# Biochemical changes during larval development in the short neck clam, *Paphia malabarica* Chemnitz

Raghavan Gireesh[1], Abraham Biju[2] & Palaniyappan Muthiah[3]

[1]Central Marine Fisheries Research Institute, Ernakulam North, Kochi, Kerala, India

[2]National Institute of Oceanography, Ernakulam North, RC-Kochi, Kerala, India

[3]Central Marine Fisheries Research Institute, Regional Centre- Tuticorin, Tamil Nadu, India

**Correspondence:** R Gireesh, Central Marine Fisheries Research Institute, Ernakulam North, PO Box 682018, Kerala, India.
E-mail: girmsr@gmail.com

## Abstract

Biochemical compositions were determined for eggs, D-shaped larvae, umbo larvae and pediveliger of the short neck clam *Paphia malabarica* Chemnitz. Spawned eggs were composed of 63.2% protein, 25.4% lipid and 11.4% carbohydrate. After 48 h of embryogenesis, 2.6% of the protein, 11.8% of the lipid and 3.2% of carbohydrate mass had been lost, providing 20.5%, 75.4% and 4.1% of the total energy expenditure of 0.2147 mJ embryo$^{-1}$. During 48 h of metamorphosis, lipid was utilized first, followed by a heavy consumption of protein; protein, lipid and carbohydrate lost 23.8%, 50.2% and 32.5% of their mass respectively. Protein and lipid supplied a comparable amount of energy for metamorphosis, 34.2% and 55.2%, respectively, whereas, carbohydrate contributed only 10.6% to the 2.733 mJ larva$^{-1}$ metamorphic energy expenditure.

**Keywords:** *Paphia malabarica*, larvae, biochemical composition, energetics

## Introduction

As it is difficult to obtain information on bivalve larval survival rate during developmental changes from the natural environment, larval rearing and hatchery production of commercially important species provides substantial information. Low survival during early developmental stages under laboratory conditions has often been found to be associated with embryogenesis and metamorphosis (Castagna & Duggan 1971; Heffernan, Walker & Crenshaw Jr 1992), with fluctuations in environmental factors (Tettelbach & Rhodes 1981; Lu 1989). In many bivalves, embryogenesis occurs in surrounding waters rather than in the female and cannot feed on particulates due to the lack of a digestive system and hence represents a negative energy balance. Endogenous reserves are utilized to supply the necessary energy till it attains D-shaped larvae, which represent a stage with a digestive tract. Metamorphosis represents the next phase of development, where stored energy reserves are consumed for metabolism during early development (Whyte, Bourne & Hodgson 1990). Energy reserves are accumulated by the planktonic larvae through feeding on organic particles and are subsequently used for supporting metamorphosis. (Rodriguez, Sedano, Garcia- Martin, Perez-Camacho & Sanchez 1990; Haws, DiMichele & Hand 1993). During this stage, larval velum disappears, and larvae lose their ability to feed until the development of gill filaments (Bayne 1965; Hickman & Gruffydd 1971). Larval survival and settlement after completing the two stages depends on the energy reserves inherited from the female and/or accumulated through feeding. High survival has been reported in *Mercenaria mercenaria* and *Argopecten irradians* (Kraeuter, Castagna & Van Dessel 1982), probably to high energy content. Other possibilities for survival may depend on the ability of embryos or larvae to complete development of feeding structures before energy reserves are depleted (Haws *et al.* 1993).

Short neck clam (*Paphia malabarica* Chemnitz) is one of the most commercially important bivalve species for mariculture along the southwest coast of India, especially in Ashtamudi estuary. It is a major fishery in Kerala coasts (Appukuttan 1993, 1996) and is commercially exploited for both meat as food and shell as raw material for industrial applications

(Appukuttan, Aravindan, Yohannan & Balasubramaniam 1999), and hence represents an important income for the local people. In recent years, there have been increasing demands on this clam, and their commercial culture has shown considerable promise. Traditionally, clam farming depends on natural seeds that are collected from intertidal zones, which is labour intensive, often unreliable and limited only to a short season. Moreover, overexploitation of undersized clams has also depleted natural resources. Recently, larval rearing and spat production of *P. malabarica* have been successful and its nutritional requirements have been studied (Gireesh & Gopinathan 2004a). Gireesh and Gopinathan (2004b) evaluated the optimum salinity and pH requirements for larval survival, growth and metamorphosis. However, information on the energy metabolism of early development in the short neck clam is lacking. The objective of this study was to investigate changes in the biochemical composition of eggs, larvae and juveniles and the energy expenditures during embryogenesis and metamorphosis of the short neck clam *P. malabarica* Chemnitz.

## Materials and methods

The present study was carried out at the Tuticorin Research Centre of Central Marine Fisheries Research Institute, India. Brood stock clams of 30–48 mm length were collected from Ashtamudi estuary (latitude 8°45′N, longitude 78°28′E) in the west coast, where *P. malabarica* is one of the major bivalve species, and packed in a wet gunny sack and transported by road to the shellfish hatchery in Tuticorin on the east coast (latitude 8°48′ N, longitude 78°11′E). These clams were kept in fibre-reinforced plastic tanks of 100 L capacity at a temperature of 22–24 °C with mild aeration. Conditioning, spawning and incubation were carried out in indoor fibre tanks feeding *Isochrysis galbana* 14–16 × $10^3$ cells mL$^{-1}$ for 2 days. Culturing of larvae and juveniles followed the methods described by Narasimham, Muthiah, Gopinathan and Gandhi (1988). Larvae were cultured at a density of 3–5 mL$^{-1}$ and fed 10–28 × $10^3$ cells mL$^{-1}$ of *I. galbana* daily depending on the larval size. Seawater was replaced every day in the amount of 1/3 of the total volume. The daily food ration for juveniles was increased gradually from 3 to 10 × $10^4$ cells mL$^{-1}$ of *I. galbana*.

Fertilized eggs were collected using 40 µm sieves. A part of the eggs were used for biochemical analysis, and the rest were released in 0.45 µm filtered seawater (salinity 30.0 ± 1) and allowed to develop at 23.2 ± 1.7 °C in three 10 L beakers. After 48 h, D-shaped larvae were collected on a 60 µm sieve. The egg and larval samples were washed with filtered seawater and pipetted to a graduated beaker. Seawater was added to bring the volume to 500 mL and total eggs or larvae were determined by counting five 0.5 mL samples. The mean egg diameter and larval length were determined by measuring 25 individuals each using a microscope fitted with a micrometer. Subsamples were drawn from the beaker, thoroughly washed with 1% ammonium formate solution isotonic with seawater to remove excess salt and frozen at − 20 °C until analysed for biochemical composition. Each sample for chemical analysis contains 10–15 × $10^4$ eggs or 15–20 × $10^3$ D-shaped larvae.

Pre-metamorphic larvae (shell length 171.0 ± 0.2 µm) from previous batches were kept in 10 L beakers containing filtered seawater. Development was followed microscopically, and juveniles were observed in the culture. Veligers that settled on the beakers were brushed into a Petri dish. Juveniles were separated from the pedivelgers under a microscope. Samples of pediveliger and juveniles were washed with 1% ammonium formate solution, quantified and frozen at − 20 °C until analysed. Each sample for chemical analysis contains 3–5 × $10^3$ pre-metamorphic larvae or 1–2 × $10^3$ post-metamorphic larvae. Eggs and larval sample were homogenized in 1.5 mL distilled water using a homogenizer. Subsamples were taken from the slurry for protein, lipid and carbohydrate analysis. Juveniles with a shell length in the range 1–5 mm were collected, and sample size ranged from 1 to 50 individuals, depending on the size. For juveniles < 2 mm in length, whole animals were homogenized in distilled water with a tissue grinder and then with a homogenizer (Omni International, Marietta, USA). For larger juveniles up to 5 mm in length, only the soft body was homogenized. All the samples were analysed immediately.

### Biochemical analysis

Protein analysis was carried out as described by Lowry, Rosebrough and Fair (1951). The optical density of the blue-coloured homogenate sample solution was measured at 750 nm with bovine serum albumin as the standard. Lipid was extracted according to the method of Bligh and Dyer (1959), and samples were measured at 375 nm on a spectrophot-

ometer with tripalmitin as the standard (Marsh & Weinstein 1966). The total carbohydrate content in samples was determined using the phenol–sulphuric acid method of Dubois, Gillies, Hamilton, Rebers and Smith (1956) and measured at 490 nm on a spectrophotometer (Perkin-Elmer AAnalyst-700, MA, USA) with glycogen as the standard.

Triplicate samples were analysed for eggs and all larval stages, with the exception of juveniles, which were analysed individually (juveniles < 2 mm in length were ground together and the shells of juveniles > 2 mm in size). Energy expenditures for embryogenesis and metamorphosis were calculated by the loss of protein, lipid and carbohydrate during those two stages. Energy conversion factors of 20.0, 39.5 and 17.5 mJ µg$^{-1}$ were used for protein, lipid and carbohydrate respectively (Brett & Groves 1979).

## Results

The spawned eggs of the short neck clam had a mean diameter of 64.0 ± 0.3 µm and were composed mainly of protein (63.2%), lipid (25.4%) and carbohydrate (9.5%). The mean energy content of an egg was 3.37 mJ. The biochemical contents at various developmental stages and shell length are presented in Table 1. During embryogenesis, all the three components decreased. Protein declined 2.6% by mass, lipid 11.8% and carbohydrate 5.0%, respectively, leading to a loss of 5.0% of the total organic matter (Table 2). Lipid was the major component utilized for embryogenesis, supplying 75.4% of the total energy expenditure of 0.2147 mJ per embryo, whereas protein and carbohydrate contributed 20.5% and 4.1% respectively.

The lipid concentration increased as the larvae developed, increasing from D-shaped larvae (23.6%) to pre-metamorphic larvae (24.4%) with a mean energy content of 7.62 mJ larva$^{-1}$. Thereafter, overall, the process of metamorphosis consumed 32.5% of the total organic substrate, contributed by 23.8%, 50.2% and 41.3% of protein, lipid and carbohydrate reserves respectively. The total energy expenditure during metamorphosis was 2.73 mJ larva$^{-1}$, with lipid and protein providing 55.2% and 34.2%, respectively, and carbohydrate contributing only 10.6%. The growth rate and larval survival during the developmental stages are represented in Table 3.

The proximate compositions of juveniles are summarized in Table 4. In contrast to the biochemical composition of larvae, juveniles had significantly higher levels of protein ($P < 0.001$) and lower levels

**Table 1** Biochemical (µg ind.$^{-1}$) changes during embryogenesis and metamorphosis of *Paphia malabarica*

| Developmental stages | Length (µm) | Protein | Carbohydrate | Lipid | Total |
|---|---|---|---|---|---|
| Fertilized eggs | 64 | 86.2 ± 0.5 (63.2%) | 15.5 ± 0.1 (11.4%) | 34.7 ± 0.4 (25.4%) | 136.4 |
| D-larvae | 80 | 84.0 ± 0.1 (64.8%) | 15.0 ± 0.1 (11.6%) | 30.6 ± 0.2 (23.6%) | 129.6 |
| Pre-metamorphic (umbo) | 171 | 196.0 ± 6.5 (62.8%) | 40.2 ± 0.4 (12.9%) | 76.1 ± 7.0 (24.4%) | 312.3 |
| Post-metamorphic | 222 | 149.3 ± 2.0 (78.3%) | 23.6 ± 0.2 (11.2%) | 37.9 ± 1.6 (19.9%) | 210.8 |

**Table 2** Losses of biochemical composition, their caloric equivalents and contribution to energy expenditure during embryogenesis and metamorphosis of *Paphia malabarica*

| | Protein | Carbohydrate | Lipid | Total |
|---|---|---|---|---|
| **Embryogenesis** | | | | |
| Weight loss (µg ind.$^{-1}$) | 2.2 | 0.5 | 4.1 | 6.8 |
| Weight loss (%) | 2.6 | 3.2 | 11.8 | 5.0 |
| Energy equivalent (mJ ind.$^{-1}$) | 0.044 | 0.0088 | 0.1619 | 0.2147 |
| Energy contribution (%) | 20.5 | 4.1 | 75.41 | 100% |
| **Metamorphosis** | | | | |
| Weight loss (µg ind.$^{-1}$) | 46.7 | 16.6 | 38.2 | 101.5 |
| Weight loss (%) | 23.8 | 41.3 | 50.2 | 32.5 |
| Energy equivalent (mJ ind.$^{-1}$) | 0.934 | 0.2905 | 1.508 | 2.7325 |
| Energy contribution (%) | 34.2 | 10.6 | 55.2 | 100% |

**Table 3** Growth rate and survival rate of *Paphia malabarica* during different development stages

| Stage | Growth rate (µm day$^{-1}$) | Survival rate (%) |
|---|---|---|
| Fertilized egg | 58.6 ± 0.09 | 98 ± 0.05 |
| D-shaped | 160.3 ± 0.6 | 96 ± 0.9 |
| Pre-metamorphic larvae | 159.6 ± 0.6 | 85.6 ± 0.8 |
| Post-metamorphic larvae | 206.4 ± 0.3 | 32.4 ± 0.7 |

of lipids ($P < 0.001$). There was no significant difference in the carbohydrate levels between larvae and juveniles. Protein was the major component in the proximate composition of juveniles, constituting 74.8 ± 5.7% of the total organic matter, and the mean lipid and carbohydrate contents were

**Table 4** Biochemical composition (µg ind.$^{-1}$) in *Paphia malabarica* juveniles

| Mean shell length (mm) | Protein | Carbohydrate | Lipid | Total |
|---|---|---|---|---|
| 1.05 | 7.89 (68.4%) | 1.3 (11.4%) | 2.3 (20.2%) | 11.4 |
| 2.0 | 57.5 (69.1%) | 10.7 (12.9%) | 15.0 (18.0%) | 83.2 |
| 3.2 | 147.3 (81.1%) | 15.4 (8.5%) | 19.0 (10.5%) | 181.7 |
| 4.0 | 233.3 (76.9%) | 34.3 (11.3%) | 44.8 (14.8%) | 303.4 |
| 5.1 | 350.1 (78.6%) | 42.1 (9.5%) | 65.2 (14.6%) | 445.4 |

$15.6 \pm 3.7\%$ and $10.7 \pm 1.7\%$ respectively. Carbohydrate was the least significant component both in larvae and in juveniles.

## Discussion

Energy reserves in the form of proteins, lipids and carbohydrates are considerably reduced at the end of embryogenesis. The present study on the eggs of *P. malabarica* shows a similar trend, where protein comprises the main biochemical constituent (63.2%), followed by lipid (25.4%) and carbohydrate (11.4%) is the smallest component. Holland (1978) reported that protein forms the main constituent in eggs, followed by lipid and carbohydrates in marine invertebrates. In *P. malabarica*, lipid was used as the principal energy source for egg development, supplying 75.4% of the total energy expenditure, more than protein (20.5%) and carbohydrate (4.1%) combined. Carbohydrate played a minor role as an energy reserve in this clam due to its low content in eggs. Holland (1978) reported that the high conversion efficiency of protein and low conversion efficiency of lipid provide evidence that protein is conserved for the formation of pelagic planktonic larvae and lipid is the major reserve fuelling this process. Most of the earlier reports on the utilization of major biochemical compositions during larval development of oysters (Gallager, Mann & Sasaki 1986), mussels (Bayne 1965) or sea scallops, *Placopecten magellanicus*, (Pernet, Tremblay & Bourget 2003) and *Argopecten purpuratus* (Nevejan, Saez, Gajardo & Sorgeloos 2003) are geographically from temperate conditions. Although some species use lipid as the major energy reserve (Pandian & Schumann 1967; Shakuntala 1977), other species depends on protein (Barnes 1965). The utilization of lipid as the dominant energy reserve in eggs was also reported for bay scallop, *A. irradians* Say (Lu, Blake & Torres 1999). In *M. mer-*

*cenaria*, 69.0% of the total lipids was lost during embryogenesis, and 71.0% in the oyster *Crassostrea virginica* (Gallager *et al.* 1986) shows heavy use of lipid. Pernet *et al.* (2003) have reported that the increase in the triacylglycerol, lipid component during larval development was due to heavy food assimilation, and conclude that this might be due to the lag phase in the metabolic process of food assimilation and storage. There were similar reports on Pacific oyster, *Crassostrea gigas* (Garcia-Esquivel, Monica & Marco 2001), scallop larvae (Nevejan *et al.* 2003) and *Tapes philippinarum* spat (Caers, Coutteau, Lombeida & Sorgeloos 1998). However, there is no information on changes in the protein and carbohydrate compositions, which does not allow estimation of the relative importance of protein and lipid. Whyte *et al.* (1990) reported that both lipid and protein substrates used the energy reserve equally during embryogenesis in rock scallop *Crassadoma gigantea*, accounting for 46.7% and 43.5%, respectively, and only 9.8% supplied by carbohydrates.

During the pelagic stage form, larvae feed on suspended particles, and their organic mass increases as larvae grow (Lu & Blake 1996). Thus, larvae build up energy reserves of protein, lipid and carbohydrate through filter feeding as represented in Table 1. The relative lipid content increased from 23.6% in D-shaped larvae to 24.4% in pre-metamorphic larvae. *Paphia malabarica* larvae use the lipid reserve at the beginning of metamorphosis, followed by heavier consumption of protein reserves. On average, lipid and protein provided energy 75.4% and 20.5%, respectively, during the late metamorphosis. As in embryogenesis, carbohydrate is the least important constituent in larval metamorphosis and contributed only 4.1% of the energy expenditure. There are several reports regarding the source of the energy substrate, which serves as the energy reserve for growth. In *Ostrea edulis*, over half of the neutral lipid reserves were used for metabolic activities during metamorphosis (Holland & Spencer 1973), but there are no sufficient data to prove the use of protein as the source. In other species of oysters, *C. virginica* and *C. gigas*, 50.2% and 51.5% of their total energy expenditure during metamorphosis came from lipid and 39.9% and 38.5% from protein respectively (Haws *et al.* 1993). A similar report by Whyte, Bourne, Ginther and Hodgson (1992) stated that protein formed 60.0% of the total energy expenditure during metamorphosis and lipid 38.7%. All these values were derived from relative values and are very difficult to interpret. In *P. malabarica*, metamorphosis

consumed 32.5% of the total organic reserves, equivalent to a total energy value of 2.733 mJ larva$^{-1}$. This may represent the optimum level of consumable energy reserve of short neck clam larvae, below which larvae cannot complete metamorphosis without obtaining energy from the environment. This value is comparable to earlier studies on oyster *C. viriginica* and *C. gigas* (2.13 and 4.65 mJ larva$^{-1}$ respectively) (Haws *et al.* 1993) and in bay scallop *A. irradians* (4.35 mJ larva$^{-1}$) (Lu *et al.* 1999), which further supports our estimation on the energy requirement for metamorphosis. As the physiological variations are species specific, it is difficult to understand the feeding activities and hence estimate the biochemical changes during embryogenesis and metamorphosis. The present study was based on the assumption that metamorphic larvae lack the capability to feed and their energy demand comes from the energy accumulated during the planktonic life. The results thus demonstrate that clam larvae are able to complete metamorphosis based solely on the accumulated energy reserves of their biochemical substrates.

In a natural environment, larvae may stop feeding at a later stage of metamorphosis, or may stop feeding for only a few hours during metamorphosis (Baker & Mann 1994). Hence, the optimum energy requirement can be substantially lower and larvae may not need much energy reserve for further development. In conclusion, therefore, the estimation in the present study may represent the better range of the metabolic requirement of the clam, *P. malabarica*.

## Acknowledgments

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