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Polychaetes - a suitable live feed for growth and colour quality of the clownfish, *Amphiprion sebae* (Bleeker, 1953)

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Abstract

Growth and colour quality of hatchery bred clownfish, *Amphiprion sebae* were monitored in three sets of feeding trials with polychaetes, trash fish and clam meat. The increment in length (4.55±0.17 cm) and weight (5.15±0.57 g) were found to be more in fishes fed on polychaetes followed by fishes fed with trash fish (4.11 ±0.08 cm and 4.95±0.5 g) and clam meat and (4.10 ±0.05 cm and 3.38 ±0.62 g). Enhanced colour quality was also noticed in fishes fed with polychaetes. Fatty acid profile showed a high level of PUFA in polychaetes compared to other two feeds. Two ñ way ANOVA indicated significant variations in length and weight in relation to days of experiment and treatments. The present study shows usefulness of polychaetes in enhancing the growth and colour quality of ornamental clownfish.

Keywords: *Amphiprion sebae*, ornamental fish, polychaetes, water quality, proximate composition, fatty acid profile

Introduction

Ornamental fish trade is a growing multibillion dollar industry. The major bottleneck in marine ornamental fish industry is inadequate feed quality which leads to stunted growth, poor coloration and mortality. Therefore, the feed given to the aquarium fishes i) should be readily acceptable to the fish and ii) should meet the nutritional requirements, which promotes growth and colour. In recent years, scientists have shown interest to find out the role of specific nutritional components such as essential fatty acids (EFA), phospholipids, vitamins C & E, carotenoids and other dietary components of the feed on the growth of fish. As protein is the most expensive part of the feed, it is imperative to accurately determine the protein requirement of the cultured species.

In aquaculture, feeds such as squid meat, trash fish, clam meat, polychaetes etc., are being used. Among these, polychaetes in general and nereids in particular are extensively used as live feed for shrimp and ornamental fish brooders, which enable successful breeding on a large scale (Olive, 1999). Nereid worms are commonly called omegaworms due to their high content of omega-3 (ω-3) polyunsaturated fatty acids (PUFA) (Lytle *et al*., 1990; Olive *et al*., 1992). The nutritional value of *Nereis* sp. was demonstrated by feeding ragworms to shrimp and fishes, which resulted in an increased number of eggs per spawning, increased egg viability and larval survival in shrimps (Briggs *et al*., 1994) besides rapid maturation in shrimps and ornamental fishes (Luis and Ponte, 1993; Gopakumar *et al*., 2001; Ignatious *et al*., 2001). Therefore, food regimes containing nereids potentially improve the reproductive fitness of cultured animals. Aquaculture farms produce huge amounts of sludge, which in turn might be recycled by feeding to the worms. In this backdrop, an attempt was made to study the efficiency of polychaete worms on the growth and colour quality of clownfish, *Amphiprion sebae*.

Material and Methods

Hundred numbers of freshly grown clownfish, *A. sebae* (length range: 2 to 2.5 cm) were collected from the ornamental fish hatchery of CAS in Marine Biology, Annamalai University and the same were acclimatised for seven days before the start of experiment. From this, 90 healthy and active fishes were chosen and 5 fishes each were stocked in three experimental tanks. The experiments were conducted for six months in 3 FRP tanks of 50 l capacity. A triplicate set of experiment with same number of fishes was also carried out simultaneously. The fishes were fed 5% of their body weight with three types of fresh feeds such as live hatchery bred polychaete, *Perenereis cultrifera* (Treatment I), trash fish, *Leiognathus* sp. (Treatment II) and clam, *Meretrix meretrix* (Treatment III). Length and weight of fish were recorded once in fifteen days. The colour quality was judged by a test panel of scientists from Central Institute of Brackishwater Aquaculture and Central Marine Fisheries Research Institute by ranking fishes having more colour and brightness as 3, for moderate colour and brightness as 2, and less colour and brightness as 1. Water was aerated and 10% was exchanged every alternate day. Each tank had a biological filter packed with activated charcoal, coral sand and synthetic sponge which helped in purification of water.

Water quality parameters such as temperature (∞C) was measured using a thermometer, salinity (psu) by using hand refractometer (ATAGO - JAPAN), dissolved oxygen by Winkleris method described by Strickland and Parsons (1972) and pH using a pH pen (Elico model). Ammonia was measured following the method of Solorzano (1969) and nutrients (phosphate and nitrite) were estimated following the methods described by Strickland and Parsons (1972).

The experimental feeds were subjected to proximate analysis. Total protein was estimated by the method of Lowry *et al*. (1976); carbohydrate by Dubois *et al*. (1956); lipid by Folch *et al*. (1957). Ash content was determined through weight loss after incinerating 30-60 mg of samples at 500∞C for 4 h. Besides this, fatty acid profile of the feeds was analysed using the methods of Anon (2000) and Sahin *et al*. (2005). For this, 100 mg of sample was added to 1 ml of 1.2 M NaOH in 50% aqueous methanol in a screw-cap test tube tightly sealed with Teflon tape and then incubated at 100∞C for 30 min in a water bath. The saponified samples were cooled at room temperature, and then acidified and methylated by adding 2 ml of 54% 6 N HCl in 46% aqueous methanol and incubated at 80∞ C for 10 minutes in water bath. After rapid cooling, methylated fatty acids were extracted with 1.25 ml 50% methyl-tart butyl ether (MTBE) in hexane. Each sample was mixed for 10 minutes and the bottom phase was removed with a Pasteur pipette. Top phase was washed with 3 ml 0.3M NaOH. After 5 minutes of mixing, the top phase was removed for analysis. Following the base wash step, the FAMEs were cleaned in anhydrous sodium sulphate and transferred into GC sample vials for analysis.

Results and Discussion

Temperature varied from 27.5 to 32∞C; salinity from 21 to 29 psu; pH from 7.6 to 8.2 and dissolved oxygen from 3.45 to 5.73 ml/l (Fig. 1). The nitrite level varied from 0.02 to 0.09 ppm with minimum in Treatment III and maximum in Treatment I; phosphate from 0.12 to 1.47 ppm with minimum in Treatment I and maximum in Treatment III. Ammonia values were from 0.02 to 0.09 ppm with minimum in Treatment I and maximum in Treatment II. The quality of water during the culture deteriorates mainly due to feed waste, animal excreta, and decaying organisms (Choudhury and Panigrahy, 1991). In confirmation of this, Allen and Maguire (1992) found that even changes in pH and salinity could affect the survival, growth and osmo-regulation in marine organisms. Likewise, Tedengren *et al*. (1998) and Ravi *et al*. (1998) reported that drop in salinity causes physiological stress in crustaceans.

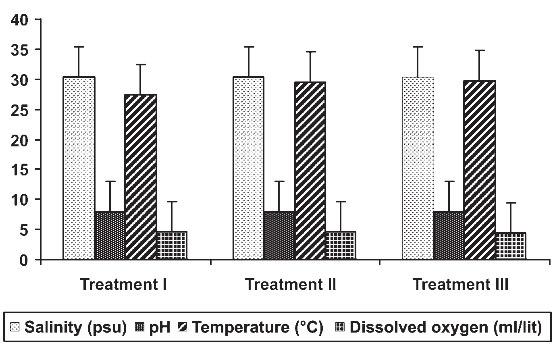


Fig. 1. Water quality parameters (mean and SE) recorded in various experimental tanks

The nutrients levels recorded in the present study were found to be comparable to that of Murugesan *et al*. (2008). Ammonia level was found to be within the permissible range even though it showed slightly higher level in trash fish and clam meat experiments. This might be due to more animal waste in that tank which normally triggers the ammonia level as has been reported earlier by Bhattacharjee (2008).

Besides this, fishes need to consume adequate amount of protein, fat, vitamins and minerals for their successful breeding and embryonic development (James and Sampath, 2004). Therefore, the feeds used in the present study were subjected to proximate analysis. The protein level ranged from 39.6 to 49.2% with minimum in clam meat and maximum in polychaetes; carbohydrates content from 8.5 to 15.6% with minimum in clam meat and maximum in trash fish; lipid content between 11.09 and 17.7% with a minimum in trash fish and maximum in polychaetes; moisture level from 58.6 to 76.2% with minimum in polychaetes and maximum in clam meat; and ash content varied from 14.2 to 27.3% with minimum in polychaetes and maximum in clam meat (Table 1). In many cases, the marine ornamental fishes fed with dry pellets did not show significant survival, growth and colouration. In natural environment, fishes have developed a wide variety of feeding specializations to acquire essential nutrients and utilize various food sources. However, the feeding behaviours of fishes in captivity are to be taught to readily accept various prepared foods which contain essential nutrients. Gardon *et al*. (1998) and Sales and Jassens (2003) studied the effect of balanced nutrition on growth and survival of clownfish larvae. Similarly, Johnston *et al*. (2003) highlighted the influence of nutrition on ornamental fishes. Olivia *et al*. (2006) studied the effect of feeding on the spawning frequency of clownfish and observed an increased spawning rate in *A. sebae* which fed on polychaetes. Similarly, Watanabe and Vassallo (2003) underscored the importance of broodstock nutrition in marine finfishes. Likewise, Booth and Alquezar (2002) worked on the supplementary feeding of damselfish, *Acanthochromis polyacanthus* larvae and found promising results using live feeds. Recently, Ignatius *et al*. (2001) and Gopakumar *et al*. (2001) studied on the growth and survival of clown fish *A. chrysogaster* fed with various feeds. Among the feed combinations, fishes fed on polychaetes and live *Acetes* grew faster and showed better colour quality. Recently, Sithara and Kamalaveni (2008) reported that growth pattern in ornamentals is largely influenced by type and quality of feed.

In light of the above, in the present study, the fishes fed with polychaetes yielded good results, in terms of growth and colour quality compared to fishes fed with the other two feeds. Above all, polychaete fed fishes were also found to be hale and healthy when compared to fishes fed on other feeds. When the results of growth were viewed, the initial average length and weight of the fishes were 2.1 cm (±0.42) and 0.85 g (±0.12) in Treatment I; 1.9 cm (±0.14) and 0.6 g (±0.14) in Treatment II and 2.15 cm (±0.14) and 1.07 g (±0.10) in Treatment III. At the end of the experiment, the average length and weight of the fishes were 6.65 cm (±0.23) and 6.0 g (±0.11) in Treatment I; 6.0 cm (±0.28) and 4.45 g (±0.35) in Treatment II and 6.25 cm (±0.13) and 5.55 g (±0.14) in Treatment III. Maximum length gain of 4.55 cm (±0.17) was obtained in Treatment I and the minimum of 4.10 cm (±0.05) in Treatment III (Fig. 2). Similarly, maximum weight gain of 5.15 g (±0.57) was recorded in Treatment I and a minimum of 3.38 g (±0.62) in Treatment III (Fig. 3). Two- way ANOVA revealed that the difference were highly significant ($p > 0.005$) between the progressive days of experiment and between feeds. In respect of colour quality, fishes fed on polychaetes showed enhanced colour quality (3) followed by clam meat fed fishes

Table 1. Proximate composition in experimental feeds (% of wet tissue weight)

Feed	Protein (%)	Carbohydrate (%)	Lipids (%)	Moisture (%)	Ash (%)
Polychaetes	49.2	12.4	17.7	58.6	14.2
Trash fish	41.1	15.6	11.09	69.2	15.26
Clam meat	39.6	8.5	13.01	76.2	27.3

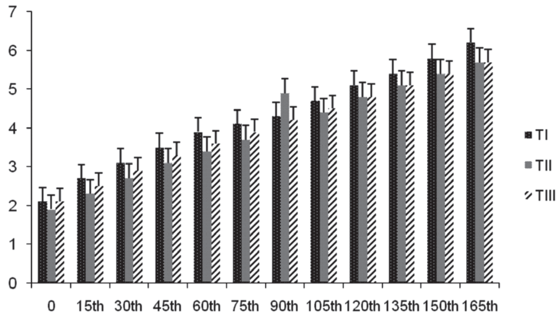


Fig. 2. Length (mean and SE) recorded in fishes fed with three different diets

(2) and fishes fed on trash fishes (1). The colour and brightness of fishes are shown in Plate I. This indicated that marine polychaetes possess high level of poly unsaturated fatty acids (PUFA-Eicosapentaenoic acid and Docosapentaenoic acid) and protein content which are considered to be very much essential for promoting growth, egg maturation and colour quality as has been reported earlier by Olive (1999). True to this, Fatty acid profile (Table 2) of the present study revealed that a higher amount of PUFA especially, docosahexaenoic acid, eicosapentaenoic acid, linoleic acid and arachidonic acid in polychaetes compared to clam meat and trash fish, which might be the plausible reason for the maximum growth and attractive color in polychaete fed fishes as reported earlier by Alasalvar *et al*. (2002); Celik and Gokce (2003) and Senso *et al*. (2007). There are two possible routes for the *de novo* generation of PUFA in polychaetes. Firstly bacteria may be breaking down the yeast and the metabolic products are then utilized by phytoplankton in the system which then synthesizes PUFA. Alternatively

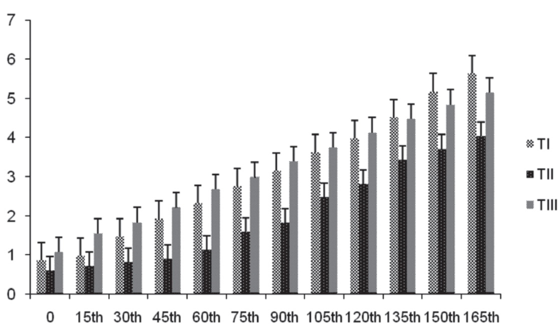
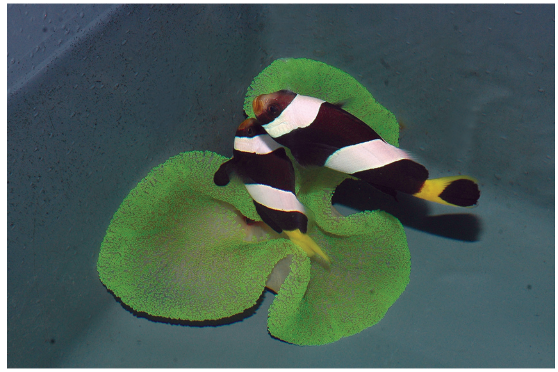
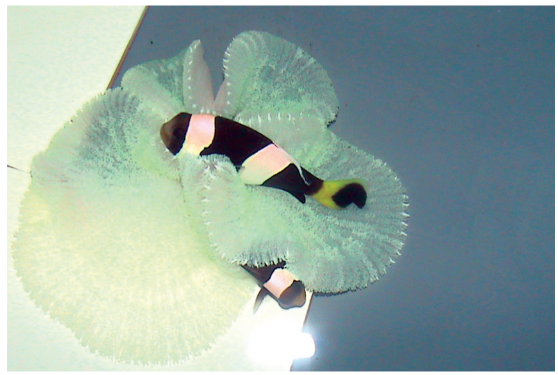


Fig. 3. Weight (mean and SE) recorded in fishes fed with three different diets



a) Fishes fed with polychaete



b) Fishes fed with trash fish

c) Fishes fed with clam meat

the accumulation of PUFA may be *via* a process in which there is bacterial degradation of organic complexes to pyruvate and *de novo* of PUFA by specific bacterial species e.g. *Shewanella* spp., in the substrate, which produce a maximum amount of EPA (Eicosapentaenoic acid), DHA (Docosahexaenoic acid) and AA (Arachidonic acid) in marine substrates

Table 2. Fatty acid profile (% of total fatty acids) in various experimental feeds

Carbon Chain	Fatty acid	Polychaete	Trash fish	Clam meat
C9:0	-	0.23	-	-
C10:0	Capric acid	-	0.13	-
C12:0	Lauric acid	0.92	0.29	0.71
C13:0	Tri decyclic acid	-	-	0.66
C14:0	Myristic acid	3.31	5.22	3.98
C15:0	Penta decyclic acid	2.34	1.25	1.83
C16:0	Palmitic acid	18.50	16.98	21.62
C17:0	Margaric acid	2.30	1.29	2.90
C18:0	Stearic acid	2.17	5.09	6.32
C19:0	Nonadecyclic acid	-	0.20	4.51
C20:0	Arachidic acid	1.32	0.13	2.64
C22:0	Pehinic acid	1.43	0.23	-
C23:0	Tricosanic acid	0.13	-	-
C24:0	Lignoceric acid	0.59	1.19	-
Σ SFA		33.24	32.01	46.09
C14:1 ω-3	-	0.10	0.02	-
C14:1 ω-7	-	0.14	0.10	-
C14:1 ω-5	Physeteric acid	0.77	0.09	-
C15:1 ω-6	-	-	0.14	-
C16:1 ω-5	-	5.16	0.22	2.24
C16:1 ω-7	Palmitoleic acid	4.12	5.14	4.86
C16:1 ω-11	-	0.72	-	-
C17:1 ω-8	-	0.69	1.69	2.20
C18:1 ω-5	-	1.06	-	-
C18:1 ω-7	Cis-7-Octadecenoic acid	3.30	-	-
C18:1 ω-9	Oleic acid	7.10	12.46	5.32
C20:1 ω-7	Cis-7- Eicosenoic acid	-	-	2.09
C23:1 ω-9	-	-	2.63	-
C24:1 ω-3	Cis-3- Tetrassenoic acid	-	2.64	-
Σ MUFAs		23.16	25.12	16.71
C16:2 ω-6	-	1.42	4.38	-
C18:2 ω-6	Linoleic acid	5.66	-	-
C18:3 ω-3	Alfa linolenic acid	4.30	9.81	13.96
C18:3 ω-6	Gamma linolenic acid	0.36	3.28	14.57
C18:4 ω-3	Stearidonic acid	2.09	5.69	-
C19:2 ω-6	-	-	3.24	-
C20:2 ω-6	Eicosadienoic acid	5.94	3.12	-
C20:3 ω-6	Dihomogamma linolenic acid	-	3.38	-
C20:4 ω-6	Arachidonic acid	4.66	4.69	8.67
C20:5 ω-3	Eicosapentaenoic acid	5.90	5.28	-
C22:4 ω-6	Docosatetraenoic acid	1.35	-	-
C22:5 ω-3	Docosapentaenoic acid	2.52	-	-
C22:6 ω-3	Docosahexaenoic acid	9.40	-	-
Σ PUFAs		43.60	42.87	37.20

(Gong and Hollander, 1997; Harvey and Macko, 1997; Nichols and McMeekin, 2002). In light of the above statements, the higher PUFA content in polychaetes is justifiable. Recent studies have also demonstrated that supplementation of docosahexaenoic acid in the live feed improved the growth, survival and stress resistance of marine fish larvae (Watanabe, 1993). Other studies done elsewhere have also found that PUFA, especially eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA, 22: 6n-3) are essential dietary components for the growth of marine fish larvae (Webster and Lovell, 1990). Therefore, the results of above said studies corroborate the findings of the present study and also underlines the need of these essential fatty acids by the fishes to ensure good growth and survival.

Thus, the present study indicated that the mass scale production of marine clown fish, *A. sebae* can be successfully carried out using polychaetes than any other feeds which contain more protein and essential fatty acids. Further, based on the results, the present study call for a detailed screening of amino acids structure of the feeds experimented in order to have a holistic picture of the role of nutrients in the growth and colour quality in the ornamentals.

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