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Yolk utilization in a marine edible crab *Charybdis lucifera* (Fabricius)

T. Kannupandi, A. Veera Ravi and P. Soundarapandian

Centre of Advanced Study in Marine Biology,
Annamalai University, Parangipettai – 608 502,
Tamil Nadu, India.

Abstract

The yolk utilization during embryonic development was monitored in the marine crab *Charybdis lucifera*. Water content enhanced from 68.60% to 90.34 %; ash content increased from 1.9% to 19.02%; whereas, all the organic constituents declined with a range from 66.88% to 56.63% in protein, from 26.58% to 20.11% in lipid and 4.42 to 27.19% in carbohydrate. Of the organic substances, lipid was the major source of energy, since nearly 50% of the total energy was stored in the form of lipid in the eggs. The protein and carbohydrate contributed only 41.94% and 8.38% of the total energy respectively. During embryonic development, the saturated fatty acids increased by 13.73% but the monounsaturated and polyunsaturated fatty acids decreased by 8.03% and 8.05% respectively. Of the two essential polyunsaturated fatty acids, the 20:5n-3 decreased linearly and the 22:6n-1 maintained about a constant level throughout the embryonic development. Proteins in developing eggs are progressively depleted for the possible utilization during embryogenesis. The decrement of fat during embryogenesis not only provides metabolic energy but also for freezing the protein from being oxidized. The carbohydrate has a dual role of supplying energy for metabolism and contributing to structural formation.

Introduction

In crabs, the utilization of yolk during embryogenesis varies based on the percentage composition of yolk material, nature of habit and size of egg. Babu (1987) reported that the protein is the major energy source for the embryonic development of rocky shore crab *Xantho bidentatus*. The eggs of freshwater crab *Paratelphusa hydrodromous* utilizes their enormous lipid reserves (Pillai and Subramonium 1985). Subramonium (1991) has also observed lipid as the chief energy source for the embryogenesis in the intertidal mole crab *Emertia asiatica*. Kannupandi *et al.* (1999) reported that protein is the main source of energy for embryonic development of estuarine crab *Thalamita cranata*. Recently Kannupandi *et al.* (in press) reported that lipid is the main food reserve used for embryonic development of mangrove crab *Sesarma brockii*. Further there is no information on fatty acid profile during yolk utilization. Hence the present study is aimed to know the possible energy source including fatty acids for the embryonic development of marine edible crab *Charbdis lucifera*.

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Material and methods

The ovigerous females of *C. lucifera* were collected from the trawlers at Parangipettai coast and were brought to the laboratory in plastic containers with filtered and aerated seawater with a salinity of 35 ± 1 ppt. The number of fertilized eggs in a brood varied from 50,000 (carapace length 2.5 cm) to 3,00,000 (carapace length 5.0 cm). For the present study even sized brooder of 4.3 cm carapace length were used. The egg mass from each crab was carefully removed. The developmental stages were noted by observing them under a stage binocular microscope (Maiji, Labex, Japan). Six distinct stages of developing embryo from egg to first zoea were identified following the method of Amsler and George (1984).

Stage I – Blastula – undeveloped eggs and clevage stages, a mass of undifferentiated cells. The diameter of the freshly spawned eggs ranges from 0.58 – 0.64 mm. The eggs are yellowish orange in colour.

Stage II – Gastrula – A portion of embryo yolk-free and transparent. The eggs are of diameter from 0.64 – 0.73 mm and orange in colour.

Stage III – Eye placode – Eye appearing as a scarlet crescent. The diameter of egg is from 0.74 – 0.79 mm. The eggs are brown in colour.

Stage IV – Pigment – Appendages of embryonic larvae pigmented. The eggs are brown in colour and diameter varied from 0.74 – 0.79 mm.

Stage V – Heart beat – Eyes round in shape; heart beating vigorously. The diameter of the egg ranged from 0.76 – 0.81 mm. The eggs are dark brown or black in colour.

Stage VI – Freshly hatched first zoea of 0.37 – 0.40 mm carapace length.

The diameter of the eggs were measured using the ocular micrometer mounted on a compound microscope. The number of eggs were counted and weighed as such, in an electronic balance for the fresh weight. The dry weight of the eggs was obtained after drying them in an oven at 60°C, until to get a constant dry weight and stored under $CaCo_3$ in a desiccator. A few healthy berried females were maintained individually in the plastic tanks with seawater with a salinity of 35 ± 1 ppt at a temperature of 28 ± 1 °C

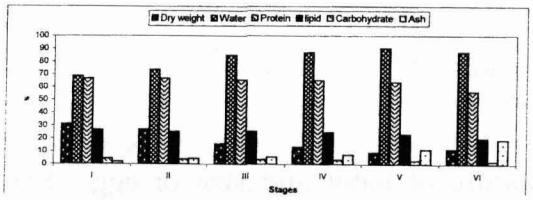

Fig. 1 *Biochemical constitutents in different stages of embryonic development in C. lucifera*

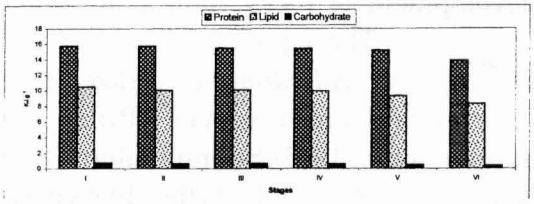

Fig.2. *Energy level in different stages of embryonic development in C. lucifera*

until the eggs were hatched into zoea. The freshly hatched active zoea were collected, dried at 60°C in an oven and used for biochemical analysis. The difference between the fresh weight and the dry weight was considered as the water content of the sample. Ash content was estimated by burning the known weight of sample in the silica crucible in a muffle furnace at 550°C for 5 hours (Paine, 1964). Protein, lipid and carbohydrate was estimated by following Raymont *et al.* (1964), Folch *et al.* (1956) and Dubois *et al.* (1956) respectively.

The calorific content was calculated from the biochemical constituents by using the conversion factors *i.e.* 23.64 J mg^{-1} for protein, 17.15 J mg^{-1} for carbohydrates and 39.54 J mg^{-1} for lipid (Winberg, 1971). For fatty acid analysis, the methyl esters of the samples were injected into the Gas chromatograph (HP. 5890) capillary column coated with 5% phenyl silicane at a temperature of 170 to 310°C for 23.33 minutes. Flame lonization Detector was used for analysis. Based on the retention time the different fatty acids of the sample were identified.

Results

Dry weight

A continuous decrease in the dry weight as well as organic substances was observed in the present study (Table 1). The rapid decline was noticed between the stages II (27.07%) and III (15.65%). However, an uniform change in the dry weight was observed in the later stages. Similar pattern of changes was also found with the organic substances.

Water

Water content changed with the stages of embryonic development (Table 1). Water content of the freshly laid eggs (68.60%) increased steadily upto 90.43% in the V stage of embryonic development

Table 1. *Percentage of fatty acid profiles during embryonic development of C. lucifera.*

Sl. No.	Name of fatty acid	% of fatty acid					
		Stages of development					
		I	II	III	IV	V	VI
1.	16 : 0	16.18	16.29	16.37	16.87	18.36	19.33
2.	16 : 1n-7	14.14	4.61	12.67	12.16	15.24	10.15
3.	16 : 2n-7	4.14	4.61	4.87	5.21	5.74	7.06
4.	17 : 0	0.93	2.17	2.34	2.84	3.24	4.00
5.	18 : 1n-5	0.10	0.89	0.43	0.14	2.47	1.20
6.	18 : 3n-6, 9, 12	0.58	1.06	1.38	1.47	2.00	2.40
7.	20 : 5n-3	14.83	13.44	11.78	11.32	9.37	6.12
8.	22 : 6n-3	10.04	10.86	11.46	10.86	10.24	9.86
9.	Saturated	32.97	42.63	34.05	35.92	36.34	46.90
10.	Monounsaturated	35.00	22.54	32.61	31.90	35.18	26.97
11.	Polyunsaturated	34.56	34.62	21.79	30.47	28.77	26.51

and then decreased to 87.12% in the VI stage. The water content in the III and IV stages showed marginal changes from 84.78% to 86.83%.

Ash

Ash content showed considerable variation in the developing eggs (Table 1). It increased from 1.91% in the freshly laid eggs to 19.02 % in the VI stage. Ash accumulated slowly in the early stages and rapidly in the later stages.

Protein

Protein was the main component of organic substances in all developmental stages of egg (Table 1). The maximum protein content, which was noticed in the stage I, decreased gradually in the subsequent stages. The protein content varied between 66.68% (I stage) and 56.63% (VI stage). Thus a total of 10.05% of the protein was utilized during embryonic development. The maximum utilization of 7.41 % was found between the V and VI stages.

Lipid

Lipid contributed the second major portion of the dry weight of the egg and larval stages (Table 1). Like protein, the lipid also showed a steady decline during the developmental stages. A maximum of 26.58% was observed at the I stage and a minimum of 20.11% at the VI stage. A total of 6.47% lipid was utilized during the whole developmental process.

Carbohydrate

Compared to protein and lipid, the carbohydrate utilization was negligible during embryonic development (Table 1). The carbohydrate content ranged between 4.42% (I stage) and 2.19% (VI stage). During the embryonic development, 2.23% of carbohydrate was utilized. A maximum utilization of 0.08% was found during the transition period between V and VI stages.

Energy

A constant decline was noticed in the calorific value from I stage to VI stage (Table 1). During the embryonic development, 4.53 $KJ.g^{-1}$ of energy was utilized. Of this, the oxidation of protein and carbohydrates contributed 38.18% and 9.8% respectively, the remaining amount of 51.94% was derived from the oxidation of lipid.

Fatty acid

The fatty acid profiles of the developing embryo exhibited a range of variations. Some fatty acids, such as 16:0, 16:1n-7, 18:1n-9, 20:5n-3 and 22:6n-3 constituted high percentages of less than 10% each (Table 2). Among these major fatty acids, 16:0 increased and 20:5n-3 decreased linearly, whereas 22:6n-3 maintained a constant level throughout the embryonic development (Table 2). Other fatty acids 16:2n-7, 17:0, 18:3n-6, which constituted only more than 10% and increased linearly from I stage to VI stage (Table 2). In general, saturated fatty acids increased by 13:93% but monounsaturated and polyunsaturated fatty acids decreased by 8.03 and 8.05% respectively during embryonic development.

Table 2. *Correlation matrix for fatty acids, dry weight and lipid composition during embryonic development of C. lucifera*

	Saturated	Mono-unsaturated	Poly unsaturated	Dry wt.	Lipid
Saturated	-	-0.8120*	-0.5301	-0.1770	-0.5505
Mono-unsaturated	-	-	-0.0292	-0.2000	0.0399
Poly-unsaturated	-	-	-	0.8025*	0.9572**
Dry wt.	-	-	-	-	0.8020*
Lipid	-	-	-	-	-

* Significant at 5% level (P<0.05)
* * Significant at 1% level (P<0.01)

Statistical analyses

The results obtained in the biochemical constitution (dry weight, water, ash, protein, lipid, carbohydrate and fatty acids) were subjected to the correlation matrix treatment and the r–values are given in the Table 3.

Discussion

Water

During the embryonic development of *C. lucifera*, a constant increase in the water content was noticed upto stage V. But when larvae were just released from egg membrane, there was a decrease in water content. This reduction in water content was due to the loss of water during hatching. The increase in water content of the developing egg as found in the present study (Table 1) is more common in aquatic organisms (Pandian, 1972). This increase in the water content of developing egg is due to the continuous imbibition of water from the environment and/or due to retention of metabolic water of organic materials (Amsler and George, 1984; Vijaykumar, 1992; Kannupandi *et al.*, 1999).

The permeability of water through the egg membrane varies with their environmental conditions. In estuarine crab *Thalamita crenata*, eggs are initially less

Table 3. *Correlation matrix for biochemical constituents during embryonic development in C. lucifera.*

	Water	Dry wt.	Portein	Lipid	Carbohydrate	Ash
Water	-	-0.9984**	-0.5751	-0.7790	-0.7734	-0.7.97
Dry wt.	-	-	0.6125	0.8029	0.7974	0.7411
Protein	-	-	-	0.8293*	0.9363**	-0.9645**
Carbo-hydrate	-	-	-	-	-	-0.9866**
Ash	-	-	-	-	-	-

* Significant at 5% level (P<0.05)
* * Significant at 1% level (P<0.01)

permeable to water and the permeability increases at later stages of development (Kannupandi *et al.*, 1999). Vijaykumar (1992) has noticed a gradual increase in permeability during the embryonic development of semi-terrestrial mangrove crab. *Sesarma brockii.* In *Paratelphusa hydrodormous,* the freshwater crab, eggs absorb water throughout their embryonic development (Pillai and Subramonium, 1985). In marine swimming crab *C. sapidus* the eggs are more permeable to water in early stages than the later stages of development (Amsler and George, 1984). Similarly in the present study also, the eggs are more permeable to water upto III stage and the permeability decreases in the later stages of embryonic development. The percentage of water absorbed versus metabolic water may vary from species to species as substrate for utilization varies (Amsler and George, 1984).

Ash

In the present study, a continuous increase in ash content was observed with the advancement of developing stage. The increase in ash content indicates an active absorption of water and salts from the environment across the embryonic membrane. Addition of salts during the stages of development is gained either from yolk or absorbed along the concentration gradient in the marine environment (Kannupandi *et al.*, 1999). Pandian (1967) has stated that the increased salt is due to absorption of water or the production of metabolic water from the oxidation of fat and protein.

In the present study, nearly a 10-fold increase was observed in the ash content from 1.91% in I stage to 19.02% in the VI stage (Table 1). In estuarine crab *T. crenata* nearly a 7-fold of increase in ash content was observed by Kannupandi *et al.* (1999). Vijaykumar (1992) has also reported nearly 6.75-fold increase in the eggs of semi-terrestrial mangrove crab, *S. brockii.* Similar increasing trend in the ash content was observed for the other crabs such as *C. sapidus* (Amsler and George, 1984), *P. hydrodromous* (Pillai and Subramonium, 1985) and *Xantho bidentatus* (Babu, 1987).

The colour of the ash is also an important criterion, changing from white colour in the undivided stage to deep blue in the zoeal stage. The blue colour is due to the presence of copper, which is an important metal in haemocyanin, the respiratory pigment of crustacean. The increase in ash content is due to accumulation of copper and calcium, as ingredients of outer exoskeleton (Babu, 1987).

Proximate composition

The present study showed a constant decline in protein, lipid and carbohydrate during the embryogenesis of egg. Holland (1978) states that protein and lipid are the major contributors, whereas carbohydrate contributes very less. Carbohydrate content of the egg is negligible as compared to that of either lipid or protein (Shakuntala and Reddy, 1982). The protein content of the egg yolk is an important source for tissue differentiation and organel formation, particularly for cuticu-

lar layers, muscles, digestive and nervous system (Babu, 1987). Pandian (1972) reported that the protein in developing eggs are progressively depleted and they also suggested the possible utilization of protein during embryogenesis to meet the metabolic energy demand. The utilization of yolk during embryogenesis varies based on the percentage composition of yolk material, nature of habitat and size of the egg. Babu (1987) found that the protein is the major energy source for the embryonic development of rocky shore crab *X. bidendatus.* Similarly Kannupandi *et al.* (1999) recently reported that protein was utilized as the major source of energy during embryonic metabolism of estuarine crab, *Thalamita crenata.* Vijaykumar (1992) has stated that the lipid is main source of energy for embryonic development of semi-terrestrial mangrove crab *S. brokii.* Similarly, the eggs of freshwater crab *P. hydrodromous* utilizes their enormous lipid reserves with a concomittent increase in protein level (Pillai and Subramonium, 1985). Amsler and Goerge (1984) have reported that marine swimming crab *C. sapidus,* during embryonic development at 16°C the lipid is the major energy source. Subramonium (1991) has also observed lipid is the chief energy source for the second half of the embryogenesis in the intertidal mole crab *E. asiatica.* In the present study, lipid was found to be the major energy source for the embryonic development.

Fatty acids

The saturated and monoethylenic fatty acids increased linearly with embryogenesis and the polyunsaturated fatty acids declined. All these changes in the fatty acids could result from either oxidative degradation of acids as source of energy, or less likely from endogenous synthesis (Whyte *et al.*, 1991). There is no chance of exogenous supply of fatty acids which result in changes of fatty acids during the process of embryogenesis and hence the changes are only endogenous occurring inside the embryo itself.

Of the principal fatty acids of the present study the eicosapentaenoic (20:5n-3) and docosahexaenoic acid 22:6n-3 are widely regarded as nutritionally essential for most marine species (Kanazawa *et al.*, 1979). In the present study the two essential fatty acids showed wide variations during embryogenesis. The 22:6n-3 was relatively constant throughout the embryogenesis indicating its role in structural function (Whyte *et al.*, 1991). However, requirement of n-3 polyunsaturated fatty acids was suggested to maintain membrane fluidity and permeability characteristics in the relatively cold marine environment, to be involved in activation of specific enzymes and in regulation of prostaglandin processes (Castell, 1970). On the contrary, the 20:5n-3 was constantly, decreasing from I stage to VI stage of embryogeneis, indicating the utilization of the fatty acid in energy contribution.

It is also evident that the enzymatic oxidation of n-3 and n-6 of C_{20} isomers of higher unsaturated fatty acids yield prostaglandins which play an active role in

water transport and osmoregulation in marine invertebrates (Freas and Grollman, 1980). The higher concentration and linear increase of 16:0 fatty acid is believed to play a major role in structural formation (Whyte *et al.*, 1993).

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