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Effects of diet, stocking density and environmental factors on growth, survival and metamorphosis of clam, *Paphia malabarica* (Chemnitz) larvae

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Abstract

A series of experiments were conducted to evaluate the effects of diet, stocking density and environmental factors on the growth, survival and metamorphosis of short neck clam *Paphia malabarica* larvae. These experiments examined the following factors: diet [*Isochrysis galbana*, *Nannochloropsis salina* and a mixture of *I. galbana* and *N. salina* (1:1 w/w)], stocking density (1, 3, 5 and 7 larvae mL^{-1}), light intensity (unshaded, partially shaded and fully shaded) and water filtration (unfiltered and sand filtered). Results indicated that *N. salina* could replace 50% of *I. galbana* as a food source for the clam larvae with an increase in growth, survival (47.2%), metamorphosis (33.5%) and early settlement. Larval growth decreased significantly with increasing stocking density. A density of 1–3 larvae mL^{-1} appeared to be optimal for normal growth of clam larvae. Neither diet nor stocking density used in the study had a significant effect on larval survival. Under partially shaded (light intensity = 1000–5000 lx) and fully shaded (light intensity < 1000 lx) conditions, larval growth was significantly faster than under direct sunlight (unshaded). Larvae grew significantly faster in the unfiltered water than in the filtered water.

Keywords: *Paphia malabarica*, diet, stocking density, illumination intensity, growth and metamorphosis, survival

Introduction

The short neck clam, *Paphia malabarica*, is naturally distributed along the south-west coast of India, especially in Ashtamudi estuary where it is a major clam fishery in Kerala coasts (Appukuttan 1993, 1996). The clam is commercially exploited for both meat as food and shell as raw material for industrial applications (Appukuttan, Aravindan, Yohannan & Balasubramaniam 1999) and hence is an important income resource for local people. The species has great market demand in Japan and other European countries. Even though the species are widely distributed along the coastal and estuarine areas of Vietnam, China and Bangladesh, there is no report on clam culture using standard protocol. In recent years, there have been increasing demands on *Paphia* clams, and their commercial culture has shown considerable promise. Traditionally, clam farming depends on natural seeds that are collected from intertidal zones. This method of larval collection is labour intensive, often unreliable and limited only to a short season. Moreover, overexploitation of undersized clams has depleted the natural resource also. Developing a larval rearing technique using hatchery protocol and nursery techniques for mass spat production is key to the success of clam farming.

Like many other bivalve molluscs, clam larvae and juveniles are filter feeders, which largely rely on algae for food. Various cultured algae have been tested as food sources for Manila clam larvae and juveniles. Muthiah, Rodrigo and Suja (2002) evaluated the effects of *Isochrysis galbana* in various concentrations on larval rearing and spat production of *Marcia opima*, another clam species, which is widely distributed in the east and west coast of India. Gireesh and Gopinathan (2004) evaluated the effects of salinity and pH on the growth, survival and

metamorphosis of *P. malabarica* larvae, and found that in clam larvae and juveniles fed with flagellate, *I. galbana* had the best growth, survival and metamorphosis for salinity 33–35 and pH 7.8–7.9. *Nannochloropsis salina* is another algal species with higher protein content and of smaller size (2 μm) than *I. galbana*, which can be easily cultured and is usually less expensive than *I. galbana* (Morizane 1991; Nelson, Guarada, Cowell & Heffernan 1992). However, no information is available on the effects of *N. salina* as a single food source or as a partial replacement of *I. galbana* on the growth, survival and metamorphosis of short neck clam larvae.

There have been several reports on the other clam species regarding effects of stocking density (Mitchell 1992) and environmental factors (Lin, Wu & Huang 1983; Numaguchi 1998; Dong, Xue & Li 2000), and all these studies have been either conducted for juveniles only or limited only to the effects of water temperature and salinity. There is a lack of information on larval development of *Paphia* as influenced by stocking density and environmental factors, such as light intensity and water filtration. Therefore, the purpose of the present study was to investigate the effects of these two environmental factors in addition to diet and stocking density on the growth, survival and metamorphosis of *P. malabarica* larvae.

Materials and methods

The present study was carried out at 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) on the west coast, where *P. malabarica* is one of the major bivalve species, 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. The conditioning, spawning and incubation were carried out in indoor fibre tanks.

Diet and stocking density

The experiment was carried out from 11 November to 1 December 2002. Three diets and four stocking densities were evaluated in a 3 × 4 factorial arrangement. There were two replicates in each of the 12 treatments. About 24 h after fertilization, D-shaped larvae were collected through a 60 μm sieve and placed in 10 L polyethylene rearing buckets covered with one layer of black cloth (partially shaded). The D-shaped larvae were stocked at densities of 1, 3, 5 or 7 larvae mL^{-1}. Larval densities were determined by counting two samples of 5.0 mL each. They were fed *I. galbana*, *N. salina* or a mixture of both algae at a 1:1 (w/w) ratio. The *I. galbana* (32.5 ± 2.6 pg $cell^{-1}$) fed group was fed at increasing rations of 4000, 8000 or 16 000 cells mL^{-1} on days 1–3, 4–9 and 10–14 respectively; the *N. salina* (4.9 ± 0.1 pg $cell^{-1}$) fed group was fed 6000, 12 000 and 18 000 cells mL^{-1}; the larvae fed both algae as mixture were fed 2000+3000, 4000+6000 and 8000+9000 cells mL^{-1}. The feeding rates were based on feeding activity of the larvae and concentration of the algae remaining in the water.

All seawater used for larval rearing was filtered through sand bag filters and a 60 μm sieve. Water was exchanged 100% once daily and continuous aeration was provided. Each screen used to sieve the larvae during the water exchange was assigned to each specific bucket to avoid cross-contamination among buckets. Water temperature ranged from 26.1 to 28.4 °C and salinity from 31 to 33. Water pH, ammonia and chemical oxygen demand (COD) in the rearing units were monitored (Table 1). At 2, 7, 11 and 15 days of age, shell length was measured with a stage micrometer on 20 larvae that were randomly collected from each bucket and preserved in a 5% formaldehyde solution. Larval survival was determined at 6 and 9 days of age. To determine metamorphosis rate, pediveliger larvae (legs appear, but the velum still remains) at 12 days of age were placed into each of 12, 500 mL polyethylene bottles at densities of 80–100 larvae $bottle^{-1}$. The larvae were reared for 8 days before metamorphosis rate was determined. During this time, the daily feeding and water exchange were the same as in the 10 L rearing buckets.

Analyses were conducted using the SPSS SOFTWARE, version11.5, and analysed by multiple comparisons of means with a one-way or two-way ANOVA using Linear Model. To stabilize the variances of errors, per cent survival and metamorphosis values were transformed to angular values. The significant level for all analyses was set to $P < 0.05$ unless otherwise noted.

Light intensity

The experiment was conducted from 4 to 19 January 2003. The three levels of light intensity tested were

Table 1 Mean dynamics of pH, ammonia and chemical oxygen demand (COD) before and after seawater exchange

	Larval density (mL^{-1})			
Diets	**1**	**3**	**5**	**7**
Isochrysis galbana				
pH	7.91 ± 0.03	7.87 ± 0.04	7.88 ± 0.04	7.91 ± 0.03
Ammonia (mg^{-1})	0.100 ± 0.02	0.100 ± 0.01	0.089 ± 0.01	0.099 ± 0.01
COD (mg^{-1})	3.90 ± 1.1	3.04 ± 0.2	3.10 ± 0.1	2.9 ± 0.3
Nannochloropsis salina				
pH	7.92 ± 0.03	7.89 ± 0.02	7.91 ± 0.03	7.95 ± 0.03
Ammonia (mg^{-1})	0.095 ± 0.02	0.098 ± 0.03	0.089 ± 0.02	0.088 ± 0.02
COD (mg^{-1})	3.80 ± 1.1	3.20 ± 0.2	3.10 ± 0.2	3.1 ± 0.2
Isochrysis galbana+*Nannochloropsis salina*				
pH	7.91 ± 0.03	7.86 ± 0.03	7.89 ± 0.04	7.91 ± 0.3
Ammonia (mg^{-1})	0.099 ± 0.01	0.093 ± 0.02	0.089 ± 0.03	0.096 ± 0.03
COD (mg^{-1})	2.99 ± 0.1	3.01 ± 0.1	2.92 ± 0.7	2.90 ± 0.01
After seawater exchange				
pH	7.48			
Ammonia (mg^{-1})	0.049			
COD (mg^{-1})	2.18			

15 000–20 000 lx (under direct sunlight), 1000–5000 (rearing units were partially shaded with a single layer of black cloth) and < 1000 lx (the units were fully shaded with black cloth). Two replicates were used for each treatment. D-shaped larvae were stocked at densities of 1.5–3.3 larvae mL^{-1}. The larvae were fed daily at rations of 2000+3000 (*I. galbana*+*N. salina*) cells mL^{-1} on days 1–4 and 4000+6000 cells mL^{-1} on days 5–9. Water temperature varied from 26.0 to 28.3 °C. Shell length was measured daily on days 1–4 and on days 8 and 12. Survival was determined on day 12. Other experimental conditions and statistical analyses of the data were the same as described for the previous experiment.

Water filtration

Another experiment was carried out during the period mentioned above. D-shaped larvae were stocked at densities of 1.5–3.3 larvae mL^{-1}. The larvae were reared in unfiltered and sand (0.2–0.7 mm)-filtered seawater with rearing units covered with black cloth. Four replicates were used for each treatment. The transparency in the unfiltered and filtered waters was approximately 30 and 90 cm respectively. Water pH was monitored on days 1 and 3. Shell length was measured on days 3, 5 and 7. Survival was determined on day 12. Other experimental conditions and statistical analyses of the data were the same as described for experiment 1.

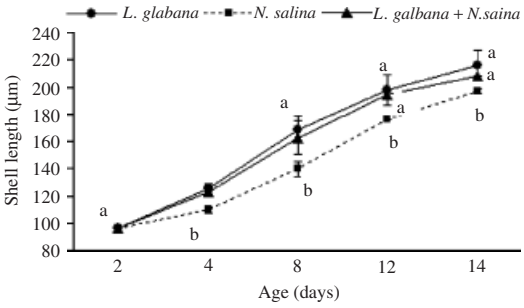

Figure 1 Mean shell length of clam larvae fed different algae. Means within age followed by different letters were different ($P < 0.05$).

Results

Diet and stocking density

Larval growth was significantly affected by both diet and larval density, but not by their interaction. At 4 days of age, the shell length of larvae fed *I. galbana* only or a mixture of *I. galbana* and *N. salina* (1:1 w/w) was significantly greater than that of larvae fed *N. salina* only (Fig. 1). However, there were no significant differences in shell length between larvae fed with *I. galbana* alone and a mixture of *I. galbana* and *N. salina*. The same trend continued until 15 days of age.

At 4 days of age, the shell length of larvae stocked at densities of 1 and 3 larvae mL^{-1} was significantly greater than that of larvae stocked at 7 larvae mL^{-1}, whereas the shell length of larvae stocked at 5 larvae mL^{-1} was intermediate (not significantly

different from other densities) (Fig. 2). The same trend continued until 8 days of age. When the larvae reached 12 days of age, the shell length of larvae stocked at 1 larva mL^{-1} was significantly greater than that of larvae stocked at 5 and 7 larvae mL^{-1}, whereas the shell length of larvae stocked at 3 larvae mL^{-1} was intermediate (not significantly different from other densities). The same trend continued until 14 days of age.

Survival of the larvae determined at 6 and 10 days of age was not significantly affected by either diet (Table 2) or stocking density (Table 3). The metamorphosis rate determined at 20 days of age was significantly high for larvae fed *I. galbana*+*N. salina* and for larvae fed *I. galbana* alone (Table 2).

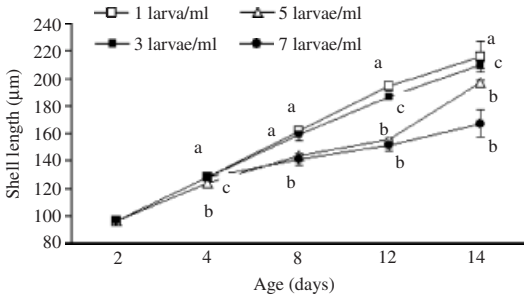

Figure 2 Mean shell length of clam larvae reared at various stocking densities. Means within age followed by different letters were different ($P < 0.05$).

Table 2 Mean (± SE) survival and metamorphosis of larvae fed with different algae in the experiment

	Days	*Isochrysis galbana*	*Nannochloropsis salina*	*I. galbana*+ *N. salina*
Survival (%)	6	48.4 ± 2.8a	55.0 ± 4.7a	52.2 ± 4.6a
	10	42.6 ± 2.0a	49.6 ± 5.9a	47.2 ± 5.1a
Metamorphosis (%)	20	31.2 ± 3.0a	23.9 ± 4.1b	33.5 ± 5.0a

Means in each row within each group followed by different letters are different ($P < 0.05$).

Table 3 Mean (± SE) survival of clam larvae reared at various stocking densities

	Survival (%)			
Age (days)	1 larva mL^{-1}	3 larvae mL^{-1}	5 larvae mL^{-1}	7 larvae mL^{-1}
6	68.2 ± 0.10	70.5 ± 0.1	65.0 ± 0.3	59.4 ± 0.3
10	58.0 ± 0.01	59.0 ± 0.2	59.0 ± 0.2	52.0 ± 0.1

Means in each row are not different ($P < 0.05$).

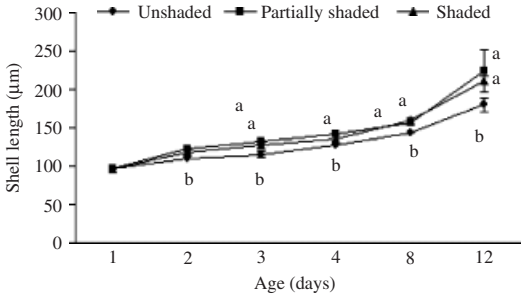

Figure 3 Mean shell length of clam larvae reared at various light intensities. Means within age followed by different letters were different ($P < 0.05$).

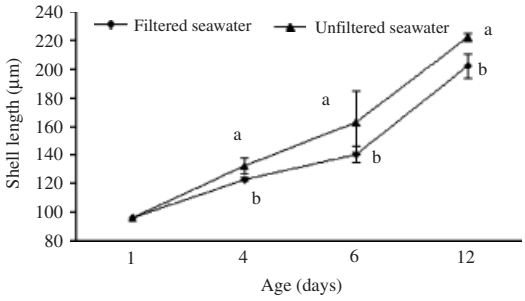

Figure 4 Mean shell length of clam larvae reared in filtered and unfiltered seawater. Means within age followed by different letters were different ($P < 0.05$).

Light intensity

At 1 day of age, the shell length of larvae reared in fully shaded culture units was significantly greater than that of larvae reared under direct sunlight, whereas the shell length of larvae reared in partially shaded units was intermediate (not significantly different from other treatments) (Fig. 3). At 2 days of age, the shell length of partially and fully shaded groups was significantly greater than that of larvae reared under direct sunlight, whereas there were no significant differences in shell length between partially and fully shaded groups. The survival (determined at 8 days of age) of larvae reared under direct sunlight was significantly lower than that of larvae in the partially or fully shaded groups. There were no significant differences in the survival between partially and fully shaded groups.

Water filtration

Sand-filtered water had a significantly ($P < 0.001$) lower pH than unfiltered water (7.59 ± 0.02 against 7.73 ± 0.06). At 4 days of age, the shell length of

larvae reared in unfiltered water was significantly greater than that of larvae raised in sand-filtered water. The same trend continued until 12 days of age (Fig. 4). Mean larval survival at 12 days of age was not significantly different between unfiltered and filtered water groups.

Discussion

The results from the present study indicated that *N. salina* could replace 50% of *I. galbana* as a food source for the yellow neck clam larvae without affecting growth, survival and metamorphosis. The use of a mixture of *I. galbana* and *N. salina* would reduce the cost compared with feeding *I. galbana* only because *N. salina* can be more easily and less expensively cultured than *I. galbana* (Morizane 1991; Nelson *et al.* 1992). However, complete replacement of *I. galbana* with *N. salina* enhanced early settlement but with reduced growth and metamorphosis rates of the clam larvae.

In the present study, larval growth was significantly influenced by stocking density. Shell length decreased with increasing stocking density. A prolonged planktonic stage was noted in the larvae stocked at densities > 5 larvae mL^{-1}. Results indicated that a density of 1–3 larvae mL^{-1} was optimal for growth of the *P. malabarica* larvae raised in the hatchery. The optimum stocking density obtained from the present study appeared to be similar to those reported for clam *Anadara granosa*, *M. opima*, bay scallop *Agopecten irradians* and *M. meretrix* (Duggan 1973; Narasimham, Muthiah, Gopinathan & Gandhi 1988; Muthiah, Narasimham, Gopinathan & Sundararajan 1992; Wei & Xu 1996; Muthiah *et al.* 2002). With increasing stocking density, more metabolic wastes may be accumulated in the water, which may be detrimental to larval growth. In addition, competition for space and food may be another reason for the slower growth rate in high stocking densities than at lower densities.

Light intensity may affect the growth and development of bivalve molluscs. High light intensity is critical for the normal growth and survival of juvenile clam *Tridacna gigas* juveniles (Lucas, Braly, Crawford & Nash 1988), and the growth pattern of clam *Mercenaria* juveniles is affected by light and dark conditions (Cenni, Cerrato & Siddall 1989). However, there is a lack of information on the effects of light intensity on the growth and survival of *P. malabarica* larvae. Results from the present study demonstrated that growth of the clam larvae raised under direct sunlight was significantly lower than that of partially and fully shaded groups. This suggests that *Paphia* clam larvae are sensitive to direct sunlight and the larvae should be reared under a light intensity of < 5000 lx by means of shading. As the natural habitat for *P. malabarica* is mudflat regions in the intertidal zone where light penetration is relatively low in the water because of high water turbidity, the animal may be adapted to the low-light environment and intensive light may be detrimental to its growth and development at the larval stage. In addition, intensive light may stimulate the growth of benthic diatoms on the wall of rearing units and the shell of larvae, making it impossible for the larvae to swim normally.

The differences in the growth and survival among the various water exchange regimens were unlikely to be caused by water ammonia, COD and pH levels because these water quality variables were not significantly different among treatments. It is not clear whether handling stress played a role in larval survival seawater exchange. More studies are needed to further evaluate the effect of water exchange rate on survival of the clam larvae.

In the present study, the larvae grew significantly slower in the sand-filtered water than in the unfiltered water. The slower growth of larvae raised in filtered water was probably because filtration reduced or eliminated the phytoplankton present in the water source that could serve as a food source for the larvae. Another possible reason is that unfiltered water had a lower pH than filtered water. In the present study, filtration significantly reduced water pH (7.59 for filtered water vs. 7.73 for the unfiltered water). A pH of 8.5 appears to be optimal for the growth and survival of bivalve larvae (Wang, Zhang, Ji & Zhang 1985; Sun, Xu, Dong, Huang & Wang 1999; Gireesh & Gopinathan 2004).

Conclusion

Nannochloropsis salina could replace 50% of *I. galbana* as a food source for *P. malabarica* clam larvae without affecting growth and metamorphosis. A density of 1–3 larvae mL^{-1} appeared to be optimal for the growth of clam larvae reared in the hatchery. Rearing the larvae under partially shaded (light intensity = 1000–5000 lx) and fully shaded (light intensity = $<$ 1000 lx) conditions resulted in better growth and survival compared with larvae reared under direct sunlight (unshaded). The larvae grew faster in

the unfiltered water than in the sand bag filtered water. The above results could have been helpful for larval rearing, spat production in a hatchery system and sea ranching of this cultivable species.

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