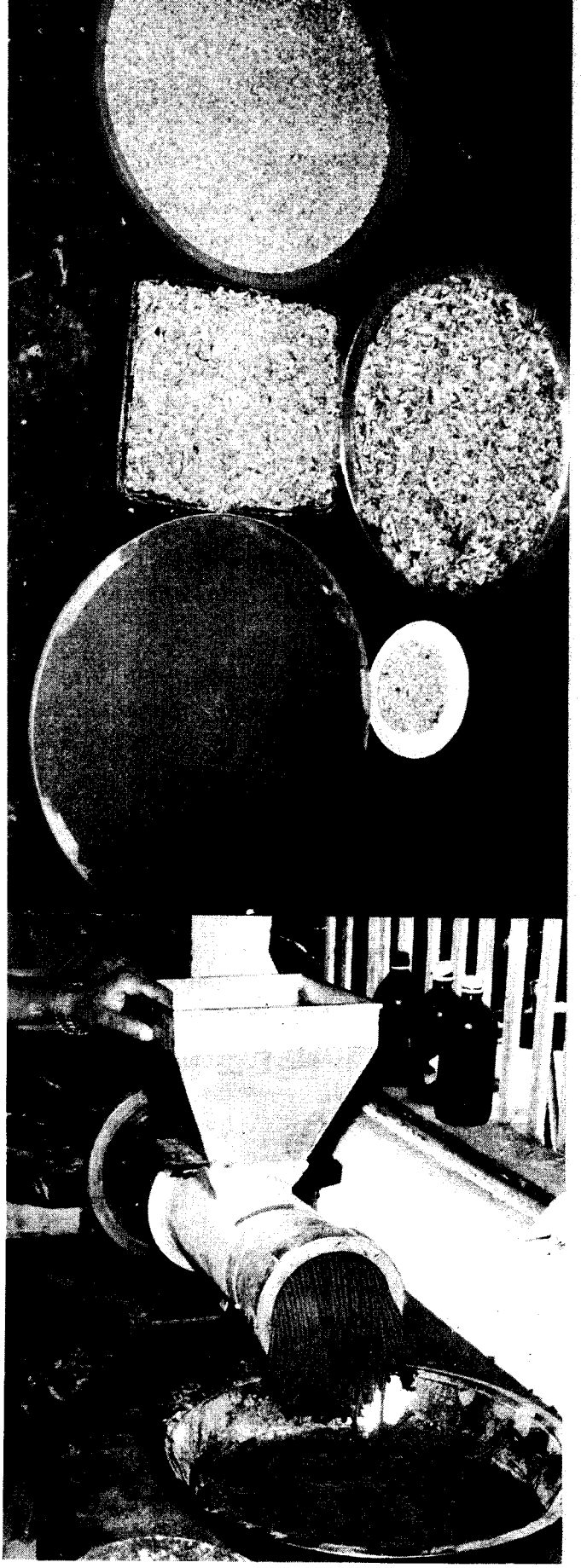
Villemur, R. 1990. The DNA sequence and structural organization of the GC 2 plasmid from the red alga *Gracilaria chilensis. Plant Mol. Biol.*, **15**:237-243.

Wheeler, W., M. Neushul and B. Harger 1981. Development of a coastal marine farm and its associated problems. *Int. Seaweed Symp.*, **10**: 631-636.

Zablackis, E., V. Veerland and B. Kloareg 1993. Isolation of protoplasts from *Kappaphycus alvarezii var. tambalang* (Rhodophyta) and secretion of *i*-carrageenan fragments by cultured cells. *J. Exp. Bot.*, **44**: 1515-1522.

Efficacy of compounded feeds prepared from fermented mantis shrimp on growth performance of *Peneaus indicus* post larvae

Manpal Sridhar and
M . Chandrashekar*
C.M.F.R.I., Cochin-682 014
**Cochin University of Science & Technology , Cochin - 682022*



ABSTRACT

Ten compounded feeds incorporating 20 - 100 % of mantis shrimp (Oratosquilla nepa), *fermented using* Bacilles licheniformis *and* Beauveria sp. *respectively and containing 32 to 49 % crude protein were fed to post larvae of* Penaeus indicus *(initial average weight 0.19 ± 0.06 g) for a period of 45 days . A significant (P< 0.05) increase in body weight was recorded in all*

the B . licheniformis *treatment groups as compared to control post larvae . The group of post larvae maintained on the diet with 80 % incorporation of mantis shrimp accorded the best growth in terms of average body weight (563.16 %). The FCR for these feeds ranged from 1.56 to 2.08 as compared to a FCR of 5.0 on the control feed, Survival in all the treatments was in the range of 86 to 98% and not significantly different from the control.*

Feeds containing 20 to 100% mantis shrimp fermented using Beauveria *sp. elicited a poor growth response as compared to feeds prepared from mantis shrimp fermented using* B. licheniformis. *At the 60 % incorporation however, a slightly better response was obtained wherein a 316 % increase was observed in comparison to 213% in case of control animals . The feed conversion ratio and survival rates also did not show any significant (P > 0.05) variation in comparison to the control group.*

Overall growth performance and carcass yields show the superiority of fermented mantis shrimp and prove its efficacy as a feed ingredient in shrimp feeds.

Introduction

In the context of increasing global demand for prawns and promotion of prawn culture the key factor becomes the provision of appropriate feed , which constitutes around 50 – 80 % of the total operational cost in aquaculture . Scarcity of high quality feed ingredients , their location specificity and cost remain critical factors in the large scale production of practical feed .

Fishery wastes (trash fish , mantis shrimp , squid waste , prawn heads and peels) along with other agro- industrial wastes are highly attractive for explotation on account of their protein , chitin, carbohydrate and cellulose contents and also for the presence of certain pigments and flavours . Most of these however , require a pre- treatment in some physical or chemical form , and are therefore uneconomical on a large scale . The use of marine micro - organisms to convert protein , carbohydrate , chitin and other low – cost , agro - industrial wastes into foodstuffs, rich in protein employing solid state fermentation seems to be promising and presents a novel and

cheaper method of compounding nutritious feeds for larval and grow-out stages of shrimp . Therefore, in the present investigation Solid State Fermentation (SSF) technology was employed for the production of microbial protein as well as protein enrichment of mantis shrimp *(Oratosquilla nepa*) using one strain of bacteria *B. licheniformis* and one strain of fungi *Beauveria* sp . in order to evaluate it's suitability for aquafeed formulation. The fermented material was incorporated at varying concentrations into a formulated feed base and fed to post larvae of *P . indicus* for a period of 45 days in order to evaluate it's effect on growth and survival and overall suitability in aquafeeds .

Materials and methods

Solid State Fermentation of mantis shrimp was carried out following the method suggested by Ramesh and Lonsane (1987) . Necessary changes were however , made in the medium composition and methodology after optimization of process parameters .

Microorganisms

Bacillus sp BTM 01 (*B. licheniformis*) and Fungi (*Beauveria* sp.) isolated from brackish water samples using ZoBell's marine agar for the former and mycological agar and streptomycin in sea water (35 ppt.) for the latter were maintained as agar slants and subcultured every week .

Preparation of solid substrate

Fresh mantis shrimp was collected from the Cochin Fisheries Harbour and transported immediately to the laboratory in polythene bags , sorted off adhering debris, washed well three to four times in running tap water and dried at 70 ° C for 24 h in an oven . The dried material with a moisture level of < 10 % was powdered in a laboratory pulveriser and sieved through a sieve of 200 μ to obtain uniform particle size. The pH was determined and so also the moisture content by using one g samples in duplicate. The solid substrate was dispensed as 5 g aliquots in petriplates and in 250 ml conical flasks and adjusted to the desired level of moisture content

(between 30-60 %) with sea water adjusted to the optimum pH (pH 8.0 and 12.0 for bacteria and fungi respectively). The flasks and petriplates along with their contents were autoclaved at 121 ° C for 60 min. and cooled down to room temperature .

Inoculum preparation

Bacteria : A loopful of 24 h old agar slant culture of *B. licheniformis* was first grown in 10 ml of ZoBell's marine broth for 18 h at room temperature (28 ± 2 ° C). One ml of this culture was then transferred asceptically to 50 ml nutrient broth and incubated on a rotary shaker at 150 rpm for 18 h at room temperature . Cells were harvested by centrifugation (10,000 rpm for 15 min. at 4 ° C) and then were made to 10 ml using sterile physiological saline (0.85 % NaCl) after repeated washings . This prepared cell suspension was used as inoculum for fermentation .

Fungi : 20 ml of sterile physiological saline containing 0.1% Tween was added to each fully sporulated (2 week old) slant culture (raised on Bennet's agar prepared with aged sea water) by means of a sterile pippete . The spores were scraped using an inoculating needle under strict asceptic conditions and the spore suspension obtained was adjusted to the desired concentration using sterile physiological saline.

Inoculation and incubation

The prepared inocula were adjusted to a concentration of 2 mg dry cell equivalent in one ml of cell suspension for bacteria and 2 ml of spore suspension per flask (inoculum level selected randomly) for fungi and added to the sterilised moist media in flasks . The contents were mixed thoroughly and incubated in a slanting position at 35 ° C and 28 ° C for bacteria and fungi respectively with 60 – 70 % relative humidity for 48 to 72 h period . After fermentation , the contents were dried to a constant moisture level and proximate composition analysed .

Feed preparation : A set of five feeds each were formulated by incorporating 20, 40, 60 , 80 and 100 % of mantis shrimp fermented using *B . licheniformis* and designated as B MS 1, B MS 2 , B MS 3 ,

B MS 4 and B MS 5 respectively and a set of five feeds each were formulated by incorporating 20 , 40, 60 , 80 and 100 % of mantis shrimp fermented using *Beauveria* sp and designated as MS 1, MS 2, MS 3, MS 4 and MS 5 respectively . The diet devoid of fermented material designated as C served as control . The % incorporation of the other feed ingredients in the feed base and their composition are given in Table 1 . All powdered and weighed ingredients excluding tapioca flour were mixed together and thoroughly blended .Tapioca was gelatinised in hot water and then mixed with other ingredients . The prepared dough was extruded through a 1mm die and the feed pellets broken manually into smaller pieces of 3-5 mm length . The pellets were sun dried to less than 10 % moisture and stored in labelled plastic containers at room temperature (28 ± 2 ° C).

Table 1. Percentage incorporation of fermented mantis shrimp * and other feed ingredients used for feed formulation.

Ingredients (g/ 100 g dry diet	Diets					
	C	MS1	MS2	MS3	MS4	MS5
Mantis shrimp*	0	20	40	60	80	100
Shrimp meal	25	05	-	-	-	-
Fish meal	20	20		05	-	-
Soyabean meal	20	20	20	05	-	-
Groundnut oilcake	15	15	15	15	-	-
Tapioca powder	12.5	12.5	12.5	12.5	12.5	3.0
Oil [1]	04	04	04	04	04	04
Vitamin mixture [2]	02	02	02	02	02	1.5
Mineral mixture [3]	01	01	01	01	01	01
Cholesterol	0.5	0. 5	0.5	0.5	0.5	0.5

* Mantis shrimp fermented using *B. licheniformis* or *Beauveria* sp.

[1] Oil – mixture of a 1: 1 combination of vegetable and fish oil.

[2] Vitamin mixture (mg/ 100 g dry diet)- para aminobenzoic acid 5.55 ; inositol 22.06 ; nicotinic acid 22.21 ; Ca pantothenate 33.31; pyridoxine – Hcl 6.66 ; riboflavin 4.44 ; thiamine – Hcl 2.22 ; menadione 2.22 ; Betacarotene 5.55 ; tocopherol 11.10 ; calciferol 6.66 ; Na – ascorbate 110.3 .

[3] Mineral mixture (g /kg dry diet) K_2PO_4 1.008 ; Na_2HPO_4-$7H_2O$- 2.167; $Ca(H_2PO_4)$ $2H_2O$ - 2.671 ; $CaCo_3$ -0.978 ; Ca-lactate -1.663 ;KCl -0.282; $MgSO_4$ $7H_2O$ - 0.048 ; $MnSO_4$ $6H_2O$ - 0.0108 ; $CuCl_2$ -0.0015 ;KI- 0.0023; $CoCl_2 6H_2O$ - 0.0141 ; Celufill -0.0216 .

Animal experiments

Post larval *P. indicus* , having an average initial length of 3.1 ± 0.3 cm and an average initial weight of 0.19 ± 0.06 g , procured from a commercial shrimp hatchery , were used for the feeding experiments. The larvae were acclimatized to laboratory conditions for a week prior to starting the feeding trial during which period they were maintained on the control feed . A set of post larvae were sacrificed and their body composition analysed before the start of the feeding experiment.

The post larvae were housed in 10 litre plastic tubs each provided with individual aeration . Each treatment was carried out in triplicate with twenty post larvae present in each replicate . Feeding was carried out at the rate of 20 % of the body weight in two divided doses at 10 and 18 h daily .The salinity of the water used was maintained at 35± 1 ppt ; temperature at 28.0 ± 2.0 ° C and dissolved oxygen at 5.8 ± 0.4 ml / lit. throughout the experimental duration of 45 days . One third water exchange was carried out daily while complete water exchange was done every third day . Left over feed and faecal matter were removed daily . At the termination of the experiment post larvae from each group were weighed and their carcasses dried , powdered and analysed for their proximate composition.

Analytical methods

Moisture , crude protein , crude fat , crude fibre and ash contents in fermented mantis shrimp , feed ingredients, prepared feeds and carcasses were determined by standard procedures of A. O . A . C . (1990). Water analysis was carried out as per the method out lined by APHA (1980).Water stability of the feeds was determined by the method of Jayaram and Shetty (1981). RNA content of the fermented mantis shrimp was measured by the method of Fleck & Munro (1962) and DNA by the method of Burton (1956). Data obtained in the feeding experiments were subjected to statistical analysis ((Snedecor and Cochran, 1973).

Results and discussion

The use of microorganisms to convert carbohydrates , lignocelluloses and other industrial wastes into protein rich food and feedstuffs has been well documented(Barbesgaard, 1977; Toyama, 1976 ; Mudgett, 1986 and Ghildyal *et., al.*,1981) . Fishery wastes on account of their high protein and polyunsaturated fatty acid contents, pigments and flavours offer great potential in aquafeed formulation. However, their high perishability under normal ambient conditions remains a hurdle in their maximum utililisation . Mantis shrimp was therefore collected and subjected to fermentation separately using both bacteria *B . licheniformis* and fungi *Beauveria* sp . respectively . In the bacterial fermentation apart from the mild change of colour obtained after fermentation was complete (48 h) , no other major physical change in appearance was observed . In the case of fermentation with fungi which took 72 h there was pronounced spore formation leading to a visible change in colour and a strong mouldy odour . Protein content increased in both the fermentations though the increase was higher in case of bacterial fermentation as compared to the fermentation with fungi . The protein content increased from an initial value of 32.40 % to 44.60

upon fermentation with *B. licheniformis* while an increase of 37.65% was recorded upon fermentation with *Beauveria* sp. Increase in protein content of banana meal for poultry feeding from 6 to 16 % was recorded (Leon , 1988) and in cassava from an initial value of 1.28 g / 100 g dry matter to 14.32 g / 100 g dry matter (Balagopalan and Padmaja , 1988). All other nutrients showed a desirable decrease in both the fermentations, which was indicative of the ability of the microorganisms to carry out bioconversion (Sridhar and Chandrashekar, 1985). The present work on fermentation of mantis shrimp - a fishery waste , is probably one of the first reported and there is no similar work to compare results .

The nucleic acid content of *B . licheniformis* fermented mantis shrimp was 2.2 % and in *Beauveria sp* fermented mantis shrimp it was 0.8 % . Both these values are within the safe limits prescribed for nucleic acids in single cell proteins (Litchfield , 1968).

The results of the proximate composition analysis of the five feeds prepared incorporating 20 to 100 % of mantis shrimp fermented with *B . licheniformis* as well as the five feeds prepared incorporating 20 to 100 % of mantis shrimp fermented with *Beauveria* sp. are presented in Table 2 A & B. The protein content of the former ranged between 40 to 49 % and the latter between 32 to 40 % . The control diet had a protein content of 47 % . Crude fat ranged from 10 to 11 . 36 % ; crude fibre from 2.94 to 3.46 % and ash from 14.98 to 17.31 % respectively for the bacterially fermentated feeds while for the diets prepared using mantis shrimp fermented with fungi crude fat ranged between 10.96 to 11.41 %, fibre from 2.42 to 3.00 % and ash from 14.92 to 17.09 % . All feeds met the nutritional specifications of shrimp feeds .

Table Proximate composition of the control and experimental diets (prepared from mantis shrimp feeds fermented with *B. licheniformis*) used for the feeding trails .

Nutrient	Diets (% dry matter)					
	C	BMS1	BMS2	BMS3	BMS4	BMS5
Dry matter	92.22	91.42	93.42	91.16	90.48	92.12
Crude protein	46.81	48.14	49.79	52.14	54.29	56.95
Ether extract	10.78	6.48	8.42	6.96	7.63	7.21
Crude fibre	2.13	2.06	2.21	2.33	2.41	2.56
NFE *	23.93	28.96	24.67	23.51	20.82	19.27
Ash	16.35	14.36	14.91	15.06	14.85	14.01

* Nitrogen free extractives calculated as 100 – (% crude protein + % crude lipid + % crude fibre + % ash + % moisture)

Table 2 B. Proximate composition of the control and experimental diets (prepared from mantis shrimp feeds fermented with *Beauveria sp.* fungi) used for the feeding trails .

Nutrient	Diets (% dry matter)					
	C	MS1	MS2	MS3	MS4	MS5
Dry matter	92.22	90.16	90.47	92.48	91.86	90.07
Crude protein	46.81	40.25	36.84	32.48	31.68	37.04
Ether extract	10.78	11.41	10.96	11.02	11.38	11.02
Crude fibre	2.12	2.86	2.42	2.54	2.81	3.00
NFE *	23.93	28.39	33.64	39.08	39.00	34.02
Ash	16.35	17.09	16.14	14.88	15.13	14.92

* Nitrogen free extractives calculated as 100 – (% crude protein + % crude lipid + % crude fibre + % ash + % moisture) .

The water stability of these feeds is presented in Figure 1A & B . The initial dry matter (DM) content of these feeds was above 95 % and only 2 –4 % loss was observed within one hour . From 2-5 h most feeds exhibited an additional loss of 8 % in DM at the rate of approximately 2 % / h . No cracking or physical disintegration of the

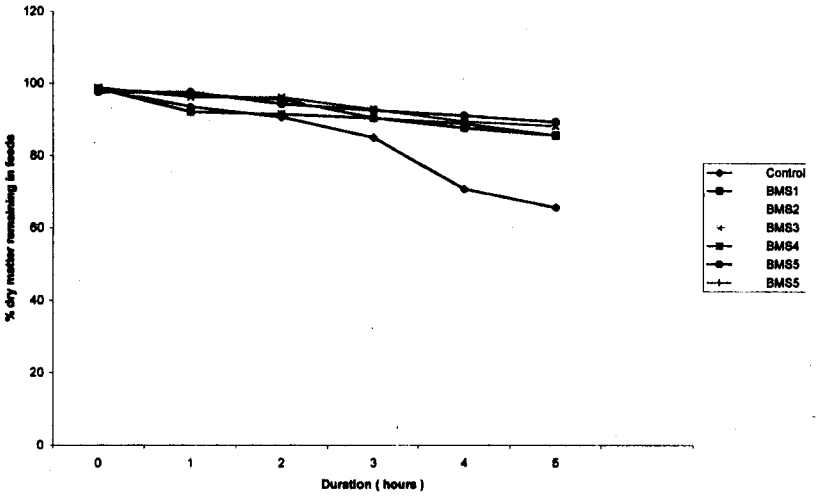


Fig. 1A Water stability of the control and experimental feeds prepared using *B.lichenIformis*

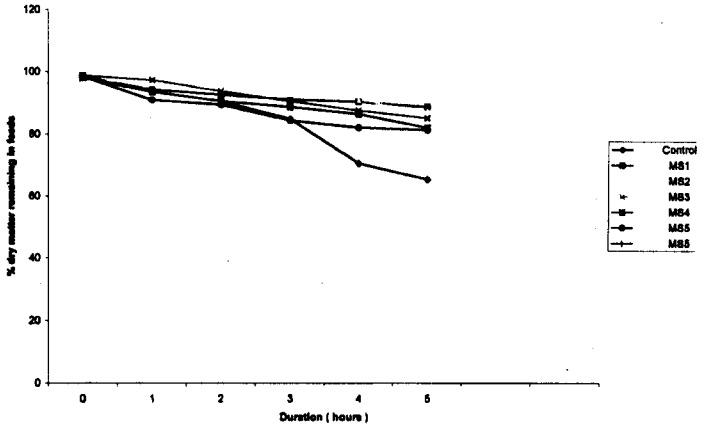


Fig. 1B Water stability of the control and experimental feeds prepared using *Beauveria sp.*

feed pellets was observed , though desirable softening was observed in all feeds and the post larvae readily accepted the pellets.

Single cell proteins have many advantages over other conventional feed ingredients (Tacon , 1990) and have been successfully used in animal and poultry feeds but their use in aquaculture so far has been limited to a few studies incorporating bacterial biomass indirectly or directly into finfish and shellfish feeds (Yasuda and Taga , 1980; Gatesoupe ,1980 , 1989 ; Mohamed , 1996 and Sridhar and Chandrashekar, 1996).The results of feeding *P. indicus* post larvae the feeds prepared incorporating mantis shrimp fermented using *B. licheniformis* are given in Table 3 .

Table 3. Growth performance of *P.indicus* post larvae reared on B. licheniformis fermented mantis shrimp feeds for 45 days.

		Experimental Diets				
Parameters	C	BMS1	BMS2	BMS3	BMS4	BMS5
Final body* length (cm)	4.7a (± 0.004	5.9 b (±0.010)	6.8c (± 0.009)	6.1 b (±0.011)	6.9 c (± 0.006)	6.1 b (±0.014)
Final body** weight (g)	0.595a (± 0.034)	0.82b (±0.04 8)	0.96b (± 0.020)	0.99b (± 0.120)	1.26c (± 0.052)	0.86b (±0.032)
Average gain in length(%)	51.61	90.32	119.35	96.77	122.58	96.77
Average gain In weight (%)	213.16	331.58	405.26	421.05	563.16	352.63
Specific growth rate(%)	0.9	1.4	1.71	1.78	2.38	1.49
Feed conversion ratio (FCR)	5.0 a	2.0 b	1.93 b	2.08b	1.56b	1.68b
Survival (%)	80a (± 2)	86a (± 2)	96a (± 4)	94a (± 6)	98a (± 2)	90a (± 4)

* Initial body length = 3.1 ± 0.3 cm

** Initial body weight = 0.19 ± 0.06 g.

Each mean ± standard deviation in parenthesis is based on measurements from triplicate tanks . Row means having the same superscripts are not significantly different ($P < 0.05$) .

There was a significant ($P < 0.05$) increase in body weight in all the treatment groups in comparison to control post larvae with group BMS 4 (80 % incorporation of mantis shrimp) giving the best growth in terms of weight gain (563.16 %) . The feed conversion ratio ranged from 2.08 on feed BMS 3 to 1.56 on feed BMS 4 and was significantly lower ($P < 0.05$) than the control FCR of 5.0 . Survival in all treatments was in the range of 86 to 98 % and not significantly different from that of the control group where 90 % survival was obtained .

The results of the feeding trial incorporating varying levels of mantis shrimp fermented using *Beauveria* sp. are presented in Table 4. These feeds elicited a poor growth response as compared to feeds prepared using *B . licheniformis* fermented mantis shrimp in terms of both increase in weight and length . At the 60 % incorporation (MS 3) however, a slightly better response was obtained wherein a 316 % increase in body weight was observed in comparison to an increase of 213 % in case of control post larvae. The feed conversion ratio and survival rates of all experimental groups also did not differ much from those of the control post larvae. The most probable explanation for this result with mantis shrimp fermented using *Beauveria* sp. may be due to the fact that the fungi was not that effective in breaking down chitin which comprises a very high percentage in mantis shrimp as effectively as *B. licheniformis* . However, as there was an increase in the protein content of mantis shrimp upon fermentation with *Beauveria* sp. standardisation of process parameters such as increase in the time for fermentation , fermentation with other fungal species having chitinolytic properties and/ or combination with a chitinolytic bacterial strain will definitely yield superior results in terms of growth performance of aquatic culture animals.

Table 4. Growth performance of *P . indicus* post larvae reared on *Beauveria* fermented mantis shrimp feeds for 45 days .

Parameters	Experimental Diets					
	C	MS1	MS2	MS3	MS4	MS5
Final body* length (cm)	4.7 a (± 0.004)	5.0 a (±0.008)	5.1 a (± 0.012)	5.8a (±0.018)	4.9 a (± 0.018)	4.6 a (±0.002)
Final body** weight (g)	0.595 a (± 0.034)	0.62 a (±0.046)	0.64a (± 0.052)	0.79a (± 0.028)	0.595a (± 0.022)	0.570a (±0.038)
Average gain in length(%)	51.61	61.29	64.54	87.10	58.06	48.39
Average gain in weight (%)	213.16	226.32	236.84	315.79	213.16	200.00
Specific growth rate (%)	0.90	0.96	1.0	1.33	0.90	0.84
Feed conversion ratio (FCR)	5.0 a	3.14a	3.60a	3.00	4.22a	3.79a
Survival (%)	80a (± 2)	88a (± 4)	80a (± 6)	92a (± 8)	70a (± 3)	94a (± 6)

* Initial body length = 3.1 ± 0.3 cm
** Initial body weight = 0.19 ± 0.06 g
Each mean ± standard deviation in parenthesis is based on measurements from triplicate tanks . Row means having the same superscripts are not significantly different ($P < 0.05$) .

The carcass body composition of *P. indicus* post larvae after feeding mantis shrimp fermented using *B . licheniformis* for a period of 45 days is elaborated in Table 5 . There was an increase in the dry matter carcass content in all treatment groups (range 22.36 – 32.60 %) as compared to the DM content of 22.36 % in case of control animals. Protein content also was higher in all groups fed fermented mantis shrimp with BMS5 (100 % incorporation) recording the highest protein content of 71%. Though a moderate increase was recorded in the lipid content of all the animals fed fermented

mantis shrimp (5.13 - 6.02 %) it was statistically insignificant as compared to the lipid content of 4.76 % obtained in post larvae maintained on the control feed. A significant decrease was obtained in the ash content of all post larvae fed varying concentrations of fermented mantis shrimp (12.50 - 15.56 %) in comparison to 20.93 % ash content of the control post - larvae. This result can again be correlated to the beneficial changes under gone by mantis shrimp, otherwise high in ash during SSF. With regard to carcass composition of post larvae maintained on mantis shrimp fermented using *Beauveria* sp. (Table 5) the increase in DM content obtained in all the experimental groups was comparatively lower to that obtained on feeds fermented with *B. licheniformis* .The increase in protein content observed in these groups (64.96 % in MS1 with 20% incorporation to 71.66 % in MS5 with 100 % incorporation) was not in keeping with the results of the growth study. Likewise an increase in lipid content and decrease in ash contents of experimental groups in comparison to control as observed in the case of post larvae maintained on diets of mantis shrimp fermented using *B. licheniformis* was also obtained in post larvae fed the diets fermented with *Beauveria sp.* Growth performance and percentage carcass yields showed that use of the enriched material did not adversely affect the growth of *P. indicus* post larvae. The growth *per se* obtained in these post larvae fed with SCP enriched feeds was superior in comparison to control, feed intake was less which resulted in a reduction in feed usage and good FCR's.

Table 5. Carcass body composition of *P.indicus* post larvae reared on *B. licheniformis* and *Beauveria* fermented mantis shrimp feeds for 45 days.

Body Composition*	Control	BMS1	BMS2	BMS3	BMS4	BMS5	MS1	MS2	MS3	MS4	MS5
% dry matter	28.79	30.11	31.46	30.85	32.00	32.60	24.62	25.32	23.70	24.36	24.10
% moisture	71.21	69.89	68.54	69.15	68.00	67.40	75.38	74.68	76.30	75.64	75.90
% crude protein	64.96	70.46	69.39	69.98	70.09	71.00	69.48	66.28	71.17	70.48	71.66
% crude lipid	5.13	5.65	5.82	5.71	6.02	5.94	5.41	5.17	6.50	6.45	6.36
% ash	12.50	15.01	15.36	14.04	15.19	15.56	11.76	11.54	11.23	12.04	11.82

* Initial values- Dry matter-22.36%; moisture-77.64%; protein- 60.79%; Lipid- 4.76 %; ash-20.93 %.

BMS1to5 feeds formulated using mantis shrimp fermented with *B . licheniformis.*
MS1to5 feeds formulated using mantis shrimp fermented with *Beauveria.*

Though one of the first works reported on the use of SSF for marine products , the results of the present study are quite encouraging and clearly indicate that mantis shrimp can be nutritionally enriched and preserved by solid state fermentation. Further experiments incorporating fermented mantis shrimp at lower levels will generate more data on the level of mantis shrimp incorporation yielding the most promising growth and survival in post larvae, juvenile and adult shrimp for future application in shrimp culture.

The studies carried out are preliminary and offer immense scope to provide beyond doubt that solid state fermentation, which is simple and economic, is the appropriate technology for the futuristic aquafeed industry.

Acknowledgement

The authors express their gratitude to the Department of Biotechnology, Govt. of India, New Delhi, for the award of the National Associateship under which this work was carried out.

References

A . O . A . C . 1990 *Official methods of analysis of A.O.A.C.* , , Vol 1 15th edition, K . Helrich (Ed.) . Association of Analytical Chemists, Inc. , Arlington VA, 684 p .

APHA 1980. Standard methods for examination of water and waste water, 15th Edn. American Public Health Association , American water works Association and Water Pollution Control Federation, Washington DC .1134 p.

*Burton , K . 1956 *Biochem . J .* , : 315-322.

Barbesgaard , P. 1977. Industrial enzymes produced by members of the genus *Aspergillus* . In *Genetics and physiology of Aspergillus* , by Smith , J. E. and Pateman, J. A(Eds) . Academic Press, London, 391-401.

*Fleck , A. and H. N . Munro. 1962 *Biochim . Biophys. Acta.* , 55 : 571- 583.

Gatesoupe , F. J. 1980, Further advances in the nutritional and antibacterial treatments of rotifers as food for turbot larvae , *Scophthalmus maximus* L. In: N. de Pauw, E . Jaspers, H. Ackefors and N. Wilkins (Eds .) ,

Aquaculture a biotechnology in Progress ,Vol. 2. European Aquaculture Society , Bredene , Belgium, pp. 721-730 .

Gatesoupe , F. J., T. Arakawa , and T. Watanabe, 1989. The effect of bacterial additives on the production rate and dietary value of rotifers as food for Japanese flounder, *Paralichthys olivaceus* . *Aquaculture*, 83 : 39-44.

Ghildyal ,N .P. , S.V. Ramakrishna , P.N. Devi , B.K. Lonsane and H.K. Asthana , 1981. Large scale production of pectolytic enzyme by solid state fermentation . *J . Fd . Sci . Technol* . **18** , 248 -251.

Jayaram , M. G and H. P . C. Shetty 1981.Formulation, Processing and water stability of two new pelleted fish feeds . *Aquaculture* , **23** : 355-359.

Mudgett , R. E. 1986. Solid state fermentation . In: Manual of Industrial Microbiology and Biotechnology Processes , by Demaain ,A.L. and Soloman , M.A. (Eds) . *Amm . Soc . Microbiol . Washington* , 66-83.

Mohamed , K. S. 1996. Heterotrophic marine bacteria as supplementary feed for larval *Penaeus monodon* . *NAGA The Iclarm quarterly* **17** (1) 23-26 .

Ramesh , M. V. and B . K .Lonsane 1987. Solid state fermentation for production of Alpha- amylase by *Bacillus megaterium* !6 m . *Biotechnol. Letters*, **9** , 323-328 .

Snedecor ,G.W . and W. G. Cochran 1973. *Stastical methods*. 6 th ed . Iowa state univ. Press Aner . IOWA , 593 p .

Sridhar , M. and M . Chandrasekar 1996. Development of aquafeeds for shrimp employing solid state fermentation (SSF). Post doctoral project report submitted to the Department of Biotechnology (Govt. of India) New Delhi.

Tacon , A. G . J .1993. Feed ingredients for warm water fish : Fish meal and other processed feedstuffs . *FAO Fisheries Circular* No 846 .Rome FAO.64 p .

Toyaman , N. 1976. Feasibility of sugar production from agricultural and urban cellulosic wastes with *T . viride* cellulase. *Biotechnol . Bioeng . Symp.*, **6** , 207-219 .

Yasuda , K and N . Taga 1980. Culture of *Brachionus plicatilis* Muller using bacteria as food . *Bull . Jap .Soc . Sci . Fish* . **46** (8) 933 - 939 .

* Not refered to in original

Biotechnological approach in *in-vitro* pearl production

S. Dharmaraj and C. P. Suja
Tuticorin Research
Centre of Central Marine Fisheries Research Institute, Tuticorin

ABSTRACT

Explant culture of mantle tissue of the pearl oyster Pinctada fucata *(Gould) was undertaken at the tissue culture laboratory of CMFRI, Tuticorin. Mantle tissues were routinely cultured in Medium 199 and Pf 35 individually and combindly supplemented with 10% foetal calf serum at 28°C, pH 7.8. Explants released numerous epitheloid - like and fibroblast - like cells. Cells were in assorted sizes and formed colonies. Nacreous secretion by the cells was observed on culture plates. The study represents a new tool for cellular approach in in-vitro pearl production.*

Introduction

The Indian pearl oyster *Pinctada fucata* is used for the production of cultured pearls. The pearl production technology was perfected and the techniques involved were standardised. Manipulation of colour and quality of cultured pearls was rather difficult in *in-vivo* culture. Hence an attempt on *in-vitro* pearl production was made

through tissue culture technique. The work on pearl oyster mantle tissue was limited. Bavelander and Martin (1949) organised organ culture of mantle of the marine mollusc *Pinctada radiata* and obtained the deposition of conchiolin crystals typical to those found in normal regenerating shells. Machii and Wada (1989) reported the secretion of organic substance by the cells dissociated from an explant culture of pearl oyster mantle tissue. A similar approach was undertaken at the tissue culture laboratory of the Central Marine Fisheries Research Institute, Tuticorin. The results obtained in the present study are presented here.

Materials and methods

A. *Depuration of oysters:* The pearl oyster *Pinctada fucata* brought from the farm was cleaned thoroughly and placed in U.V. treated running seawater in a fibreglass tank for depuration for 3-7 days. Everyday the oysters and the tank were cleaned to remove the organic waste.

B. *Preparation of tissues :* The depurated oysters were dipped in 70% ethanol for 15 seconds and taken to clean room for further processing. They were cut open by a sterile knife and the mantle tissue was removed. The pallial organs at the free end and the connective tissue at the distal end were cut and removed. The mantle strip thus obtained was cut into pieces of 1 sq.mm.

The mantle pieces were washed six times in 10 ml of sterile seawater (SSW) or in a balanced salt solution (BSS) in petri dishes. The composition of BSS is shown in Table 1.

Table 1. Balanced salt solution for marine mollusc (MM BSS)

Components	MM BSS (g/l)
NaCl	26.22
KCl	1.08
Mg SO_4	3.18
Mg Cl_2	2.20
Ca Cl_2	1.12
Na HCo_3	0.30
Na H2- PO_4	0.044
Glucose	0.30

(After Machii and Wada, 1989)

Table 2. Composition of antibiotics in a sterile seawater

Antibiotics	Quantity
Gentamycin	125 ug/ml
Polymyxin B sulfate	100 ug/ml
Neomycin sulfate	100 ug/ml
Kamamycin sulfate	100 ug/ml
Mycostatin	200 ug/ml
Fungizone	5 ug/ml
Penicillin	200 ug/ml
Streptomycin	200 ug/ml

(After Stephens and Hatrick, 1979)

The mantle pieces were treated in 10% ethanol for 15 seconds and again washed three times in 10 ml of SSW or BSS. They were given treatment in a mixture of antibiotic solution four times each 30 mintues for 2 hours (see Table 2). After this treatment they were washed three times in 10 ml of SSW or BSS. Now the pieces are ready for inoculation.

C. *Explant culture* : The explants were inoculated in petri dishes.

2 ml Medium 199; 2 ml Pf 35; 1 ml Foetal Calf Serum (FCS) and 5 ml of SSW were combined together as medium. No antibiotic was used in the cultures. They were incubated at 28°C, pH 7.8 and the medium was changed once in two days.

Results

At this combination of culture media (Medium 199 - 2ml; Pf 35 - 2 ml ; SSW - 5 ml and FCS - 1 ml) the cells proliferated from the explant in good numbers on day 2 onwards (Fig.1). A mass of epithelial - like and fibroblast - like cells dissociated from the explant (Fig. 2). The epithelial - like cells were spherical in shape and have short pseudopodia. As large

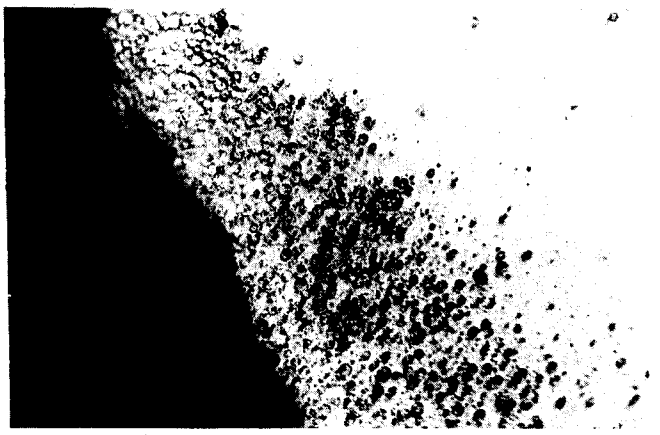


Fig. 1. Cells dissociating from the mantle explant on day 2

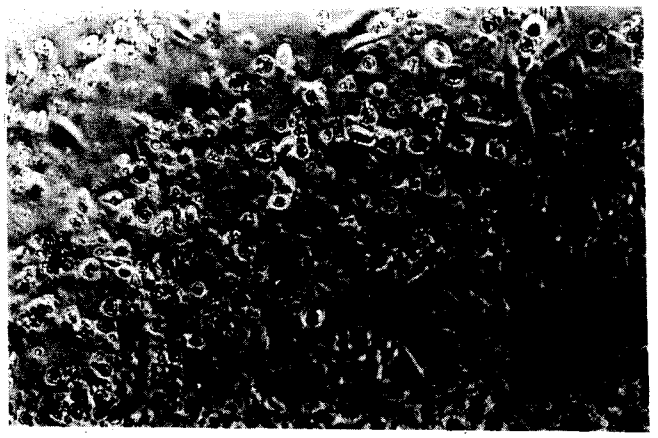


Fig. 2. Epithelial-like and fibroblast-like cells on day 8.

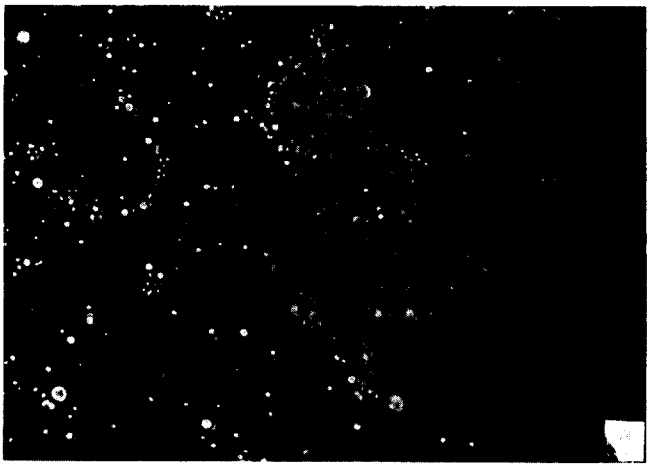


Fig. 3. Formation of colony by the mantle cells on day 15.

Fig. 4. Secretion of crystals by the cells in *in-vitro* culture.

number of cells were emerging, the cells at the distal end had moved away from the explant on day 4. On day 8 a mixture of spindle shaped, elongated, string like and spherical cells were seen. Meanwhile the explant was found to have undergone a noticeable change from a flesh

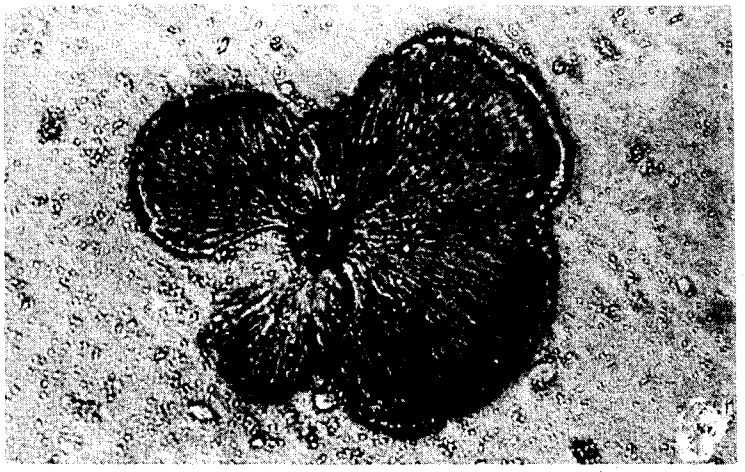


Fig. 5. Secretion of alveolar material on day 20.

colour to a dark brown colour on day 10. The number of cells had increased since day 13 and the colony formation had occurred on day 15 onwards (Fig.3). The colony of cells secreted an organic substance (Fig.4) and deposited on the culture plates. Prolonged culture of these cells resulted in trefoil - like crystals in the colony. The crystals were in different sizes. Apart from these crystals there were alveolar materials secreted by the colony of cells on day 20 (Fig.5). The alveolar materials showed no birefrigency. They were subjected to acid testing and found to be calcareous matter. On day 14-15 the explant became fragile as large number of cells had dissociated from it.

Discussion

Although research on *in-vitro* culture of marine invertebrates was undertaken, establishment of cell lines from these animals was rather limited. However some studies were carried out on the culture of mantle tissue from pearl oysters. Bevelander and Martin (1949) could demonstrate the deposition of conchiolin and formation of crystals through an organ culture of mantle from the marine mollusc *Pinctada radiata.*

The nature of conchiolin and crystals was typical to those found in normal and regenerating shells. Subsequently Machii (1974) reported that the secretion of organic substance through an organ culture of mantle from the pearl oyster *Pinctada fucata* was nothing but calcium crystals. Later the secretion of calcium crystals was also reported from the cell line culture of pearl oyster larvae (Machii, 1985) and from an explant culture of pearl oyster mantle tissue of *P.fucata* (Machii and Wada, 1989). A similar result was obtained in the present study on the explant culture of mantle tissue of *Pinctada fucata*. In our study large number of cells had dissociated from the explant and a cell sheet formed on day 4. It was identical to the study by Machii and Wada, 1989 where the formation of cell sheet took place between day 3 and 7. On day 12 the explant underwent considerable change in colour and since then the secretion of organic substance occurred. It was quite similar to our study where the change of colour and the secretion of organic substance occurred on day 10. The deposition of alveolar material was obtained on day 30 in both the studies. The high content of calcium in the crystal as reported by Machii and Wada (1989), indicated that it must be a prism, a kind of pearl formed *in vitro*.

Acknowledgements

The authors are grateful to Dr.M.Devaraj, the former Director, CMFRI and Dr. V. N. Pillai, Director, CMFRI, Cochin for their encouragement in the study. We are indebted to Dr. K. K. Appukuttan, Head MFD, CMFRI, Cochin for the valuable support given to the project and to Shri.D.C.V.Easterson, Officer-in-charge, Tuticorin Research Centre, for the facilities to the work. We express our gratitude to the Department of Biotechnology, New Delhi for the financial assistance to the development of full fledged marine invertebrate tissue culture laboratory at CMFRI, Tuticorin.

References

Bevelander, G. and J. Martin, 1949. Culture of Mantle tissue of marine molluscs. *Anat. Rec.*, **105**: 614.

Machii, A. 1974. Organ culture of mantle tissue of the pearl oyster *Pinctada fucata* (Gould), *Bull. Natl. Pearl Res. Lab.*, **18**, 2111-2117.

Machii, A. 1985. Establishment of cell line from veliger larvae of the pearl oyster, *Pinctada fucata. Tissue Cult. Res. Commun.*, **4**: 18.

Machii, A and K.T. Wada, 1989. Some marine invertebrates tissue culture. Mitsuhashi, J.Ed., *Invertebrate Cell System Applications*, **2**, 225-233.

Stephens, B.E. and M.F.Hatrick, 1979. Decontamination of the American oyster tissues for cell and organ culture. *TCA Manual*, **5** (1): 1029-1031.

Bioremediation technology for the treatment of shrimp farm effluent : an ecofriendly approach

A.P. Sangamaheswaran,
R. Santha Kumar, M.J. Prince Jeyaseelan and V. Sundararaj

Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Tuticorin - 628 008

ABSTRACT

India holds a key position in global aquaculture production, including shrimp. Unorganised growth in shrimp farming sector is at present facing a stiff challenge to sustain the productivity. The rapidly expanding aquaculture industry could pave the way to water quality degradation. Bioremediation comes in handy to tackle the problems posed by aquaculture effluents and to exercise ecofriendly mariculture. Various commercial bioremediators available in the market to effect bioremediation are summed up. The results of the laboratory trails conduced for the treatment of shrimp farm effluent by using a bioremediator is presented. The changes in the effluent characters in terms of BOD_5, COD, NH_3-N, NO_2-N, NO_3-N, total heterotrophic count, total heterotrophic ammonia oxidisers, total autotrophic ammonia oxidisers, total heterotrophic nitrite oxidisers and total autotrophic nitrite oxidisers were analysed and presented. The importance of bioremediation in the culture system for establishment of ecofriendly mariculture are discussed.

Introduction

During the process of intensification shrimp farmers resort to multiple feeding of high energy complete feed to stocked shrimp. In order

to keep the valuable stock away from stress and disease outbreak, they provide good aeration and adequate water exchange to culture system. Intensive shrimp culture systems in South East Asian countries receive as high as 670 Kg of feed per ha. per day and as high as 200 m^3 of sea water is utilized in terms of water exchange for production of one kilogram of shrimp. To maintain desirable water quality and to bring down the organic nutrient load in the culture system, water exchange is done effectively. During this process, sanitation of the pond / farm alone is of prime concern to the shrimp farmers and hazardous and long term repercussion of any effluent discharge to the delicate ecosystem have been over looked because of unorganised farming. The damage that accrued out of effluent disposal to the coastal ecosystem has now been realized by the farming community because of disease outbreak at epizootic proposition.

The unbridled growth of shrimp farming industry in a haphazard manner immediately after liberalization era witnessed effluent of one farm, serving as the source water of another farm, thus led to rapid spread of dreadly pathogen especially in select coastal states, including Tamil Nadu and Andhra Pradesh.

There are three basic methods of treatment of waste accumulated in shrimp farm. They include physical, chemical and biological means. Among the three methods, the biological means of treatment of waste is considered as the best. Seaweeds and bivalves are used for biological means of waste treatment. Bioremediators developed out of biotechnological advancement could also be used for the biological treatment of shrimp farm effluent.

Bioremediators are single or mixed cultures of live microorganisms(selected strains of bacteria) which utilise or convert toxic forms of nitrogen like ammonia to bacterial biomass and / or less toxic forms of nitrogen such as nitrates and nitrogen gas (Briggs, 1995). Many such beneficial bacterial products are currently being marketed.

There are three principal steps of bacterial water purification. The first is the solubilisation of organic solids(eg. dead algae, excess feed,

faces) which produce biochemical oxygen demand(BOD) and ammonia. This is followed by removal of the soluble organic matter. In the final step, the harmful ammonia and nitrite thus formed should be removed by nitrification. While the above processes could take place naturally, the artificial environment in aquaculture systems could delay the process, because of inadequate availability of specific strains of bacteria required for a particular process. Bacterial bioaugmentation, could help the process by facilitating the various steps involved in water purification by means of addition of heterotrophic and nitrifying microbes into the system.

A variety of commercial bioremediators are available in aquaculture market viz, Aqua bacta Aid, Aqua Cleaner, BFR-2, BMS series, Epicin, Environ AC, Super SPO, Super NB, Super PS, Wunapuo-15, etc. In this study Epicin was used for the treatment of shrimp farm effluent.

Materials and methods

Effluent water sample was collected from a shrimp farm adjacent to Tuticorin and brought to the laboratory in carbouys. It was transferred to 15 lit. plastic troughs and subjected to bioremediation treatment with and without aeration using three different concentrations (5 ppm, 10 ppm, 20 ppm) of Epicin, a product of Epicore Ltd., UK. Two controls (with and without aeration) were also maintained.

The Nitrogenous nutrients such as ammonia - nitrogen (NH_3-N), nitrite - nitrogen (NO_2-N) and nitrate - nitrogen (NO_3-N) were determined daily for the entire period of experiment (6 days) following the method described by FAO (1975). Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were estimated by using standard methods (APHA, 1971) during the initial and final period of the experiment.

Total heterotrophic bacterial count was determined following the procedure of APHA (1976). To enumerate the total heterotrophic bacterial population, Tryptic Soy Agar (with 1.5% NaCl) was used. In order to estimate total heterotrophic ammonia oxidising bacterial count and total heterotrophic nitrite oxidising bacterial count, the ATCC media 438 and

480 respectively were used by carrying out the modifications suggested by Rittmann *et al.* (1994) and solidification through addition of bacteriological grade agar (by adding 1.5 g agar to 100 ml of broth solution). For the above mentioned three categories of bacterial estimation, spread plate technique was followed. The plates after inoculation were incubated for 48 hours at ambient temperature (30 ± 2°C).

The total autotrophic ammonia oxidising bacterial count and total autotrophic nitrite oxidising bacterial count were estimated adopting the "3 tube Most Probable Number (MPN) technique" (APHA, 1971 and Ritmann *et al.*, 1994).

Results and discussion

Ammonia level reduction in effluent was remarkable in treatments and control subjected to aeration and not so in treatments and control which did not receive aeration (Fig. 1a & b). Drastic reduction in ammonia from 196.42 μg at /l to mere 6.63 μg at /l was recorded in effluent sample which received 6 days of aeration and 20 ppm Epicin (T_3) while the reduction was only marginal for the treatment (T_6) which received same dose (20 ppm) but without aeration (from 196.42 to 141.27 μg at /l). The remarkable reduction in ammonia level in effluent sample which received aeration and Epicin at relatively higher dosage (20 ppm) also coincided with highest population level of both total heterotrophic ammonia oxidizers count (1.38×10^4 cfu/ml) and total autotrophic ammonia oxidizers count (2.10×10^3 MPN/ml) among all treatments and control (Table 1). The initial level of these two groups of ammonia oxidizers were 1.42×10^2 cfu/ml and 9.0×10^2 MPN /ml respectively.

Nitrite level reduction was also remarkable in effluent sample which received aeration and 20 ppm Epicin treatment. The nitrate level in this treatment showed a reduction from 166.25 μg at NO_2 -N/l to 96.47 μg at NO_2-N/l. In aerated control (C_1) the reduction in nitrate level was only marginal from 166.25 μg at NO_2-N/l to 143.31 μg at NO_2-N/l. The bioremediation at 20 ppm level in the absence of aeration (T_6) did not show promising reduction in nitrite in effluent sample (Fig. 2a & b). The population of total hetetrophic

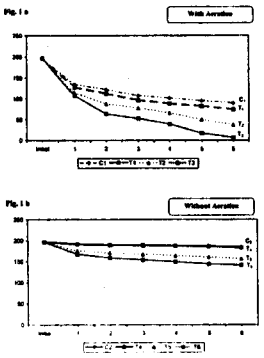


Fig. 1a, b. Ammonia levels in effluent recorded during the period of experiment in different treatments and control

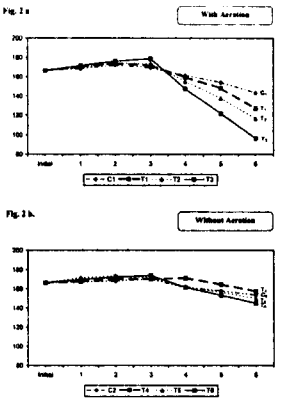


Fig. 2a, b. Nitrite levels in effluent recorded during the period of experiment in different treatments and control

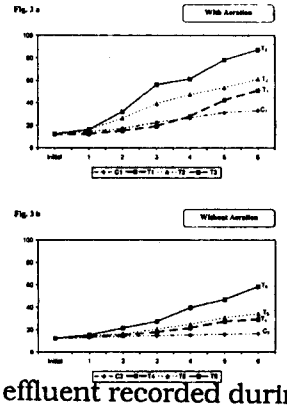


Fig. 3a, b. Nitrate levels in effluent recorded during the period of experiment in different treatments and control

nitrite oxidizers and total autotrophic nitrite oxidizers also recorded highest values of 5.8 x 10^3 cfu/ml and 2.90 x 10^3 MPN/ml respectively in effluent on final day of experiment (6^{th} day) which received aeration and 20 ppm Epicin dose (T_3) (Table 1).

Increase in nitrate level in effluent was remarkable which received aeration and 20 ppm Epicin treatment. The nitrate level in this treatment showed an increase from 12.34 μg at NO_3-N/l to 87.19 μg at NO_3-N/l. In aerated control (C_1) the increment in nitrate level was less, from 12.34 μg at NO_3-N/l to 33.08μg at NO_3-N/l. The bioremediation at 20 ppm level in the absence of aeration (T_6) did not show promising increment in nitrate level in effluent sample (Fig. 3a & b).

Recording reduction in BOD level of effluent, the treatment (T_3) which received aeration and Epicin at relatively high doze (20 ppm) showed appreciable rate of reduction from 5.54 mg/l to 1.36 mg/l. Aerated control (C_1) showed BOD reduction from 5.54 to 4.43 mg/l only in six days duration. COD reduction is also highly promising in effluent (T_3) which received aeration and 20 ppm Epicin addition (15.48 mg/l to 2.42 mg/l in six days). The aerated control (C_1) showed COD reduction from 15.48 mg/l to 12.54 mg/l only. Epcin addition at 20 ppm level in the absence of aeration did not show COD reduction as promising as that of aerated one (15.48 mg/l to 8.46 mg/l only as against 2.42 mg/l in the case of treatment with aeration) (Table 2).

Conclusion

Bioremediation are useful not only in shrimp ponds but also in treating shrimp farm effluents. Harmful substances such as ammonia and nitrite showed promising level of reduction at 20 ppm Epicin incorporation level in the presence of aeration. Mere addition of bioremediation without aeration would result in waste of money and resources. Appreciable reduction of B.O.D. and C.O.D. in effluent subjected to 20 ppm Epicin addition and good aeration would vouch for the usefulness of the beneficial microbes –

Table 1. Bacterial populations recorded during the period of experiment in different treatments and control

Parameters	Initial	Sixth day							
		C_1	T_1	T_2	T_3	C_2	T_4	T_5	T_6
Total Heterotrophic bacterial count CFU/ml	$3.3x10^5$	$1.32 x 10^5$	$4.6 x 10^5$	$3.7 x 10^5$	$8.9 x 10^5$	$1.67 x 10^4$	$4.2 x 10^4$	$4.6 x 10^4$	$6.3 x 10^4$
Total heterotrophic ammonia oxidisers CFU/ml	$1.42x10^2$	$2.11 x 10^2$	$5.3 x 10^2$	$2.19 x 10^3$	$1.38 x 10^4$	$1.03 x 10^2$	$1.16 x 10^2$	$1.71 x 10^2$	$2.38 x 10^2$
Total heterotrophic nitrite oxidisers CFU/ml	$1.67x10^2$	$4.2 x 10^2$	$2.41 x 10^2$	$1.49 x 10^3$	$5.8 x 10^3$	$9.7 x 10^2$	$1.09 x 10^2$	$1.56 x 10^2$	$1.97 x 10^2$
Total autotrophic ammonia oxidisers MPN/ml	$9.0x10^2$	$9.0 x 10^2$	$1.2 x 10^3$	$2.0 x 10^3$	$2.10 x 10^3$	$3.0 x 10^2$	$7.0 x 10^2$	$9.0 x 10^2$	$1.2 x 10^2$
Total autotrophic nitrite oxidisers MPN/ml	$6.0x10^2$	$7.0 x 10^2$	$1.6 x 10^3$	$2.6 x 10^3$	$2.90 x 10^3$	$3.0 x 10^2$	$4.0 x 10^2$	$6.0 x 10^2$	$9.0 x 10^2$

Table2. BOD and COD levels in effluent recorded during the period of experiment in different treatments and control

Parameters	Initial	Sixth Day							
		With Aeration				Without Aeration			
		C_1	T_1	T_2	T_3	C_2	T_4	T_5	T_6
BOD (mg/l)	5.54	4.43	3.34	1.92	1.36	5.41	5.36	3.71	2.88
COD (mg/l)	15.48	12.54	8.37	5.09	2.42	14.31	13.62	10.73	8.46

especially the heterotrophic and autotrophic nitrite and ammonia oxidizers in shrimp farm waste reduction and containment of organic pollution. This would in turn lead to sustainable shrimp farming by establishing clean coastal environment and containment of pathogenic microbes.

Acknowledgement

Authors are thankful to the Dean, Fisheries College and Research Institute, Tuticorin for providing necessary laboratory facilities.

References

FAO, 1975. Manual of methods in aquatic environment measurement and monitoring of water pollution. FAO. *Fish. Tech. Paper. No. 137.* pp. 238.

APHA, 1971. Standard methods for the examination of water and waste water, 13th ed. *Amer. Publ. Health Ass.* New York, pp. 1100

APHA, 1976. Compendium of methods for the microbiological examination of foods. M.L. Speck, (Ed.), *Amer. Publ. Health Ass.*, New York, pp. 70.

Rittmann, B.E., J.M. Regan and D.A. Stahl, 1994. Nitrification as a source of soluble organic substrate in biological treatment. *Water Science and Technology*, **30**(36) : 1-8.

New horizon of fisheries development - mariculture through credit

M.A. Upare
NABARD, Mumbai - 400 018

ABSTRACT

Capture fishery resources of India are almost fully exploited barring a few species specific resources. To meet the widening gap in demand and supply, there is an urgent need to explore new horizons of fisheries development. In contrast to capture fisheries, culture fisheries offer more scope for stock manipulation to achieve higher production per unit area. The freshwater aquaculture has potential for expansion albeit competing with other land based agriculture activities. Mariculture is a new horizon of fisheries development in India where much development has not taken place despite vast porential both on landward and seaward side of our coastal line with the exception of shrimp farming. Many technologies involving pearl oyster culture, edible oyster culture, mussel culture, clam culture, lobster farming, crab culture, seaweed culture, sea-cucumber culture and culture of many finfishes were developed by the research institutes. However, the fact that there is no commercial level sea farming activity indicate the need for concerted efforts to commercialise them and to resolve various bottlenecks encountered. The National Bank for Agriculture and Rural Development (NABARD) had been taking various measures to commerialise mariculture in India. The Bank in addition to assisting pilot scale demonstration of mariculture technologies under its Research and Development grant scheme, has been encouraging commercial scale projects through its refinance machanism. The paper

reviews the status of the mariculture in India, constraints in development, efforts taken by the NABARD to develop the sector, and call for improved co-ordination among the research institutes, developmental agencies and financial institutions to commercialise new technologies through their combined efforts.

Fisheries development - scenario

Fisheries from time immemorial has a unique distinction in the economy. Marine fisheries made significant progress since the inception of Five Year Plan with the fish production of 5.96 million tonnes from marine and inland sectors together. Against the estimated potential of 3.9 mt. in the marine sector, currently 2.95 mt. is exploited. Earnings from export of marine products have been Rs. 46268 m. (1106.91 million US $) in 1998-99 as against 10 m. US $ in 1960-61. Contribution of fisheries sector to the net domestic product has shown an eight fold increase from Rs.8.06 billion in 1980-81 to Rs.67.5 billion in 1993-94 at current price compared to only four fold increase in agriculture during the same period. An average growth in the marine sector during the period from 1987-88 to 1997-98 is around 5.9 per cent.

The basic objective of fisheries development is to augment fish production and export earnings thereby strengthening the economy. However, the seventh and eighth Five Year Plan stressed emphasis on the welfare of the fishermen considering that the sector provides employment for 57.70 lakh fishermen with an equally impressive segment of population engaging in fisheries associated activities. The development strategy was accordingly oriented to modernization of traditional and mechanised sector and introduction of a judicious mix of resource specific deep sea fish vessels, charters, joint ventures, 100% Export Oriented Unit (EOU), etc. for optimum exploitation of fishery resources in EEZ and increasing export besides improving of living standards of traditional fishermen by opening new horizon of fisheries development through mariculture.

Fisheries infrastructure

The fishing fleets consist of about 1,91,207 traditional crafts of which 31,726 are motorised, 46,918 mechanised boats and about 70 deep sea fishing vessels using different gears, combination of varying levels of

investments. Construction of fishing harbours and landing centres has been included as an important development activity under the planned scheme. Infrastructure has also been created for post harvest facilities, such as, processing and marketing of fish and fish products. However, the major efforts have been aimed at creation of landing and berthing facilities for different kinds of fishing vessels. These facilities have contributed significantly to the development of marine fisheries and increased fish production from the marine resources.

Exports

The export of fish and fish products reached all time high of 3.858 lakh tonnes in 1997-98 with a value of Rs. 46,974 m. In 1998-99 it has been 3.029 lakh million tonnes at an estimated value of Rs. 46,268 m. This is almost eight times the export made in 1988-89 of Rs.5980 million. Marine product export crossed one billion $ mark in 1994-95. The export earnings from fisheries sector in India has been mostly forthcoming from marine products. Frozen shrimp constituted about 67 per cent of the total export in fishery sector. Most of India's export is in bulk packing. Only value added product exported from India is quick frozen shrimp (IQF).

Fish production

The marine fish production in the country has increased from 1.658 m.t. in 1987-88 to about 2.95 m.t. in 1997-98 showing an average growth of around 5.9% during the period. However, production of mariculture is negligible.

Global mariculture situation

A recent report of the Consultative Group on International Agriculture Research (CGIAR) states: "within 15 years, fish farming and sea ranching could provide nearly 40% of all fish for the human diet and more than half of the value of global fish catch". According to a report of the FAO, the world aquaculture production is projected to increase by 2.69 times by 2025, growing from 19.3 mt. in 1992 to 26.9 mt. in 2000 and to 51.8 mt. in 2025. Marine finfish production by farming is expected to increase from 0.36 mt. to 1.0 mt. crustaceans from 1.0 mt. to 4.1 mt. molluscs from 3.5 mt. to 8.9 mt. and seaweed from 5.4 mt. to 9.8 mt.

Indian mariculture potential

India, as a leading country in Asia in aquaculture production, should be able to achieve atleast a production of 2 mt. through mariculture by the year 2025, i.e. 3.9% of the projected global aquaculture production of 51.8 mt. Besides, with the improvement in the domestic marketing, diversification of marine products exports, availability of a large number of culture technologies and different hydroclimatic zones for coastal mariculture and seafarming, India could become a major player in world mariculture production.

Role of NABARD in fisheries development

In fisheries, the bank's refinance assistance is available for all sub sectors i.e. marine, coastal fisheries, freshwater fisheries projects and infrastructure support like fish processing plants, ice plants, cold storages, fish seed hatcheries, market yards etc. NABARD's contribution for the growth of fisheries sector has been spectacular in the last ten years. The sector which availed Rs.10 crores in 1986 has made quantum jump and attained a level of Rs. 107 crores in 1995-96. However, due to the problems faced by the shrimp farming sector the disbursements declined in subsequent years to Rs.33 crores in 1997-98. The bank has so far financed more than 5408 fisheries schemes with total refinance disbursement of Rs. 583.43 crores.

NABARD has been reviewing its policies from time to time keeping in view the national priorities. In the early eighties major share of bank finance was allotted to marine capture fisheries but later the attention was shifted to freshwater aquaculture and fish seed hatcheries. Now with the advancement of technical knowledge and standardisation of technologies newer areas like shrimp farming, integrated fish culture projects, mariculture etc. are being brought under institutional finance.

Freshwater sector

In fresh water, approximately 0.3 million hectare area has been developed with credit support for increasing fresh water aquaculture production. The national bank can rightly claim successful implementation of the IDA assisted inland fisheries project for the development of freshwater fish ponds of 1.7 million ha and establishment of 14 modern

fish seed hatcheries in five states of Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal and Orissa. We doubly feel proud because we disbursed more than 12 million US Dollars and achieved a target of 120% of the stipulated target in physical terms. The project has created general awareness on the economics of carp farming and has helped in increasing the average fish production per unit area from 500 kg/ha to 2200 kg/ha. The co-ordinated efforts in the establishment of indigenous technology of composite fish culture through this project with the efforts of banking sector, state fisheries departments, central project unit and research institutes proved our capability to work jointly and paved the way for future such collaboration.

Collaboration with other agencies

In its effort to develop the sector in a scientific manner the bank has been actively associated itself with the regional and international agencies like Network of Aquaculture Centres in Asia-Pacific (NACA), Asian Development Bank (ADB), International Development Association (IDA), Food and Agricultural Organisation of United Nations (FAO) etc. Recently the NABARD had associated in a study on aquaculture sustainability and the environment sponsored by NACA/ADB/Govt of India. Besides, the bank also collaborates with ICAR and other related research and development agencies on a continuous basis to remain up to date on the latest developments and to prioritise research problems for R&D support.

Brackishwater sector

In the last five years brackishwater fish culture has also been getting focused attention. The bank supported about 7000 hectares for developing coastal aquaculture and popularised the shrimp farming technology, before the environmental degradation issues and social conflicts put a stop in 1996 for further development. However, efforts are on to remove the bottlenecks and the sector would continue to get focused attention within the Supreme Court's guidelines during the IXth plan as well.

Going by the trend of fisheries growth the bank is also enthusiastic about its promotional role for fisheries sector and has prepared its IX five year plan credit document accordingly. We have provisionally kept Rs.8062 crores as total financial outlay for fisheries of which Rs. 6041 crores would be the share of bank credit. Out of the above total outlay, the coastal

aquaculture and fresh waterfisheries development will have 25% and 65% respectively. These enthusiastic projections have been made keeping in view the available potential and our experience of financing. However, this allocation may undergo change as per national policies decided from time to time.

The present level of fish production in the country is only 5.1 million tonnes as against the envisaged target of 6.37 million tonnes by the end of IX five year plan. Since only 15% of the total available potential is under utilisation NABARD has no doubt about achieving the above target. However, recently emerged social conflicts, environmental problems, technical issues and disease outbreak need immediate attention of scientists, planners and administrators to remove the bottlenecks in achieving eco-friendly sustainable production and propagate new technologies.

Banking sector particularly NABARD is keen to propagate and adopt newer scientific technologies for increasing national fish production and rural prosperity. It is therefore seeking assistance from the scientists in the endeavor. NABARD in order to diversify, have been supporting research projects and studies from its R&D grants. In fisheries sector nine research projects with financial assistance of Rs.91 lakhs have been supported for standardising field level technologies.

NABARD'S focus in mariculture

In the field of mariculture the bank had sanctioned a soft loan assistance to Tamil Nadu Pearls, a joint venture between Southern Petrochemical Industries Corporation (SPIC) and Tamil Nadu Fisheries Development Corporation (TNFDC). One Research and Development Project to demonstrate pilot scale operations of edible oyster culture was sanctioned to CMFRI with a total grant assistance of Rs. 6.44 lakhs and has been completed successfully. The bank has great expectations from such research projects as we are interested to propagate such technologies by linking with institutional finance. As an outcome of such efforts the bank has sanctioned refinance assistance for two projects for on - farm culture of pearl oysters in Andhra Pradesh with technical support from the CMFRI. Two schemes for mussel culture has been sanctioned in Kerala with NABARD refinance commitment of Rs. 13.55 lakhs.

The bank has taken lead in supporting innovative on-shore culture of pearl oysters for the first time in the country. Two such projects with a total outlay of Rs. 534.5 lakhs has been sanctioned by NABARD through refinance assistance.

Culture of marine algae, *Dunaliella* with a total financial outlay of Rs.44 lakhs was received for refinance. The project envisaged production of dry algal powder for application in various industries.

Open sea cage culture

One scheme on open sea cage culture of sea bass and grouper was sanctioned for implementation in A&N Islands. The scheme involved an outlay of Rs. 178 lakhs and production capacity of 120 tonnes per annum. Large scale development of marine cage culture are hindered due to various constraints.

Constraints

Large scale development of mariculture has not yet taken its roots though the technology has been developed by research institutes. The following issues may be considered to ensure commercial scale of adoption of technology under mariculture.

Mapping of suitable areas for mariculture

There is need to identify the locations suitable for mariculture of different species through out the coastal line. Once published information is available on this the entrepreneurs may come forward to take up mariculture activities suitable in each location.

Appropriate leasing policy for coastal areas

The legal system for leasing of coastal areas/open seas for mariculture is to be reviewed and announced. The Central govt. may work out a guideline for adoption by the state govts. for the areas under their control. For areas beyond the control of the states there should be a national policy to encourage open sea mariculture keeping in view the interests of various user groups.

Appropriate system for participation of local fishermen

Considering the over capitalisation/over exploitation of marine

capture fisheries and the consequent declining Catch Per Unit Effort, the fishermen are often not able to get remunerative catch. The govt. may encourage formation of co-operative societies/SHGs of coastal fishermen who may be allotted suitable areas on lease and provided with suitable mariculture technology. This will help the fishermen to realise additional income and avoid disguised/under employment in capture fisheries.

Lack of awareness

There is lack of information about potential scope for mariculture in our country. Though we celebrated 1998 as International Year of the Oceans there is further need for awareness programmes.

Need for co-ordinated approach

Joint efforts with ICAR institutes and Agricultural Universities, have helped in establishing several new technologies and therefore we wish to continue such support. But what we need is identification of key areas of applied research and prioritise them and prepare detailed guidelines. It is expected that the fishery scientists have vital role in assessing the cost-benefits of various technologies for commercial applications. For sustained production there is need to diversify our production systems. In this regard many new and innovative technologies are developed by scientists which need to be commercialised. Few such technologies are pearl culture, fresh water prawn culture, cage culture in open water bodies, culture and use of blue green algae, suitable technologies for cold water regions, culture of fin fishes like sea bass etc. in coastal areas, mussel culture, edible oyster culture, culture of sea cucumber etc.

Fishery scientists and planners, GOI and State Govt. should come out with suitable strategies for fisheries which can become a major economic activity in the years to come and would set an era of blue revolution exploring new horizon on fisheries development with co-ordinated approach.

The views expressed in this paper are the personal views of the author. The institution with which he is associated is in no way responsible for the same.

Brackishwater shrimp farming boom in Andhra Pradesh- technical and policy shortcomings

C. M. Muralidharan
AFPRO Field Unit VI,
Secunderabad-500017

ABSTRACT

Brackishwater shrimp farming boom in coastal Andhra Pradesh in the early nineties gave a bad reputation to aquaculture, mainly because of certain unregulated and unplanned development. The paper looks into the circumstances of a Government promoted programme going beyond its control both from the policy and technical point of view. Improper site selection, development beyond the carrying capacity of the system, unplanned farm layouts, technical risks of intensive and semi-intensive shrimp farming, feed and farm input suppliers turning as technical experts, use of groundwater, etc. are the major reasons for the chaos created. The policy makers including the Aquaculture Authority has to take these points into serious consideration apart from various other socio-economic impacts.

Introduction

Brackishwater shrimp farming has attained a bad reputation, thanks to the unregulated poorly planned development. Brackishwater shrimp farming could be a good way to make use of the exact unutilised tide effected brackishwater lands productively, only if the right sites are developed in a planned way with sustainable technology for or by the right people. It was the failure in the latter aspect that shrimp farming boom in Andhra Pradesh ended up in chaos. In the light of the Supreme Court Judgement of December 11, 1996, the "Aquaculture Authority" is to take up regulatory measures and enforce them. Accordingly the authority is formed. This paper would be dealing on some of the important points, the authority and the various Government bodies should be taking care of based on the present situation in Andhra Pradesh.

Background of shrimp farm development in Andhra Pradesh

Andhra Pradesh does not have a traditional shrimp farming culture as of Kerala and West Bengal. In the eighties, the promotional activities of Marine Products Export Development Authority triggered off the interest in brackishwater shrimp farming. With MPEDA's initiative quite a few scientifically managed sustainable improved extensive (please see annexure for classification of shrimp farms) shrimp farms did come up at the right sites.

Seeing the success of shrimp farms, especially its high returns, quite a few local farmers and entrepreneurs from other places took interest and started converting their own lands, lands on lease, encroached lands, purchased lands -legally or illegally- into shrimp farms. Quite often these neo-entrants did not take technical assistance from MPEDA or other agencies. Many could not get financial assistance from formal sources. Hence the development had been based on individual farmers/entrepreneurs whims and fancies, convenience and financial capacity. Examples of such types of farms located at scientifically unsuitable sites or designed poorly can be seen plenty in Krishna, Guntur and West Godavari districts (Table 1).

Table 1. District-wise no.of shrimp farms in AP.

District	No.of Shrimp Farms	Land (Ha.) taken by Shrimp Farms	Water spread area (Ha)
Srikakulam	37	183	137
Vijayanagaram	4	61	43
Visakhapatnam	61	500	350
East Godavari	2,119	4,747	3,798
West Godavari	14,753	14,020	11,216
Krishna	34,337	29,601	23,644
Guntur	10,667	8,808	7,046
Prakasam	1,577	4,253	3,426
Nellore	479	4,199	3,054
Total	64,634	66,369	52,714

Source: MPEDA, (1996)

Another initiative by MPEDA and the Government was to promote 100% export oriented units under the corporate and big entrepreneur sector, which took off by late eighties and early nineties. Many of the Nellore and Prakasam based farms, few in Visakhapatnam, East Godavari districts and one in Vijayanagaram falls under this category.

The technologies used by them were for semi-intensive or intensive shrimp culture. Many of them took technologies from consultants abroad or private Indian consultants. Some of them have vertically integrated systems with hatcheries, feed mill, farm and processing plants. The area required (at least procured) for such farms ran into hundreds of acres or more. Land were purchased, took on lease and reportedly some even encroached upon. The number of such farms especially in Nellore district

grew. Often farms got crowded around same source of water intake/ drainage points or too crowded around certain villages.

In 1991, Government of Andhra Pradesh brought out the brackish water land leasing policy wherein 60% of the Government brackishwater lands were supposed to be leased out to the fishermen cooperatives, 20% to local entrepreneurs and 20% to technocrats. There had been lengthy procedures including interviews with fishermen cooperatives, asking for margin money for development to be deposited. But after six years now hardly a few cooperatives had been allotted lands. In fact most of the lands that had to be leased out had been encroached already. Being desperate, some fishermen too encroached some of these lands and started shrimp farming in whatever technology they thought was good and affordable.

Another development was the booming up of shrimp feed industry and a number of chemical suppliers both imported and indigenous. Of late these suppliers/agents took over the technical support part of shrimp farming for good or bad. To maximise their sales and profits the necessary and unnecessary inputs in large quantities went into the shrimp farms.

For all the unregulated mismanagement done, the nature itself took control, through the large-scale virus infection that affected most of the shrimp farms in 1994-95. Subsequently many have fully or partly abandoned shrimp farming.

The socioeconomic and general environmental problems that such a development led to have been well documented by many other groups.

Technical reasons for the negative impact of shrimp farming in Andhra Pradesh

I. Site selection

Many of the sites selected and converted for shrimp farming in Andhra Pradesh were not the ideal sites. The various types of sites taken up for shrimp farming fall under 4 major categories. The advantages and disadvantages and impacts of taking up shrimp farming in such sites are de-

scribed in the Table-2. The diagrammatic representation of the 4 types of sites is also given in Fig.1.

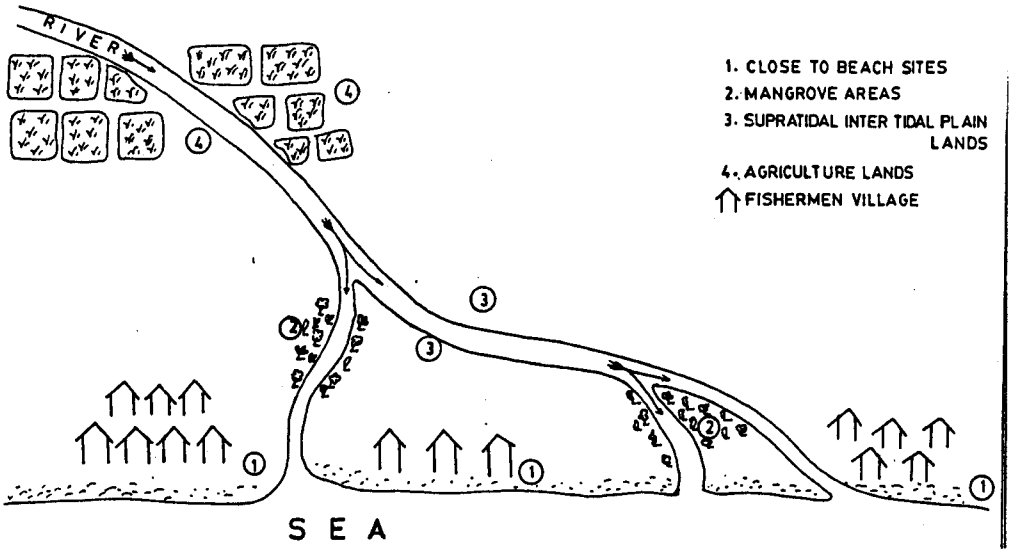


Fig. 1. Various types of sites taken up for shrimp farming

Most of the site selections were done by the entrepreneurs/farmers themselves based on the land available. The percentage of shrimp farms located in approved sites by any recognized technically competent authority would be very small. There was and is no regulation (except the recent Supreme Court Judgement and the temporary stay before that) of any sort limiting shrimp farm in any site.

II. Farms developed beyond the carrying capacity of the system

This has happened in many cases. The most important system that supports a shrimp farm is the water source for the ponds and the system that is impacted the most is that which receives drainage water from the ponds.

In the farms developed by the private entrepreneurs and corporate bodies in Nellore, Prakasam and few in other districts, the technology

Table 2. Advantages/disadvantages of various type of sites taken up for shrimp farming

	Site	Advantages To Shrimp Farmer	Disadvantage To Shrimp Farmer	Advantage To Environment	Disadvantage To The Environment	Other Implications	Examples In Andhra Pradesh
1	Beach area and just above the beach area.	Nearness to sea facilitates pumping in sea water directly, with little chances of pumping in drained out water from other ponds. Almost constant salinity.	Soil usually percolative. Hence seepage loss. Natural fertility less. Seawater salinity 32-34 ppt not best for the fast growth especially of Tiger Shrimp.	Nil	Otherwise potential areas for shelter belt plantation keeping fishing craft & gear and accessibility of fishermen to the beach lost/disturbed. Seepage of saline water into shallow aquifers, on which coastal fishermen depend for drinking and other purposes. If ponds are more seepage may even cause dampness of ground, floor and walls of fishermen huts.	Alienation of common lands for fishermen.	Many farms developed by the corporate and private sector in Prakasam & Nellore districts.
2	Mangrove areas	Low lying clayey area. Brackish water within desired	Reclamation cost high. Remaining rootstock and crab menace cause leakage of water from ponds. Usually acidic soil. After 2-3 crops the oxidation of soil causes very acidic conditions not suitable for shrimp culture.	Nil	Loss of the rich mangrove ecosystem, which supports various marine, brackish water & fresh water, live forms along with other flora & fauna. A natural protection barrier to coastal areas being lost. Natural fishing grounds lost.	Once shrimp farms are established in such areas Fishermen fishing in the creek in these systems are denied access.	Outside Koringa Reserve Forest in E.Godavari Dt. Some areas in Krisna, W.Godavari & Guntur Dt. Very few cases in Prakasam & Nellore dt.

	Site	Advantages To Shrimp Farmer	Disadvantage To Shrimp Farmer	Advantage To Environment	Disadvantage To The Environment	Other Implications	Examples In Andhra Pradesh
3	Supratidal / Intertidal plain mud flats or vacant lands, surrounded by natural brackish water sources (Estuarine creeks river mouths, back water)	Ideal site for shrimp culture. If supratidal, good pump fed ponds can be developed. If intertidal, tide fed ponds can be developed. Natural brackish water usually available. Drainage easy if it is supratidal.	Comparatively limited area available. If farms are not planned & laid out well reasons for polluting each other's farm.	Utilisation of an otherwise unutilised area more productively.	Only if the number of farms and intensity of culture goes beyond the carrying capacity of the system. i.e., Siltation, high BOD etc. may affect the biota in the creeks, rivers.	If well planned, designed and managed by locals, hardly any negative effects.	Some sites along Kadaleru creek in Nellore Dt. Very few sites in Krishna, W.Godavari & E.Godavari districts. Some sites at Varaha & Sarada river mouth in Visakhapatnam Dt.
4	Upstream lands including agriculture lands	Easier to convert to shrimp ponds if it is a paddy field. Easy excuse of the agriculture lands low productivity.	Usually in upstream areas (often tail end of irrigation canals), the salinity will be very low and often fresh water for larger part of the year, Shrimp undercontinuous stress and easily susceptibled to disease. Pesticide residues, from the nearby agriculture farms also add to the stress on shrimp.	Nil	Loss of agriculture lands. Chances of rendering other nearby lands saline and unproductive. Quite many farmers drill bore wells for subterranian saline water. This is extremely harmful to the surrounding aquifers.	Many of such lands converted in AP is not legally permitted to be done so, Many benami transactions have taken place.	Majority of shrimp farm lands in Koduru, Divi and other parts of Krishna Dt. Major part of W.Godavari & Guntur district.

adopted is the semi-intensive culture or intensive culture. Many of them depend on seawater directly. In this case the problem of polluting their own ponds with pond drainage hardly occurs. But invariably almost all farms drain out the pond effluents into the creeks/channels. Many farms draw water from the creek and drain farm effluents also to the creek.

In semi-intensive and intensive shrimp culture the increased stocking density necessitates larger quantity of feed and metabolic waste would be more. Use of aerators though may take care of oxidation to some extent, large volume of water exchange is inevitable. The feed waste, metabolic waste and silt from the ponds need to be flushed away from the system by natural flow from upstream and tides. But quite often the flushing capacity of the system falls short of the large volumes from increased number/area of ponds, resulting in siltation in creeks/canals, metabolic waste accumulation, high BOD, eutrophication, etc. This affects the natural flow, depth of the creek, natural biota of the system, etc. If the pond effluents also contain antibiotic or other chemical residues such as copper based compounds. the effect is still worse.

If the same creek is the water source for other ponds, the untreated effluent will have a negative effect on the shrimp culture and situation becomes worse as the crop progress and crop after crop. Mortalities, poor growth and susceptibility to disease increase.

The situation mentioned above are available in some areas of Kandaleru creek and Buckingham canal in Nellore and in Prakasam districts.

The situations are really worse in Krishna, Guntur and West Godavari where small and marginal farmers have developed the shrimp farms in agriculture lands. Many of the ponds depend on the tail end of the irrigation canals, which serve both as water feeder source as well as drainage point. The total area of the ponds dependent on these source is much more than the carrying capacity of the system. The effluent water from one pond becoming intake water of the other is quite common.

III. Unplanned farm layouts

As mentioned earlier the maximum area under shrimp farming in

Andhra Pradesh have been developed in an unplanned manner. In most cases no master plans or layouts were made. First the sites very close to the creeks were developed into ponds completely closing the creek sides. Later on when more ponds were developed behind the initially developed ponds, they didn't have direct access to the creek/canal. Very narrow channels or quite often drainage water from the ponds in front formed intake water for any of these ponds. This situation again is worse in Krishna district. This situation is because, a farmer or entrepreneur/ private firm converts or procures and converts sites of its choice in the way he/ they wants without bothering about what will happen to his/ their neighbour. Government should have had master plans, developed and allotted farms.

IV. Technical risks of intensive and semi-intensive farming

As the classification indicates, it involve high stocking density and higher production per unit area. Naturally all the inputs including feed and water management is high. Inputs in many cases have to include algicidal, fungicidal or bactericidal chemicals and antibiotics. It calls for high quality management. The ratio between the input cost and the returns narrow down, though the overall returns will be high. But any change in the critical factors may mean major losses. The picture after the 1994-95 virus attack is not very encouraging for many of the large entrepreneurs in Andhra Pradesh.

The farm effluents if untreated (in many cases even if treated) may contain algicide or antibiotic residues apart from the other organic wastes and silt with negative effects on the system around.

The question now is, how desirable is it for us to go for intensive or semi-intensive shrimp farming.

V. Compromises on minimum technical requirements

Well above 50% of the total area under shrimp culture in Andhra Pradesh, have been developed without any proper technical guidance or support. If the farmers knew of the minimum requirements, many did not have any financial support from any formal sources so as to invest sufficiently.

In the case of Krishna, West Godavari and Guntur farmers the system followed is the trench system. While the requirement is 1M water column, for scientific shrimp culture the water level maintained over the pond bed is hardly 30-50cm. The salinity is quite often very low. Scope for water exchange is minimum. The water intake and drainage system is quite often a mockery to scientific shrimp farming methods. Many depend on underground saline water through borewells to maintain salinity. The ground water would quite often be contaminated by iron.

Who is responsible for totally unscientific approach to shrimp farming as above? What can be done?

VI. Feed and farm input suppliers - technical experts?

Realising the pace at which shrimp farms went up in the recent past, all the well known international brands of shrimp feed manufacturers and a large number which mushroomed here itself took up the technical support by providing more and more of their products as the solution to all problems and for bumper crops.

A critical analysis will reveal that majority of the farms which practice extensive culture do not need any of these inputs. The risk here again is the unnecessary use of chemical inputs, antibiotics etc. with its harmful effects on both the shrimp farms as well as the system.

Who will regulate? How? Who is to be regulated? When?

VII. Use of groundwater

Though many deny that groundwater is hardly used in shrimp farming, there are considerable number of farms which depend on fresh ground water to dilute sea water or saline groundwater to keep up salinity in ponds, in various parts of coastal AP. The negative effect of this is mainly due to the large volume of water extracted/to be extracted for shrimp farming. Even if 20% of the water in a 1 Ha pond is groundwater the volume of groundwater required is about 2 million litres. Multiply this by the total water area of farms using groundwater. Also multiply the figure with the number of times this water would be exchanged.

If this is allowed, what will happen to our delicate coastal aquifers?

Conclusion

The objective of this paper was to bring to light some very important technical points on the recent shrimp farm boom, often ignored/over looked by the planners, policy makers, and even the technologists, and also to make the environmental activists to be aware of the facts if they are not aware.

The solution could be only a very planned and regulated development keeping in mind the major points on Brackishwater shrimp farming like

Why? Where? How? For/By whom? Who gains? Who losses?

Profits Vs
a) Monetary cost?
b) Ecological cost?
c) Social costs?

Acknowledgements

The author wish to thank Mr.N.C Bose Croos, Executive Director, Mr.C.Udayshankar, Programme Coordinator, Mr.K.Sivaprasad, Unit Manager and all colleagues of Action for Food Production (AFPRO) who had been a continuous encouragement, and cooperated in various works and studies which has been the background of this paper. Special thanks to Mr.V.Vivekanandan, South Indian Federation for Fishermen Societies who had helped develop an analytical approach to the problem in an earlier study headed by him. Marine Product Export Development Authority and The AP Department of Fisheries is also, acknowledged with thanks for the various information given.

References

Anon. 1996. *Handbook of Fisheries*, Department of Fisheries, Andhra Pradesh.

Muralidharan C.M. and C.Udayshankar, 1995. Shrimp Farming Boom in Andhra Pradesh -Effect on the coastal poor and their Environment: *National conference on Sustainable Aquaculture;Anna* University, Madras : 4-5 April 1995

Vivekanandan V.,C.M.Muralidharan, and M.Subba Rao 1997. A Study of the Marine Fisheries of Andhra Pradesh (To be published)

Note: The views mentioned in this paper are that of the Author, which need not necessarily be that of his employer "Action for Food Production.

Annexure-1

Level of Intensity of Shrimp Culture

Based on the stocking density, management measures - including feed and feeding levels, water exchange level, other inputs level of monitoring, etc. shrimp culture system can broadly be classified as extensive, semi - intensive and intensive as given below. But the classification varies with different agencies/authors.

	Extensive	Semi-intensive	Intensive
Feed	Natural	Natural Supplement	Formulated
Water management	Tidal	Tidal + Pump	Pump + Aeration
Pond size (Ha.)	2-20 Ha.	1-5 Ha.	0.1-1 Ha.
Stocking density (PL/m^2)	0.1-1	1-5	5->2.5
Production (Kg/Ha.)	100-500	500-4000	4000->15000

(Based on Apud *et.al.*, 1989)

Note: (a) The above production levels are for one year i.e., in 2 or 2½ crops.

(b) It would be convenient classification if we include a level of "Improved extensive" covering the first half range of semi-intensive system - i.e., upto a production level of 2000 Kg./Ha./Year in the years.

Export of finfish- impact on domestic trade and production

R. Sathiadhas and
R. Narayanakumar
Central Marine Fisheries Research Institute, Cochin-682 014

ABSTRACT

Finfish export from India is growing rapidly since 1991-92 and currently forms the single largest commodity in our sea food market. The major varieties exported are ribbon fish, pomfrets, seerfish, mackerel, reef cod and snappers. The total quantity of all finfishes exported during 1995-96 is to the tune of 1 lakh tonnes fetching a revenue of Rs.372 crores. This has far reaching impact on the domestic prices of almost all varieties of fish. The present analysis based on data from CMFRI and MPEDA intends to assess the trend in production and export trade of selected varieties of finfish, to compare the export and domestic market price structure and to evaluate the potentials of aquaculture to maintain the domestic

supplies and price stability. The study reveals that inspite of exporting the top quality selective fishes, the unit value realised by them from abroad are not appreciable and even less than that of the prevalent domestic prices for some varieties. The high grade seer fish exported during 1996-97 realised Rs.64 per kg as against the average domestic retail price of Rs.70 per kg. Expansion of export trade of finfish without enhancing the internal supply of quality fishes will be detrimental to the interest of domestic consumers and aquaculture alone is the viable alternative for the same. Diversified fresh and brackishwater aquaculture production of quality fishes and sea farming should be intensified to bridge the gap between demand and supply in the domestic market and to maintain the tempo of export trade of finfishes.

Introduction

Liberalisation and globalisation of Indian economy has been initiated to promote competitiveness in all spheres of production and trade mainly for the elimination of monopoly profit. The *laissez fair policy* of the country paved the promotion of export trade and forex earnings leading to comfortable balance of payments position. The seafood export trade is also not an exception to this phenomenon and it increased both in terms of volume and value. The export promotion measures coupled with the devaluation of rupee has enhanced the forex earnings of marine products which has crossed one billion dollar for the fourth consecutive time during 1998-99. Though the country's share in the global seafood export is just 1.5 per cent now, the scope for its expansion is high. The global fish eating population has enhanced to about 40 per cent during 1996-97, the consumer preference for fish and fishery products, being in an increasing trend year after year. Diversified product development and market penetration are very essential for the growth of internal and external sea food trade.

The sea food export from India has grown rapidly from a mere four crore rupees in the 60's to Rs. 4627 crore in 1998-99. There is a gradual diversification of the products exported over the years. The dried and

1993-94	Q: 93213	+19137	25.83	3.21
	V: 289.12	+67.02	30.18	11.5
1994-95	Q: 122529	+29316	31.45	39.78
	V: 446.57	+157.45	54.46	12.50
1995-96	Q: 100093	-22436	-18.31	33.78
	V: 372.26	-74.31	-16.64	10.63
1996-97	Q: 173005	+72912	72.84	45.74
	V: 636.92	+264.66	71.10	15.45
1997-98	Q: 188029	+15024	8.68	48.74
	V: 726.73	+89.81	14.10	15.47
1998-99	Q: 108556	-79473	-42.27	35.80
	V: 495.03	-231.7	-31.88	10.69

Structure of finfish export

Of the different varieties of finfish, ribbon fish, pomfrets, yellow fin tuna, fresh water fish and reef cods are the major ones that are exported (Table 3). Among them, ribbon fish ranks first in quantity and value followed by pomfrets. Though ribbon fish ranks first in volume/value pomfrets fetch high per unit price compared to ribbon fish, tuna and fresh fish (Table 4). The unit value realisation of the different varieties has generally shown an increasing trend between 1991 and 1998 except for a drop in 1995-96 which was because of the poor landings in the west coast.

Table 3. Structure of fin fish export (Percentage share)

Varieties		1991-92	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98
Ribbon fish	Q	41.92	51.83	74.64	37.66	38.83	57.76	64.36
	V	18.97	23.30	52.65	15.15	23.00	38.03	45.52
Pomfrets	Q	14.15	12.42	10.59	7.40	9.27	5.39	4.41
	V	35.59	37.75	36.39	30.09	27.33	22.24	19.74

Tuna (YF)	Q	0.43	0.88	1.26	0.45	0.56	0.24	0.37
	V	0.38	0.76	2.40	0.33	0.47	0.19	0.36
Fresh water	Q	0.01	0.02	0.07	0.20	0.58	0.47	1.39
fish	V	0.03	0.03	0.11	0.20	0.82	0.79	1.78
Reef cod	Q	0.00	1.82	1.94	1.16	1.78	2.18	1.14
	V	0.00	2.05	2.35	1.46	2.56	2.78	1.56
Fish fillets	Q	0.68	1.53	1.58	0.77	1.22	0.65	0.48
	V	1.31	3.01	2.76	1.45	2.70	1.77	1.30
Others	Q	42.80	31.52	9.92	52.36	47.76	33.42	27.84
	V	43.72	33.11	3.33	51.31	43.12	34.20	29.73
TOTAL	Q	100.00	100.00	100.00	100.00	100.00	100.00	100.00
		(49333)	(74076)	(93213)	(122529)	(100093)	(173005)	(1888029)
	V	100.00	100.00	100.00	100.00	100.00	100.00	100.00
		(143.20)	(222.10)	(289.12)	(446.57)	(372.26)	(636.92)	(726.73)

Source: Marine Product Export Review, (Various issues), MPEDA, Cochin

Note: Figures in the brackets indicate the totals of the quantity and value.

Table 4. Unit value realisation of selected finfish varieties (Rs./kg.)

Varieties	1991-92	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98
White pomfrets	52.54	66.57	106.59	148.10	109.72	152.03	172.81
Black pomfrets	73.00	91.17	0.00	86.93	55.93	60.51	65.41
Ribbon fish	13.14	13.47	14.66	21.88	22.03	24.28	27.33
Yellowfin tuna	25.93	25.92	28.64	27.15	30.65	29.65	37.65
Finfish fillets	55.45	59.05	54.18	68.73	82.15	100.48	105.24
Fresh water fish	82.00	45.78	46.53	37.50	52.44	62.39	49.59

canned items, which were the important components during 60's, gave way to frozen and live items in course of time. Even among the frozen items, shrimps dominated the other species until early nineties when finfish over took it in terms of volume of trade. The share of frozen fin fish has increased over the years and has contributed to 40 per cent of the volume of Indian sea food export during 1998-99. (1.08 lakh tonnes exported, earning Rs.495 crores) with far reaching implications in the domestic availability of certin quality fishes and price structure of all fish varieties. Good quality finishes like seer fish, pomfrets, tunas were diverted for export market leaving the domestic consumers devoid of their preference. Besides, the little quantity of finfish that are available in our country are concentrating only the major urban and commercial centres leaving the rural markets. With this idea in focus, the present paper makes a modest attempt to (a) asses the trend in production and export trade of selected varieties of fin fish. (b) compare the export and domestic market price structure for various varieties of fish. (c) evaluate the potentials of aquaculture to maintain the domestic supplies and price stability.

Data and methodology

The data for the study were collected from the publications of Central Marine Fisheries Research Institute (CMFRI) and Marine Products Export Development Authority (MPEDA), Cochin. Percentage analysis was employed for the study.

Findings

Trends in fin fish production and export

Table 1 presents the finfish production and export from India during the period 1993 to 1998.

Table 1. Finfish production and export from India 1993-1998

(in tonnes)

Year	Production	Exports	Percent
1993	1823452	93219	5.11
1994	1816484	122529	6.74
1995	1790645	100093	5.58
1996	191103	173005	9.05
1997	2109345	188029	8.91
1998	2063309	108556	5.26

The export of finfish has been increasing both in terms of quantity and in value in our sea food exports since 1991-92 (Table 2). The share of finfish export has been increasing both in terms of quantity and value in our sea food export increased from 28.7 per cent (1991-92) to 35.80 per cent (1998-99) with the maximum share being 48.47 per cent (1997-98). The share in terms of value increased marginally during the corresponding period. The importance of finfish in Indian seafood export is increasing. This may be because of the change in consumption pattern or consumer preference of the importers, product diversification and promotion measures of MPEDA and other agencies involved in sea food export.

Table 2 Growth of finfish export in India 1991-92 to 1998-99

(Q=Quantity in tonnes, V=Value in Rs. Crores)

Year	Fin fish export	Growth in export (annual)	Percentage of growth	Share in the total sea food export (%)
1991-92	Q: 49119	+6779	16.01	28.7
	V: 142.66	+51.84	57.08	10.41
1992-93	Q: 74076	+24957	50.81	35.44
	V: 222.10	+79.44	55.68	12.56

Export price and domestic price of finfish varieties

The average retail domestic price and the unit value realised for the selected finfish varieties are presented in the Table 5. It is found from the Table that the unit value realised through export is higher than the domestic retail price in all the cases. Hence the traders are concentrating on the export market leaving the domestic market deprived of such varieties. In the local market also, only those who have the purchasing power can get them. Sometimes, even if the consumers are ready to pay, the availability of such varieties is nil. Hence, development of internal market is also an essential component of the sea food trade.

Table 5. Average export and domestic retail prices of selected fin fish varieties during 1997-98

Varieties	Export Price[a]	Domestic Retail Price[b]
Ribbon fish	27	16
Pomfrets	172	120
Tuna	38	25
Mackerel	40	30
Oil sardine	34	25
Shark	41	38
Seer fish	67	73
Snappers	51	38

Source: [a] Marine Products Export Review, 1997-98, MPEDA
[b] CMFRI : SEETT Division

Aquaculture potential, domestic supplies and price stability

The production of all fish varieties from the Exclusive Economic Zone (EEZ) especially from the inshore zone 10-50m depth has reached

stagnation level. Further scope for expansion lies in the off-shore and deep-sea only (Murty and Rao, 1996, Devaraj *et al.*, 1998). With the demand for sea food including-domestic and global- and the scope for increasing production from capture fisheries limited, the attention has now turned seriously on aquaculture. The potential area available for aquaculture in India has been estimated at 11.90 lakh hectares, out of which 1.4 lakh hectares (11.9%) has been brought under shrimp culture (Table 6). It is found from the Table that, states like AP, Karnataka, Kerala and Orissa have comparatively higher area under aquaculture than other states. This indicates the vast scope existing for expansion of aquaculture in India. But it is to be noted that so far shrimp is the only species that is cultivated to a greater extent, which makes the enterprise a risky affair. Though the shrimp farming has been a highly remunerative one, presently the Supreme Court's ruling on aqua farms and the viral diseases have virtually stopped the future expansion. Under such circumstances, diversification of aqua farms to cultivate finfish varieties like groupers, snappers and mullets is the best alternative depending upon their location-specific, techno-economic feasibility. Sakthivel (2000) reported that it is time for diversification to other species like seabass, groupers, snappers and mullets because of the killer virus infection problem in shrimp culture. He added that technological development to culture the above species to be taken up on war footing to continue coastal aquaculture. He said that though the profit margin is less in finfish culture, it will be an ideal alternative to shrimp culture. Ravichandran *et al.*, (2000) expressed that the finfish fishery in the lagoons can be enhanced significantly, provided appropriate culture sites and species are selected.

Table 6. Brackish water aquaculture potential and shrimp culture in India, 1998-99

States.	Potential area (In hectares)	Area under Shrimp culture (in hectares)	Percentage of potential area	Shrimp production (tonnes)	Average productivity (kg/hectare)
West Bengal	405000	42067	10.40	18326	435.64
Orissa	31600	8000	25.30	6000	750.00
Andhra Pradesh	150000	71000	47.30	44856	631.78
Tamil Nadu	56000	1087	1.90	1820	1674.30
Pondicherry	800	22	2.80	27	1227.00
Kerala	65000	14705	22.60	7660	520.90
Karnataka	8000	3564	44.60	2690	754.77
Goa	18500	650	3.50	590	907.69
Maharashtra	80000	426	0.50	409	960.09
Gujarat	376000	316	0.10	256	810.13
TOTAL	11909000	141837	11.9	82364	580.69

Source: Aqua International, 1999

Conclusion and policy implications

The growth in the sea food export market in India has been increasing gradually which is a welcome sign in economic development of the country. At the same time, the domestic market should also be condsidered as it caters to the millions of the people. In finfish export, a substantial share of good quality fishes like seerfish, tunas, pomfrets are available in major commercial centres only leaving the rural consumers devoid of them. Such an unbalanced supply of quality fishes in the internal markets should be regularized. The existing trend of catching even the juveniles of quality finfishes to meet the domestic market demand need to be abolished preferably through law. The lifting of quantitative restrictions on imports as per the guidelines of World Trade Organisation (WTO) will boost the domestic sea food industry. The Sea food Exporters Association of India has expressed hope of constructively deploying the tremendous idle capacity in the seafood processing industry in India with the lifting of quantitative restrictions on imports. Our sea food exports mainly consist

of frozen raw materials, which the importing country processes and re-exports as value added products (VAP's). Our sea food exports presently comprise only 10 per cent of VAP, which should be increased further. As the supply from capture fishery is stagnant, the attention on coastal aquaculture should be intensified. Here, there is a need for diversification to finfish culture instead of concentrating shrimps alone. This will help to maintain the domestic supply of finfish as well as their price structure.

References

CMFRI, *Annual reports*, 1991-1999, Central Marine Fisheries Research Institute Cochin.

Devaraj. M., V.Sriramachandramurty, R. Sathiadhas, and K.K. Joshi, 1998, The New Economic Policy and Perspective for Marine Fisheries Research and Development in India *Fishing Chimes*, 18 (5):18-29.

MPEDA, *Marine Products Export Review*, 1991-1999, Marine Products Export Development Authority, Cochin.

Murthy, V.Sriramachandra and Rao, P. Vedavyasa, 1996. Marine Fishery Resource of India - Present status and management concerns. In *Marine Biodiversity Conservation and Management* (Eds.) Menon, N.G. and Pillai, C.S.G. Central Marine Fisheries Research Institute, Cochin.

Ravichandran, S., R. Kannan and T. Kannupandi, 2000. Aquaculture in Coastal Lagoons of India. *Aqua International*, 7(9):26-29.

Sakthivel, M., 2000. Aquaculture to have bright prospects in the next millennium, *Aqua International*. 7(9):4.

Export of finfish- impact on domestic trade and production

R. Sathiadhas and
R. Narayanakumar
Central Marine Fisheries Research Institute, Cochin-682 014

ABSTRACT

Finfish export from India is growing rapidly since 1991-92 and currently forms the single largest commodity in our sea food market. The major varieties exported are ribbon fish, pomfrets, seerfish, mackerel, reef cod and snappers. The total quantity of all finfishes exported during 1995-96 is to the tune of 1 lakh tonnes fetching a revenue of Rs.372 crores. This has far reaching impact on the domestic prices of almost all varieties of fish. The present analysis based on data from CMFRI and MPEDA intends to assess the trend in production and export trade of selected varieties of finfish, to compare the export and domestic market price structure and to evaluate the potentials of aquaculture to maintain the domestic

supplies and price stability. The study reveals that inspite of exporting the top quality selective fishes, the unit value realised by them from abroad are not appreciable and even less than that of the prevalent domestic prices for some varieties. The high grade seer fish exported during 1996-97 realised Rs.64 per kg as against the average domestic retail price of Rs.70 per kg. Expansion of export trade of finfish without enhancing the internal supply of quality fishes will be detrimental to the interest of domestic consumers and aquaculture alone is the viable alternative for the same. Diversified fresh and brackishwater aquaculture production of quality fishes and sea farming should be intensified to bridge the gap between demand and supply in the domestic market and to maintain the tempo of export trade of finfishes.

Introduction

Liberalisation and globalisation of Indian economy has been initiated to promote competitiveness in all spheres of production and trade mainly for the elimination of monopoly profit. The *laissez fair policy* of the country paved the promotion of export trade and forex earnings leading to comfortable balance of payments position. The seafood export trade is also not an exception to this phenomenon and it increased both in terms of volume and value. The export promotion measures coupled with the devaluation of rupee has enhanced the forex earnings of marine products which has crossed one billion dollar for the fourth consecutive time during 1998-99. Though the country's share in the global seafood export is just 1.5 per cent now, the scope for its expansion is high. The global fish eating population has enhanced to about 40 per cent during 1996-97, the consumer preference for fish and fishery products, being in an increasing trend year after year. Diversified product development and market penetration are very essential for the growth of internal and external sea food trade.

The sea food export from India has grown rapidly from a mere four crore rupees in the 60's to Rs. 4627 crore in 1998-99. There is a gradual diversification of the products exported over the years. The dried and

1993-94	Q: 93213	+19137	25.83	3.21
	V: 289.12	+67.02	30.18	11.5
1994-95	Q: 122529	+29316	31.45	39.78
	V: 446.57	+157.45	54.46	12.50
1995-96	Q: 100093	-22436	-18.31	33.78
	V: 372.26	-74.31	-16.64	10.63
1996-97	Q: 173005	+72912	72.84	45.74
	V: 636.92	+264.66	71.10	15.45
1997-98	Q: 188029	+15024	8.68	48.74
	V: 726.73	+89.81	14.10	15.47
1998-99	Q: 108556	-79473	-42.27	35.80
	V: 495.03	-231.7	-31.88	10.69

Structure of finfish export

Of the different varieties of finfish, ribbon fish, pomfrets, yellow fin tuna, fresh water fish and reef cods are the major ones that are exported (Table 3). Among them, ribbon fish ranks first in quantity and value followed by pomfrets. Though ribbon fish ranks first in volume/value pomfrets fetch high per unit price compared to ribbon fish, tuna and fresh fish (Table 4). The unit value realisation of the different varieties has generally shown an increasing trend between 1991 and 1998 except for a drop in 1995-96 which was because of the poor landings in the west coast.

Table 3. Structure of fin fish export (Percentage share)

Varieties		1991-92	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98
Ribbon fish	Q	41.92	51.83	74.64	37.66	38.83	57.76	64.36
	V	18.97	23.30	52.65	15.15	23.00	38.03	45.52
Pomfrets	Q	14.15	12.42	10.59	7.40	9.27	5.39	4.41
	V	35.59	37.75	36.39	30.09	27.33	22.24	19.74

Tuna (YF)	Q	0.43	0.88	1.26	0.45	0.56	0.24	0.37
	V	0.38	0.76	2.40	0.33	0.47	0.19	0.36
Fresh water	Q	0.01	0.02	0.07	0.20	0.58	0.47	1.39
fish	V	0.03	0.03	0.11	0.20	0.82	0.79	1.78
Reef cod	Q	0.00	1.82	1.94	1.16	1.78	2.18	1.14
	V	0.00	2.05	2.35	1.46	2.56	2.78	1.56
Fish fillets	Q	0.68	1.53	1.58	0.77	1.22	0.65	0.48
	V	1.31	3.01	2.76	1.45	2.70	1.77	1.30
Others	Q	42.80	31.52	9.92	52.36	47.76	33.42	27.84
	V	43.72	33.11	3.33	51.31	43.12	34.20	29.73
TOTAL	Q	100.00	100.00	100.00	100.00	100.00	100.00	100.00
		(49333)	(74076)	(93213)	(122529)	(100093)	(173005)	(1888029)
	V	100.00	100.00	100.00	100.00	100.00	100.00	100.00
		(143.20)	(222.10)	(289.12)	(446.57)	(372.26)	(636.92)	(726.73)

Source: Marine Product Export Review, (Various issues), MPEDA, Cochin

Note: Figures in the brackets indicate the totals of the quantity and value.

Table 4. Unit value realisation of selected finfish varieties (Rs./kg.)

Varieties	1991-92	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98
White pomfrets	52.54	66.57	106.59	148.10	109.72	152.03	172.81
Black pomfrets	73.00	91.17	0.00	86.93	55.93	60.51	65.41
Ribbon fish	13.14	13.47	14.66	21.88	22.03	24.28	27.33
Yellowfin tuna	25.93	25.92	28.64	27.15	30.65	29.65	37.65
Finfish fillets	55.45	59.05	54.18	68.73	82.15	100.48	105.24
Fresh water fish	82.00	45.78	46.53	37.50	52.44	62.39	49.59

canned items, which were the important components during 60's, gave way to frozen and live items in course of time. Even among the frozen items, shrimps dominated the other species until early nineties when finfish over took it in terms of volume of trade. The share of frozen fin fish has increased over the years and has contributed to 40 per cent of the volume of Indian sea food export during 1998-99. (1.08 lakh tonnes exported, earning Rs.495 crores) with far reaching implications in the domestic availability of certin quality fishes and price structure of all fish varieties. Good quality finishes like seer fish, pomfrets, tunas were diverted for export market leaving the domestic consumers devoid of their preference. Besides, the little quantity of finfish that are available in our country are concentrating only the major urban and commercial centres leaving the rural markets. With this idea in focus, the present paper makes a modest attempt to (a) asses the trend in production and export trade of selected varieties of fin fish. (b) compare the export and domestic market price structure for various varieties of fish. (c) evaluate the potentials of aquaculture to maintain the domestic supplies and price stability.

Data and methodology

The data for the study were collected from the publications of Central Marine Fisheries Research Institute (CMFRI) and Marine Products Export Development Authority (MPEDA), Cochin. Percentage analysis was employed for the study.

Findings

Trends in fin fish production and export

Table 1 presents the finfish production and export from India during the period 1993 to 1998.

Table 1. Finfish production and export from India 1993-1998

(in tonnes)

Year	Production	Exports	Percent
1993	1823452	93219	5.11
1994	1816484	122529	6.74
1995	1790645	100093	5.58
1996	191103	173005	9.05
1997	2109345	188029	8.91
1998	2063309	108556	5.26

The export of finfish has been increasing both in terms of quantity and in value in our sea food exports since 1991-92 (Table 2). The share of finfish export has been increasing both in terms of quantity and value in our sea food export increased from 28.7 per cent (1991-92) to 35.80 per cent (1998-99) with the maximum share being 48.47 per cent (1997-98). The share in terms of value increased marginally during the corresponding period. The importance of finfish in Indian seafood export is increasing. This may be because of the change in consumption pattern or consumer preference of the importers, product diversification and promotion measures of MPEDA and other agencies involved in sea food export.

Table 2 Growth of finfish export in India 1991-92 to 1998-99

(Q=Quantity in tonnes, V=Value in Rs. Crores)

Year	Fin fish export	Growth in export (annual)	Percentage of growth	Share in the total sea food export (%)
1991-92	Q: 49119	+6779	16.01	28.7
	V: 142.66	+51.84	57.08	10.41
1992-93	Q: 74076	+24957	50.81	35.44
	V: 222.10	+79.44	55.68	12.56

Export price and domestic price of finfish varieties

The average retail domestic price and the unit value realised for the selected finfish varieties are presented in the Table 5. It is found from the Table that the unit value realised through export is higher than the domestic retail price in all the cases. Hence the traders are concentrating on the export market leaving the domestic market deprived of such varieties. In the local market also, only those who have the purchasing power can get them. Sometimes, even if the consumers are ready to pay, the availability of such varieties is nil. Hence, development of internal market is also an essential component of the sea food trade.

Table 5. Average export and domestic retail prices of selected fin fish varieties during 1997-98

Varieties	Export Price[a]	Domestic Retail Price[b]
Ribbon fish	27	16
Pomfrets	172	120
Tuna	38	25
Mackerel	40	30
Oil sardine	34	25
Shark	41	38
Seer fish	67	73
Snappers	51	38

Source: [a] Marine Products Export Review, 1997-98, MPEDA
[b] CMFRI : SEETT Division

Aquaculture potential, domestic supplies and price stability

The production of all fish varieties from the Exclusive Economic Zone (EEZ) especially from the inshore zone 10-50m depth has reached

stagnation level. Further scope for expansion lies in the off-shore and deep-sea only (Murty and Rao, 1996, Devaraj *et al.*, 1998). With the demand for sea food including-domestic and global- and the scope for increasing production from capture fisheries limited, the attention has now turned seriously on aquaculture. The potential area available for aquaculture in India has been estimated at 11.90 lakh hectares, out of which 1.4 lakh hectares (11.9%) has been brought under shrimp culture (Table 6). It is found from the Table that, states like AP, Karnataka, Kerala and Orissa have comparatively higher area under aquaculture than other states. This indicates the vast scope existing for expansion of aquaculture in India. But it is to be noted that so far shrimp is the only species that is cultivated to a greater extent, which makes the enterprise a risky affair. Though the shrimp farming has been a highly remunerative one, presently the Supreme Court's ruling on aqua farms and the viral diseases have virtually stopped the future expansion. Under such circumstances, diversification of aqua farms to cultivate finfish varieties like groupers, snappers and mullets is the best alternative depending upon their location-specific, techno-economic feasibility. Sakthivel (2000) reported that it is time for diversification to other species like seabass, groupers, snappers and mullets because of the killer virus infection problem in shrimp culture. He added that technological development to culture the above species to be taken up on war footing to continue coastal aquaculture. He said that though the profit margin is less in finfish culture, it will be an ideal alternative to shrimp culture. Ravichandran *et al.*, (2000) expressed that the finfish fishery in the lagoons can be enhanced significantly, provided appropriate culture sites and species are selected.

Table 6. Brackish water aquaculture potential and shrimp culture in India, 1998-99

States.	Potential area (In hectares)	Area under Shrimp culture (in hectares)	Percentage of potential area	Shrimp production (tonnes)	Average productivity (kg/hectare)
West Bengal	405000	42067	10.40	18326	435.64
Orissa	31600	8000	25.30	6000	750.00
Andhra Pradesh	150000	71000	47.30	44856	631.78
Tamil Nadu	56000	1087	1.90	1820	1674.30
Pondicherry	800	22	2.80	27	1227.00
Kerala	65000	14705	22.60	7660	520.90
Karnataka	8000	3564	44.60	2690	754.77
Goa	18500	650	3.50	590	907.69
Maharashtra	80000	426	0.50	409	960.09
Gujarat	376000	316	0.10	256	810.13
TOTAL	11909000	141837	11.9	82364	580.69

Source: Aqua International, 1999

Conclusion and policy implications

The growth in the sea food export market in India has been increasing gradually which is a welcome sign in economic development of the country. At the same time, the domestic market should also be condsidered as it caters to the millions of the people. In finfish export, a substantial share of good quality fishes like seerfish, tunas, pomfrets are available in major commercial centres only leaving the rural consumers devoid of them. Such an unbalanced supply of quality fishes in the internal markets should be regularized. The existing trend of catching even the juveniles of quality finfishes to meet the domestic market demand need to be abolished preferably through law. The lifting of quantitative restrictions on imports as per the guidelines of World Trade Organisation (WTO) will boost the domestic sea food industry. The Sea food Exporters Association of India has expressed hope of constructively deploying the tremendous idle capacity in the seafood processing industry in India with the lifting of quantitative restrictions on imports. Our sea food exports mainly consist

of frozen raw materials, which the importing country processes and re-exports as value added products (VAP's). Our sea food exports presently comprise only 10 per cent of VAP, which should be increased further. As the supply from capture fishery is stagnant, the attention on coastal aquaculture should be intensified. Here, there is a need for diversification to finfish culture instead of concentrating shrimps alone. This will help to maintain the domestic supply of finfish as well as their price structure.

References

CMFRI, *Annual reports*, 1991-1999, Central Marine Fisheries Research Institute Cochin.

Devaraj. M., V.Sriramachandramurty, R. Sathiadhas, and K.K. Joshi, 1998, The New Economic Policy and Perspective for Marine Fisheries Research and Development in India *Fishing Chimes*, 18 (5):18-29.

MPEDA, *Marine Products Export Review*, 1991-1999, Marine Products Export Development Authority, Cochin.

Murthy, V.Sriramachandra and Rao, P. Vedavyasa, 1996. Marine Fishery Resource of India - Present status and management concerns. In *Marine Biodiversity Conservation and Management* (Eds.) Menon, N.G. and Pillai, C.S.G. Central Marine Fisheries Research Institute, Cochin.

Ravichandran, S., R. Kannan and T. Kannupandi, 2000. Aquaculture in Coastal Lagoons of India. *Aqua International*, 7(9):26-29.

Sakthivel, M., 2000. Aquaculture to have bright prospects in the next millennium, *Aqua International*. 7(9):4.

Social audit - an ideal method for neutralising conflict situations in aquaculture industry

K.Vijayakumaran

Visakhapatnam Research
Centre of Central Marine
Fisheries Research Institute, Visakhapatnam - 530 003

ABSTRACT

The environmental issues hampering the development of aquaculture in India is briefly reviewed. The genesis of conflicts among different stakeholders is analyzed. The concept of social audit in aquaculture industry is introduced. The process by which social audit could catalyze neutralization of factors of conflict and evolve a transparent environment is discussed in the background of the information obtained from a preliminary survey.

Introduction

Coastal aquaculture expanded rapidly in a very short span of time in our country, especially in the states of Andhra Pradesh and Tamil Nadu. The consequences of this unbridled expansion brought in its wake a number of problems similar to those experienced in many South East Asian countries. Concentration of farms in certain localities had compounded the issues related to disposal of waste and enhanced the rapid spread of diseases. The industry suffered heavy loss during a few years

shortly after the boom and many corporate farms, unable to cope up with the uncertainties, wound up their business.

Increased awareness of the adverse impacts of coastal aquaculture among the stakeholders manifested in the form of protests and representations calling for legal intervention. The obvious lacunae in the existing legal regime for protecting the coastal zone were more than filled by the now famous Supreme Court Judgment dated 11-12-1996. As directed in the judgment, Aquaculture Authority has been established to implement the 'precautionary principle' and 'polluter pays principle'. With powers for drastic measures such as demolition of farms violating the CRZ notification bestowed on the Authority, The explosive development of aquaculture has come to a grinding halt. Modern society is becoming more and more conscious about the environmental issues and the impact of this could be seen all over the world. All activities having environmental impact are being critically viewed for control and regulation. Entities engaged in activities causing environmental damage are either restricted or are forced to seek preventive measures. Environmental audit is becoming mandatory for all enterprises in our country and currently there are about eighty firms practicing the same (Anon, 1999).

The inevitable consequence of any system that gets stuck up with legalities is that sooner or later the system will move towards inactivity (Fig. 1). In a typically bureaucratic set up like ours, the system reaches inactive phase much faster. The development of aquaculture in the Zone-1 (Srikakulam, Vizianagaram and Visakhapatnam districts) of Andhra Pradesh in the post-judgment period could be cited as a typical example of this phenomenon. After the aquaculture authority was set up, as on December 1998, 314 application forms were sold of which only 176 was received by the Fisheries Department. Out of this, 68 are with the ADFS, 37 had been sent to the state level screening and only has been forwarded to Aquaculture Authority (information revealed by K. Satyam, RDD,

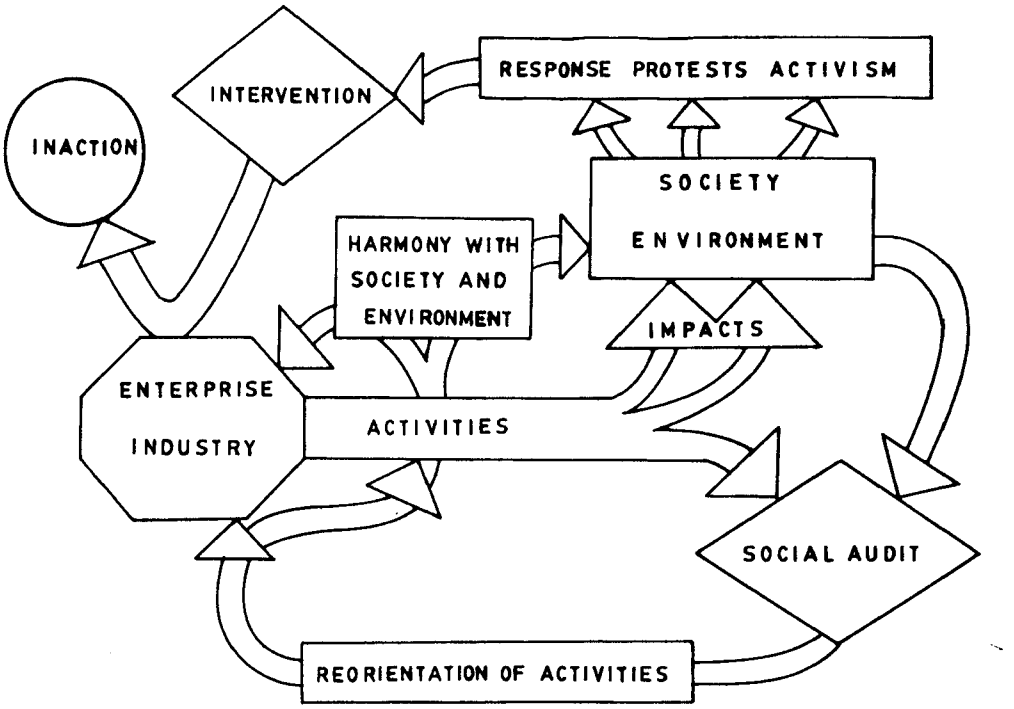


Fig. 1: Conceptual diagram showing the dynamics of the enterprise in its environment and the role of social audit in the process of bringing harmony in the system

Fisheries, Govt.of AP at the monthly meeting of the Forum of Fisheries Professionals, Visakhapatnam).

The question of evolving an eco-friendly sustainable aquaculture system will remain elusive as long as the system is tied up in the legal tangle. Unless some attempts are made to analyze the dynamics of the causative factors, and reorient actions in harmony with them, these problems will remain perennial. Fortunately, however, the situation has not drifted into an irreversible state and a transformation is quite feasible within a reasonable time horizon. The basic requirement is the evolution of transparent environment where the activities (and their repercussions) of every component is open for unbiased observation and

evaluation by every other component in the system. Social audit is identified as an ideal method for bringing in the transparency and effecting the transformation. This paper attempts to identify and examine the activities of the aquaculture industry having an impact on the immediate environment and to elaborate how social audit would help to neutralize the conflict situation in aquaculture industry.

Methodology

An overview of the current environment and social issues in the aquaculture industry is made by heavily drawing from literature and information gathered from personal contacts with people in the field. The idea of social audit as relevant to the aquaculture industry was developed based on the available literature. The conceptual elaboration is mainly done based on the author's perception of the issues. A questionnaire method was adopted to collect preliminary information from the farmers and to assess their awareness of the concept of social audit.

Social audit

Though Bowen (1953) first proposed the notion that business should make social audit of its activities more than forty years ago, the idea remained dormant for many years and has gained significant attention in the past two decade in the developed countries, particularly in USA. However, the concept is yet to make any visible influence on the corporate philosophy in most of the developing countries. As far as India is concerned, the idea is yet to catch up with the corporate world. Tata Iron and Steel Company (TISCO) has set an example in this direction by initiating some work in this area in 1980. The idea of social responsibility of business and relevance of social audit in aquaculture industry was elaborated in a recent work (Vijayakumaran, 1999). This approach will help the industry to analyze the dynamics of the causative factors, and orient the actions in harmony with them.

Social audit has been variously defined, but the one by Belkaoui (1984) appears to be more comprehensive. "Social audit-much like financial audit- is an identification, and examination of the activities of the firm in order to assess, evaluate, measure, and report, their impact

on immediate social environment"

Identification, assures tracking down and inventory of all the firm's activities having potential impact on the firm's environment. Identification will result in a definition of the social dimensions of the firm's activities in terms of social costs or social benefits depending on the nature of their impact on social environment. Assessment and evaluation, imply the categorization of the firm's impact on its environment as either positive social benefits or negative social costs. Measurement, implies the assignment of a quantitative or qualitative score to the social costs and benefits identified in assessment and evaluation. Reporting, assumes the disclosure of the firm's performance as measures.

The issues in aquaculture industry

Conflicts generally arise due to differences in perception of different stakeholders. Conflicts are more common in a rather ill informed or misinformed environment. Vijayakumaran (1999) stated that the major issues in coastal aquaculture, which drew the attention of the environmentalists, NGOs and Government were the environmental and social problems related to:

* Destruction of mangrove and other vegetation.
* Multi-user conflicts (with agriculture).
* Hyper-nutrification, and discharge of heavy load of organic and suspended matter.
* Use of chemicals, fertilizers, piscicides, antibiotics, chemotheraputants etc.
* Depletion of ground water, increase in salinity in the ground water and soil salinity of agriculture lands.
* Obstruction of access to sea front and other common resources.

No doubt, all these issues pertain to coastal aquaculture industry. They, however, give only a one sided view of the scenario. The issues

such as whether the industry is faithfully discharging its responsibility towards the stockholders are seldom discussed in any public forum. Similarly the social benefits by way of development of backward area, employment opportunities for local population, valuable foreign exchange earnings etc. were always eclipsed by the major issues listed above. On examining the activities of the coastal aquaculture industry, a set of factors contributing to social cost and social benefits could be identified (Table-1). It must be noted that though some of the factors are common to all farms, majority does not have universal applicability. More over

Table-1. A preliminary list of factors contributing to the social costs and social benefits with regard to coastal aquaculture industry.

Activities contributing to	
Social costs	**Social benefits**
Discharge of effluents with heavy loads of organic and inorganic pollutants.	Conversion of uncultivable land like sandy wasteland, abandoned salt pans, marshy unproductive areas etc.
Felling of trees providing windbreak etc.	Planting of tree cover.
Obstruction of passage to fishermen and local people to seafront.	Construction of roads which is also used by the public.
Conversion of paddy fields.	Employment to local community.
Destruction of mangroves	Construction of facilities for local community.
Contamination of source of water intake of the neighbouring farm.	Supply of seed, feed, and shrimp to the neighbouring farm/plant.
Depletion of ground water.	Providing processing facility to/ purchase of seed, feed and shrimp from neighbouring farm/plant.
Salinity changes in the soil and water.	Contribution to foreign exchange.
Import of inputs/technology.	Development of backward area.
Effect of antibacterial drugs	Benefits to shareholders.

the magnitude of these factors may vary from farm to farm. The questionnaire survey revealed these aspects.

Activities contributing to social costs

Destruction of mangroves

The importance of mangroves in sustaining the coastal ecosystem is well known. The repercussions of destruction of mangroves may manifest in various forms such as decline in the catches of some important fishes or decline in the fishery as such in the long run. Though none of the farms responded to the survey in the present study indicated any destruction of the mangroves, large areas of mangroves are reportedly converted into shrimp ponds in Andhra Pradesh and Orissa.

Conversion of paddy fields

The yield from the prawn culture being much more lucrative than the yield from paddy cultivation, many enterprising farmers have converted their paddy fields into shrimp ponds. This process, though very much beneficial to the individual farmer, is not desirable from the social point of view as it deprives the people of a primary wage good-the staple food. Only one farm had reported conversion of paddy field in the present study. However, reports from reliable sources indicate that nearly 35,000 ha of paddy fields might be under prawn culture in Godavary-Krishna belt of Andhra Pradesh. The capacity of *P. monodon* to survive and grow in zero ppt salinity has come as boon to these farmers. Incidentally these farms which are not registered with MPEDA or other regulatory authorities are constructed in simple way, allowing reconversion to paddy cultivation without much difficulty.

Another consequence, which is not apparent, of conversion of paddy fields is the displacement of labour. It was reported that there is about 25% reduction in the labour input when a unit area of paddy field is converted into shrimp pond (Nirmala, 1998). Women are the losers as they contribute major share oi labour in the paddy cultivation. Though women are employed in the shrimp processing, the labour generated is comparatively less than the labour lost.

Felling of trees and obstruction of passage

The issues and options related to the management of Casuarina plantations which acts as protection against cyclonic winds had been discussed in some recent works (Vivekanandan *et al.*, 1997; Vijayakumaran, 1998a). However none of the farmers responded in the present study reported such destruction. Obstruction of passage to seafront was reported to be another area of conflict in isolated cases. However, the farms responded to the present survey did not cause any obstruction to the passage of fishermen and other people to the seafront.

Effluents, drugs, pollution

This is one of the important points being raised against coastal aquaculture. The situation in certain segment of Nellore area where many farms draw and discharge water from/to the same creek has become a classical example of how pollution load can affect the immediate neighbourhood directly. The external costs associated with the pollution from the environmental angle was discussed in an earlier work (Vijayakumaran, 1998b). The total quantity of waste produced in the system and which load the environment is closely related to the culture system used (Bergheim and Asgard, 1996). Selection of appropriate system need to be made in order to reduce the costs identified. The uneaten and undigested feed being the major factor, the improvement in feed production technology and feeding methods and practices are likely to play a significant role in mitigating this problem. Widespread use of antibacterial drugs, which gets immediately transported to the environment, is an area of concern for all. Paucity of scientific data on the aquatic fate and effects of antibacterial drugs is one of the reasons for this concern (Weston, 1996).

Water, soil and salinity

Changes in the soil and water salinity of the nearby water bodies, depletion of ground water etc. are problems reported to be encountered in some areas. None of the farms responded to the present study indicated any such incidents.

Activities contributing to social benefits

Conversion of uncultivable land

The use of unproductive land for some productive purpose is always beneficial to the society. Two of the farms, which responded, had converted sandy area into ponds.

Planting trees, construction of roads & facilities

Planting trees as wind shelters has not been done by any of the farmers who responded. The neighbouring communities sometimes use roads constructed as approaches to the farms, at least for short distances. Construction of facilities for the local communities is a common feature of the business field. However, prawn farmers are yet to think in that direction.

Backward area development & employment generation

This is an area where the farming sector has contributing a lot to the society. Majority of the farms being located in the backward areas, the development of aquaculture activities had facilitated the development of the area. The land value had shot up very high in certain localities. The farming activities had indirectly brought in some ancillary activities also. The farm utilizes quite significant amount of local labour during the construction period and provides employment to the local community on a continuing basis.

Trade with neighbouring units

A farm may buy seeds from a nearby hatchery and sell its produce to a neighbouring processing plant. If the farm is vertically integrated, other neighbouring units use the facilities of hatchery and processing units. Thus giving direct support to the neighbouring business units.

Benefits to foreign exchange & share holders

Foreign exchange earning is the largest contribution from the aquaculture industry to the society. As the commodity is meant for export markets the foreign currency earning helps to some extent to miti-

gate the problem of trade balance. As far as the corporate farms are concerned, the value earned per share is an important benefit. However, if the market values of the shares are to be considered as an indication of the performance, the corporate farms have totally betrayed the shareholders.

The process of neutralisation

The need of the time is a transition from the regulatory regimes to a regime of voluntary action motivated by an innate desire for harmonious existence. This calls for a basic change in the business ethics. Understanding and discharging of social responsibilities is fundamental aspect of ethically sound business. The damaging and degraded state of most aquatic systems combined with public concerns about adding new sources of pollution to the already overburdened ecosytem will require aquaculture to develop ecosystem approaches and sustainable operating procedures (Costa-Pierce, 1996). If the criterion is sustainability of an activity at micro level, there will be very few activities, which would qualify perfectly. As Muir (1996) has suggested, large-scale sustainability does not require small scale sustainability to be universally attained. That is, it could be feasible to accept a few unsustainable activities such as high intensive aquaculture as long as their net deficit (such as depletion of renewable energy, longer terms qualitative and quantitative diminution of fish stocks) could be compensated by other activities (e.g. use of aquaculture) for rehabilitating damaged environment or indeed any other activity which has a suitably positive effect. Social audit is an ideal tool for this balancing exercise.

Once the factors contributing to the social costs/benefits are indentified, their objective assessment, evaluation and measurement and presentation will give an idea of the net cost/benefit of the farm. The process will help the management to determine the areas where it is vulnerable to public criticism. The audit report will inform the public of what the farm is doing in the area of social responsibility. Presenting a true picture about the social and environmental accountability to the public is likely to change the attitude of the stakeholders. Awareness of

the net social benefits will certainly justify the existence of an entity and nullify the factors causing conflicts.

For aquaculture industry to accept this idea, it is necessary to create an awareness of the underlying principles and the expected benefits. The present survey revealed that none of the farms were aware of the concept of social audit. However, they expressed their willingness to adopt such measures if beneficial to the sustainability of their business. The understanding of the principles and adoption of the practices will not yield any tangible benefits in the short-run. But the system will certainly move towards sustainability in the long-run.

References

Anon, 1999. Environment Ministry wants Green Audit made mandatory. *Economic Times*, 14 January 1999. 38(310):4

Belkaoui, A, 1984. *Socio-Economic Accounting.* Quorum Books, Westport, p.261.

Bergheim, A. and T. Asgard, 1996. Waste production from aquaculture. In:Baird, D.J.,Beveridge, M.C.M., Kelly, L.A. and Muir J.F.*Aquaculture and Water Resource Management.* Blackwell Science, Oxford. pp.50-80.

Bowen, H.R.1953. *Social Responsibilities of Businessman.* Harper & Brothers, New York.

Costa-Pierce, B.A.1996. Environmental impacts of nutrients from aquaculture: Towards the evolution of sustainable aquaculture systems. In:Baird, D.J., Beveridge, M.C.M., Kelly, L.A. and Muir J.F. *Aquaculture and Water Resource Management.* Blackwell Science, Oxford. pp.81-113

Muir, J.F.1996. A Systems approach to aquaculture and Environmental management. In:Baird, D.J., Beveridge, M.C.M., Kelly, L.A. and Muir J.F. *Aquaculture and Water Resource Management.* Blackwell Science, Oxford.pp.50-80.

Nirmala, A.K. 1998. Coastal Aquaculture in Visakhapatnam District of Andhra Pradesh. Report of a study sponsored by ICAR, New Delhi. IDPS, Visakhapatnam. 148p.

Vijayakumaran, K. 1998a. On propagation of two species of palm as a part of Integrated Coastal zone Management. In: *Eastern Ghats:* Proceedings of the National Seminar on Conservation of Eastern Ghats, 24-26 March 1998. EPTRI, Hyderabad. pp.92-97.

Vijayakumaran. K. 1998b. Incorporating environmental dimension in the economic evaluation of aquaculture practices. Paper presented at the National Seminar on Aquaculture Economics, 6-8, October 1998 CIFA, Bhubaneswar.

Vijayakumaran K 1999. The relevance of social audit in aquaculture industry. *Fishing Chimes* 18 (12): 50-52.

Vivekanandan V. and Muralidharan C.M. Subba Rao M. 1997. A study of the Marine Fisheries of Andhra Pradesh (Draft Report).

Weston, D.P. 1996. Environmental considerations in the use of antibacterial drugs in aquaculture. In: Beard, D.J., Beveridge, M.C.M., Kelly, L.A. and Muir J.F. *Aquaculture and Water Resource Management.* Blackwell Science, Oxford. pp.140-165

AUTHOR INDEX