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Effect of starvation and temperature on gonad development of baby clam, *Marcia opima* (Gmelin)

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Abstract

For studying the influence of feed and temperature on gonad growth and maturity of the baby clam *Marcia opima*, experiments were conducted for 45 days at temperatures of 23°C and 28°C. The progress of gonadal recovery was followed by periodic determination of gonadosomatic index, condition index, digestive gland index and oocyte diameter. There was significant difference in the gonadosomatic index between fed and unfed clams at 23°C and 28°C. No significant difference in the gonadosomatic index was noted in the fed treatments at 23°C and 28°C, but the time of conditioning had significant influence on the gonadosomatic index. A significant relationship was found between the gonadosomatic index and condition index of the clams in all the treatments.

Keywords: Feed, temperature, gonad development, *Marcia opima*

Introduction

In intensive bivalve aquaculture, the objective of broodstock conditioning and induced maturation is to maximize the production of high quality gametes in order to obtain maximum number of viable larvae.

Temperature plays a very important role in the development of sex cells and spawning in many bivalves (Lannan *et al*., 1980; Ruiz *et al*., 1992). Conditioning of *Crassostrea gigas* in the laboratory at 24° C resulted in production of gametes 2 - 4 weeks earlier than at 20° C (Robinson, 1992). Sastry (1968) studied the role of nutrient reserves in gonad growth and gametogenesis of the bay scallop, *Aequipecten irradians* and reported that gametogenesis is initiated when the animals are exposed to a minimum threshold temperature with food. Laing *et al*. (1987) studied the effect of diet and temperature on the growth of juvenile clams such as *Tapes decussata, T. semidecussata* and *Mercenaria mercenaria*.

The condition factor of bivalves can act as an indicator of reproductive activity and the condition of clam meat is dependent on the gametogenic activity (Martinez *et al*., 2000). The relationship between condition factor and gonadosomatic index in bivalves has been studied by Sastry (1979) and Abraham (1996). The changes in the gonad index and digestive gland index were studied in *A. irradians* by Sastry (1968). It has been suggested that in bivalves, the nutrient reserves were transferred apparently from the digestive gland to the gonad and utilized for developing gametes in the synthesis of various biochemical constituents (Griffond *et al.,* 1992).

The average oocyte diameter and the stages of gametogenic cycles are related to each other (Sastry, 1968; Borcherding, 1995; Lango-Reynoso *et al*., 2000).

The study of gonad growth and dynamics of its index with relation to gametogenic stages and its influence on meat condition of the clam is very useful both for natural seed collection and also for harvesting the clams at the right season. The effect of temperature on the conditioning and gonad maturity of bivalves has been extensively studied by Newell *et al*. (1982), Mac Donald and Thompson (1985) and Martinez and Perez (2003). Such a study on clams has not been attempted in tropical countries.

Hence the present study was designed to evaluate the influence of starvation and temperature on the gonad development of the baby clam, *Marcia opima*. For this, study of the indices such as gonadosomatic index, digestive gland index and condition index and also the oocyte diameter, which are significant in the gonad growth of clams, were carried out.

Material and methods

Eighty clams (length: 25.8 to 30.2 mm) were collected from the Tuticorin Bay on the southeast coast of India (08° 45' N lat. and 78° 12'E long.) during August-September 1999. For studying the influence of starvation and temperature on gonad growth and maturity, experiments were conducted for 45 days at water temperatures of 23°C and 28°C. The clams were segregated into four sets of twenty each and kept in rectangular FRP tanks of 100-litre capacity. Three replicate tanks were arranged for each treatment. Five clams from the stock were initially taken for analysing the mean values of gonad index, digestive gland index, condition factor and gonad stages. Two sets were transferred to water temperature of 23°C, maintained in a room of 4.5x3.25 m size with the help of an air conditioner. Out of these treatments at 23°C, one was kept as control and the other as unfed. Two sets of clams were kept at the room temperature (28°C), one treatment was control and the other was kept under starvation. The control sets were fed with cultures of *Isochrysis galbana* and *Chaetoceros* sp. Gentle aeration was provided in all the four tanks. Seawater was changed daily and filtered through charcoal and sand filter. The salinity of seawater ranged between 32.0 and 34.8 ppt throughout the experimental period. After water change, the clams of the control group were given a mixed algal culture of *Isochrysis* and *Chaetoceros* at the rate of one million cells/ animal/ day. Once in 15 days, five clams from each tank were taken at random and the gonadosomatic index, digestive gland index, condition index and oocyte diameter were determined.

To study the indices, the clams were blotted dry with tissue paper. The shell was opened by severing adductor muscles and the soft tissues were separated. They were blotted to remove the excess moisture and the wet meat was weighed in a Sartorius microbalance to the nearest of 0.01 gm. The gonad and digestive gland were carefully cut from the visceral mass and their weight was taken separately. The gonadosomatic index (GSI) and digestive gland index (DGI) were calculated by the formula as followed by Giese (1959).

$$\text{Gonadosomatic index} = \frac{\text{Gonad weight (g)} \times 100}{\text{Wet weight of meat (g)}}$$

$$\text{Digestive gland index} = \frac{\text{Digestive gland weight (g)} \times 100}{\text{Wet weight of meat (g)}}$$

To find out the condition index, the soft tissues and shells were dried at 60°C for 48 hours and weighed to obtain the dry shell weight and dry meat weight. The condition index was calculated (Walne, 1976) as follows:

$$\text{Condition index} = \frac{\text{Dry meat weight (g)} \times 100}{\text{Volume of shell cavity}}$$

For measuring the oocyte diameter, a drop of female gonad smear was taken by using a micropipette and observed under the microscope. The diameter of at least 50 eggs was measured along the largest and smallest axis passing through the nucleus, and the average value was taken for further estimation. The measurement was taken using an ocular micrometer, pre-calibrated using a stage micrometer. The measurements were classified into 10 mm class intervals.

The gonadal maturity stages were designated (Suja and Muthiah, 2007) following a modified version of the maturity scale described by Nagabhushanam and Mane (1975).

The maintenance and mass culture of algae, *Isochrysis* and *Chaetoceros* were carried out following the serial dilution (Gopinathan, 1982). The average cell concentration varied from 1.5 to 1.8 million cells / ml.

The gonadosomatic index values of the clams under different treatments were subjected to Two Way Analysis of Variance (ANOVA). The relationship between condition index and gonadosomatic index and between gonadosomatic

index and digestive gland index were assessed by Pearson Product Moment Correlation. Diameters of oocytes were compared between treatments using a non-parametric Kruskal Wallis, Turkey test.

Results and Discussion

At the time of stocking, the mean value of gonadosomatic index was 13.7 ± 1.6 and digestive gland index was 3.0 ± 0.7. The average condition index was 83.2 ± 6.9. All the clams were in maturing stage with a mean oocyte diameter of 22.0 µm. The condition of five fed and unfed clams was assessed at an interval of fifteen days. Variations of gonadosomatic index, condition index, digestive gland index and oocyte diameter for the fed and unfed clams at 23°C and at 28°C are given in Table 1.

clams was 18.1±0.9 on 45th day. The gonadosomatic index of unfed clams was 11.0 ± 1.9 at the end of 45th day, which was less than the unfed clams at 23°C by an index value of 2. The condition index of fed clams was 92.3 ± 3.0 and that of the starved ones was 65.2 ± 5.9 on 45th day. For the fed clams at 28°C, the digestive gland index at the end of 45th day was same as that of the fed clams at 23°C (3.1).

By 45th day, the oocyte diameter of the fed clams reached a maximum of 46.2 ± 9.2 µm showing maturity, whereas the oocyte diameter of the unfed clams was 26.4 ± 4.9 µm.

In the unfed clams, the gonadosomatic index was significantly affected by temperature differences ($p < 0.05$) (Table 2). No significant difference in the

Table 1. Variations of GI, CF, DGI and OD between fed and unfed treatments at 23°C and 28°C

Temperature	Days	Unfed				Fed			
		GI	CF	DGI	OD(µm)	GI	CF	DGI	OD(µm)
23° C	0	13.7±1.6	83.2±6.9	3.0±0.7	22.0±0.0	13.7±1.6	83.2±6.9	3.0±0.7	22.0±0.0
	15	13.8±0.6	75.4±5.8	2.7±0.2	22.3±1.2	14.8±1.0	83.3±5.7	3.1±0.3	24.0±1.2
	30	12.8±2.5	60.2±13.0	2.3±0.2	24.2±4.9	16.1±1.2	89.2±3.7	3.1±0.5	28.6±6.0
	45	12.6±1.9	58.7±7.7	2.1±0.1	25.1±1.3	17.1±1.0	92.1±5.7	3.1±0.8	42.2 ±6.7
28° C	0	13.7±1.6	83.2±6.9	3.0±0.7	22.0±0.0	13.7±1.6	83.2±6.9	3.0±0.7	22.0±0.0
	15	11.8±1.2	65.9±3.0	2.5±0.4	24.2±4.9	14.6±1.5	88.8±4.2	3.2±0.4	26.4±4.3
	30	11.5±1.9	63.9±13.2	2.2±0.2	25.6±2.3	15.5±0.9	89.8±5.7	2.9±0.3	37.4±6.0
	45	11.0±1.9	65.2±5.9	2.0±0.4	26.4±2.5	18.1±0.9	92.3±3.0	3.1±0.4	46.2±9.2

GI - Gonad Index DGI – Digestive Gland Index
CF- Condition Factor OD – Oocyte Diameter

The gonadosomatic index of fed clams at 23°C increased from 14.8 ± 1.0 on 15th day to 17.1 ± 1.0 on 45th day. The gonadosomatic index of the unfed clams reduced to 12.6 ± 1.9 on 45th day.

The average values of condition index also increased in fed clams and were represented by a maximum value of 92.1 ± 5.7 by 45th day. The digestive gland index of the control group showed constancy at a value of 3.1 ± 0.8 throughout the experimental period. For the starved group, the condition index and digestive gland index were 58.7 ± 7 and 2.1 ± 0.1 respectively on 45th day.

During the final observation, the oocyte diameter was maximum (42.2 ± 6.7 µm) in the fed clams, showing ripeness.

At 28°C, the gonadosomatic index of the fed

gonadosomatic index was noted in fed treatments at 23°C and 28°C, but the time of conditioning had significant influence on the gonadosomatic index (Table 3). The gonadosomatic index was significantly affected between fed and unfed treatments at 23°C and 28°C (Tables 4 and 5) ($p < 0.05$). A significant relationship ($p < 0.05$) was found between the gonadosomatic index and condition index of the clams in all the treatments (for the fed clams: $r = 0.9557$ and 0.8752; for the unfed clams: $r = 0.9405$ and 0.9614 at 23°C and 28°C respectively). The relationship between the gonado somatic index and digestive gland index was not significant only in the fed clams reared at 28°C. The diameters of oocytes obtained from the fed and unfed clams on 45th day showed significant difference (Kruskal Wallis, Turkey test $p < 0.05$).

Table 2. Two way ANOVA on the influence of temperatures (23° and 28° C) on GSI for unfed treatments

Source of Variation	SS	df	MS	P-value	F
Between days	0.60	2	0.30152	0.32	2.15
Between temperature	5.06	1	5.06653	0.026*	36.61

Table 3. Two way ANOVA on the influence of temperatures (23° and 28° C) on GSI for fed treatments

Source of Variation	SS	df	MS	P-value	F
Between days	8.43	2	4.218051	0.044*	12.34
Between temperature	0.003	1	0.003651	0.92	0.0106

Table 4. Two way ANOVA on the influence of feed on GSI at 23° C

Source of Variation	SS	df	MS	P-value	F
Between days	0.87	2	0.435658	0.72	0.371
Between feed	11.41	1	11.41812	0.039*	9.734

Table 5. Two way ANOVA on the influence of feed on GSI at 28° C

Source of Variation	SS	df	MS	P-value	F
Between days	1.88	2	0.943745	0.72	0.386
Between feed	32.36	1	32.36868	0.047*	13.24

* Significant at $p < 0.05$

It was observed that in *M. opima*, the indices of gonad and digestive gland, condition factor and oocyte diameter, which are the indicators of reproductive activity, showed variation at 23°C and at 28°C and also between the fed and unfed treatments. Studies on *C. gigas* showed a significant correlation between the gonadosomatic index, temperature and chlorophyll content of the environment (Ruiz *et al*., 1992). In the present investigation, it was observed that the gonadosomatic index, digestive gland index and condition index in *M. opima* are significantly affected by starvation. Condition factor and gonad index tend to be directly related in many bivalves (Abraham, 1996). A direct relationship between condition index and gonadosomatic index was obtained for baby clams in all the treatments. Sastry (1968) suggested that the digestive gland index was higher during vegetative and rearing stages and the changes in the monthly gonad and digestive gland indices in some marine invertebrates show a reciprocal relationship. Abraham (1996) explained a negative relationship between gonad index and digestive gland index in an experiment conducted to induce the maturation of edible oyster *C. madrasensis*. In the present investigation, such a reciprocal relationship between the gonad and digestive gland indices was observed only in the fed clams kept at 28°C. As suggested by Sastry (1968), the nutrients from the feed are perhaps stored in the digestive gland and utilized for the gonad growth.

When diets of good to moderate nutrient value were fed to *T. semidecussata* juveniles, along with an increase of temperature to 25°C, the animals grew faster because of the availability of more energy. When food was absent, the clams suppressed their oxygen consumption in the conservation of energy and the maintenance of some growth (Laing *et al*., 1987). In the present study, the lack of sufficient nutrients might have suppressed the gonadal activities and maturation process in the clams, which were kept as control. The time required to reach a condition ready for spawning is dependent on the initial physiological condition of the animal and availability and quantity of food during conditioning (Lannan *et al*., 1980).

Water temperature is the environmental factor, most often cited as influencing bivalve reproduction. Sastry (1979) comments that the period of gonadal growth and gametogenesis in various bivalve species has been positively correlated with seasonal changes in temperature, and in some cases, seem to occur with declining temperatures in autumn or with increasing temperatures in spring and summer. Different *Mytilus edulis* populations from North America cultured in nearly identical thermal environments exhibit distinct gametogenic cycles which are apparently related to temporal differences in food availability among localities (Newell *et al*., 1982). The reproductive output of *Placopecten magellanicus* is well correlated with food availability and temperature (Mac Donald and Thompson, 1985). In *M. opima*, significant difference in the gonadosomatic index was not observed in the fed clams at two different temperatures, whereas there was significant difference in the gonadosomatic index of the unfed clams at two different temperatures. So the availability of food can be considered as a critical factor, which influences the gonad maturation in *M. opima*.

Relative size of oocytes has often been considered as a valid indicator of gamete quality in bivalves (Lango-Reynoso *et al*., 2000). An increase in oocyte diameter in the freshwater mussel *Dreissenia polymorpha* was observed with increasing water temperature and food availability (Borcherding, 1995). A similar observation was made in the present study where, the oocyte diameter of the fed clams was almost double to that of the unfed ones and significant difference was observed in the oocyte diameters of fed and unfed clams. It can be concluded that the best laboratory conditioning of baby clams can be achieved under stable conditions of temperature and food, which permit an adequate accumulation of nutritional reserves.

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