E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2021; 9(3): 340-344

www.fisheriesjournal.com
Received: 01-03-2021
Accepted: 03-04-2021

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Effect of salinity and temperature on broodstock conditioning of mangrove clam *Polymesoda erosa* (Solander, 1786)

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DOI: https://doi.org/10.22271/fish.2021.v9.i3e.2507

Abstract
Culture of molluscan is clearly limited by the availability of seed, as this production proceeds almost exclusively from natural recruitment. Artificial spawning and larval rearing programs could provide an alternative source of spat. This study was designed to evaluate the effect of different conditioning temperature and salinity on the broodstock maturation and Condition Factor of mangrove clam (*P. erosa*) collected from different locations of Ratnagiri coast. Batches of clams from different sites were collected in February, and conditioned at temperature 26 °C, 28 °C, 30 °C and 32 °C. For conditioning at different salinity, it was kept at 10,15,20,25,30 and 35 psu, The results showed that the oocyte maturation was faster at salinity 35 psu and the best conditioning temperature was 32 °C. The Condition Factor (CF) was higher at 32 °C temperature and at 30 psu salinity, whereas, no significant difference was observed in CF at 30 and 35 psu. These results suggest that the efficient conditioning temperature and salinity is related to the natural spawning season.

Keywords: temperature, salinity, condition factor, gonad maturation, *Polymesoda erosa*

1. Introduction
The mangrove clam *Polymesoda erosa* (Solander, 1786) belongs to the Corbiculidae family. Species from this family usually inhabit in estuarine zone. Most of the coastal areas near to the estuaries have sediment consisting of sand and mud which are suitable for *Polymesoda* species to breed. *Polymesoda* species burrows itself shallow in the mudflat at the estuaries. The high nutrient content in this habitat is able to support the growth of *Polymesoda* clams.
Polymesoda erosa is one type of mud clam inhabiting the mangrove forests in tropical and subtropical regions. The clam has oval and dome-shaped shells [2]. The inner part of the shells are white and covered by thick periostracum. The periostracum is yellow with green spots in young spat and dark brown in adult clams [5]. Somatic and reproductive growth occurs from larvae to adulthood [11], and gonadal maturity is determined based on the increase in the length of the shells, the total mass, and the age [12].
The reproductive behavior plays an important role in the continuity of the population [1], and therefore, understanding the reproductive biology is central to providing basic information in order to plan a better conservation strategy for *P. erosa*. However, the reproduction cycle of this species is not well understood. Thus, characterization of *P. erosa* gonad is necessary to contribute a basic knowledge of the reproductive biology of this species. The environmental factors responsible for bringing a population to a mature stage so that spawning can be coordinated thereby synchronizing the release of gametes has not received much attention. Although some of these factors affecting the reproduction of mussels have been investigated experimentally but most of the information have been derived through field observations [17].
In view of the significance of the species as one of the potential candidate species for mariculture in the region, an attempt has been made to study the broodstock conditioning of *P. erosa* from Ratnagiri coast.

2. Materials and Methods

2.1 Broodstock collection

Clams were collected fortnightly over a period of 18 months from October 2016 to April 2018 from Mirya –Zadgaon (17° 00' 82''N, 73° 28' 72''E), Aare Ware (17° 07' 37''N, 73° 29' 83''E) and Waravade (17° 19' 87''N, 73° 24'84'' E) estuaries, Ratnagiri, Maharashtra, India.

2.2 Maturation of Broodstock

Size of the clams was not restricted for a standard size and specimens representing all the size classes were analysed. Size classes were based on 10 mm intervals. During both the experimentation, daily ration of *Isochrysis galbana* was provided once a day at a rate of 15 L having concentration of 1.5 lakh cells $ml^{-1.}$ Fortnightly samples were collected and 10% of clams from 6 replications (maturation experiment) were sacrificed to study the gonadal maturity based on the oocyte diameter, GI and CF. The male to female ratio was determined from the colour of the gonad. The gonad was then excised and fixed in Bouin's solution for a period of 24 h. Fixed gonads were weighed (GW) to the nearest 0.1 g and a portion of tissue was removed from the gonad. Samples were dehydrated using a graded ethanol series, blocked in paraffin wax and sectioned at 4mm thickness. All sections were stained with haematoxylin and counterstained with eosin. The gametogenic development of clam was investigated by microscopic observations. Each gonad was examined under the microscope (Nikon E 200) and assigned to one of the six stages. For female clams, fortnightly mean oocyte diameters and number were determined microscopically. Three small portions (0.01 g) of the ovary were taken from the anterior, posterior and the middle region, respectively. The oocytes present were counted from all the three regions of the gonad. Quantitative analyses were not used for male clams. Spawning activity was recognized as recently spawned gonads containing much water, decrease in gonad indices, as an evidence of spawning and recovery from microscopic observation. Condition Factor (CF) was used to characterize the physiological activity of the animals (growth, reproduction, secretions, etc.) under given environmental conditions. The CF was determined according to Walne and Mann (1975) [21].

2.3 Microalgae Culture

The maintenance and mass culture of algae, *Isochrysis galbana* and *Chaetoceros calcitrans* were carried out following the serial dilution [3]. The average cell concentration varied from 1.5 to 1.8 million cells ml^{-1}.

2.4 Effect of temperature on broodstock maturation

The effect of temperature on broodstock maturation of *P. erosa* was studied by rearing them at different temperatures: 26 °C (T_1 – control), 28 °C (T_2), 30 °C (T_3) and 32 °C (T_4) for 60 days duration. Temperature of treatment T_2, T_3 and T_4 was kept at desired levels using an immersion heater system. The experiment consisted of 6 replicates. Fibreglass-reinforced plastic (FRP) tanks of 100 L capacity were used for the experiment. Size of the clam ranged between Standard Length (SL) 36 mm to 52 mm. The salinity of all the treatments was kept between, 22- 25 ppt [2]. Observations on oocyte developmental stages and their diameter (µm) were recorded fortnightly.

2.5 Effect of salinity on broodstock maturation

The experiment on salinity standardization for maturation of broodstock was conducted with six treatments and four replications for a period of 60 days. The mangrove clams ranging from 36 to 52 mm of Standard Length (SL) were studied by exposing the them to varied salinities, viz: 10 (T_1), 15 (T_2), 20 (T_3), 25 (T_4), 30 (T_5), and 35 ppt (T_6 - control). The low salinity seawater for the experiment was prepared by diluting the seawater by adding freshwater. The salinity was measured using a refractometer (ATAGO 0-100 ppt, Japan). The experiment was conducted in 100 litre capacity fibreglass tanks at a stocking density of 40 numbers in 40 litres of seawater. Reproductive stages of the clam were observed during the maturation experiment. Observations on oocyte developmental stages and its diameter (µm) were recorded fortnightly.

2.6 Data analysis

Gonads from both male and female clams were placed into six qualitative categories, viz. 1= primordial, 2 = developing, 3 = maturing, 4 = ripe, 5 = partially spawned and 6= spent [12]. The gonadal state of each clam was described as one of the six stages based on the most dominant stage present in the clam samples.

Oocyte proportion corresponding to each reproductive stage (according to Morton, 1985) was calculated for each treatment. All statistical analysis was carried out at significance level of $\alpha = 0.05$.

Condition Factor was used to characterize the apparent 'health' or, in other words, the physiological activity of the animals (growth, reproduction, secretion, etc.) under given environmental conditions. The Condition Factor (CF) was determined according to Walne and Mann (1975) [21].

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

3. Results

3.1 Temperature

The highest oocyte diameter was found in the treatment T_3 followed by T_2, T_1 and Control, respectively. One Way ANOVA showed significant difference (P<0.05) in all the treatments. Larger oocyte diameter was observed in treatment T_3 after 60 days (79.37 ± 1.53) whereas smaller oocyte diameter was found in Control (38.39 ± 0.64).

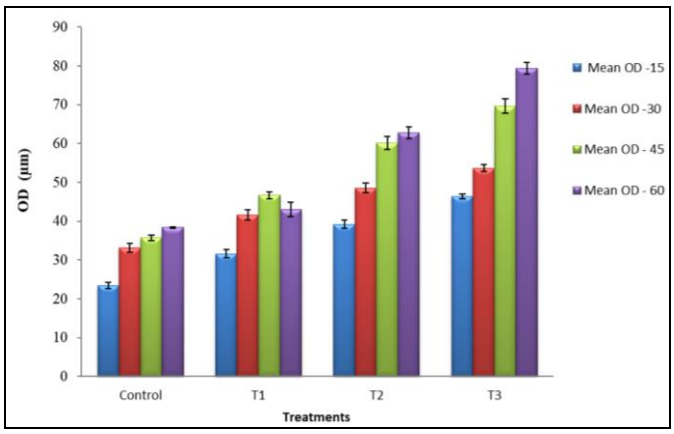

Fig 1: Effect of temperature on Oocyte Maturation of *P. erosa*

3.2 Salinity

The highest oocyte diameter was found in the Control (T_0) followed by T_5, T_4, T_3, T_2, and T_1, respectively. *Post hoc* Tukey HSD test revealed significant difference (P<0.05) among the treatments. Treatment T_3 differs significantly from all other treatments, while Treatment T_1 and T_2 differ significantly from treatment T_4, T_5 and Control. No significant difference was observed between treatment T_4, T_5 and Control. After 60 days larger oocyte diameter was observed in Control (80.26 ± 1.22) whereas smaller oocyte diameter was found in treatment T_1 (47.89 ± 2.08).

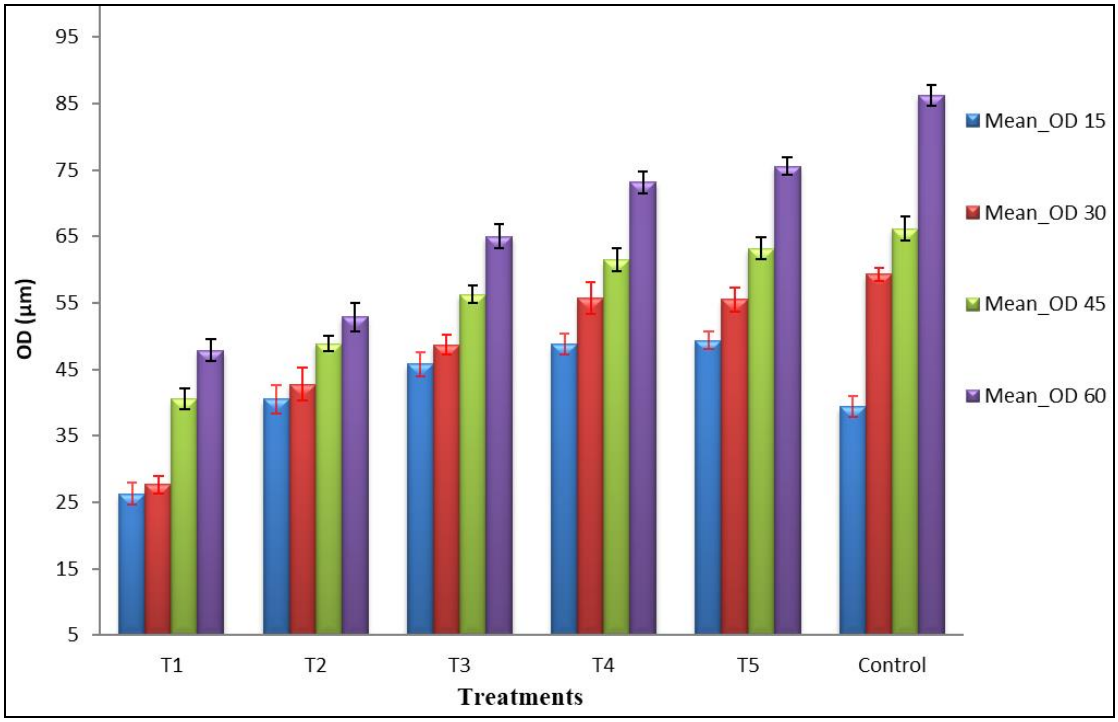

Fig 2: Effect of Salinity on Oocyte Maturation of *P. erosa*

3.3 Effect of Temperature on Condition Factor of *P. erosa*

The highest Condition Factor was observed in the treatment T_3 followed by T_2, T_1 and Control, respectively. One Way ANOVA showed significant difference (P<0.05). Highest CF was 55.15 ± 1.81 in treatment T_3 whereas lowest CF was denoted in Control (27.4 ± 0.95). *Post hoc* Tukey HSD test indicated significant difference among all the treatments.

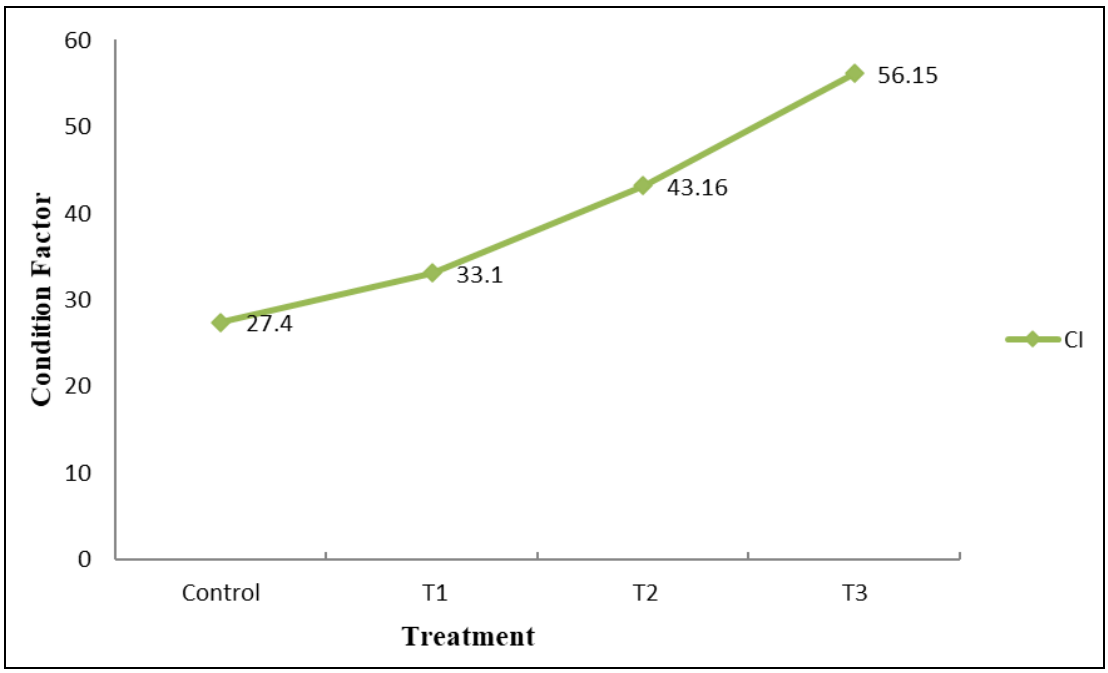

Fig 3: Effect of temperature on Condition Factor of *P. erosa*

3.4 Effect of salinity on Condition Factor of *P. erosa*

Effect of salinity on Condition Factor is shown in Fig. 4. The highest CF was found in the treatment T_5 followed by T_4, Control T_0, T_3, T_2, and T_1 respectively. *Post hoc* Tukey HSD test revealed significant difference (P<0.05) among the treatments. Treatment T_1 differs significantly from all other

treatments, while Treatment T_2 and T_3 differ significantly from treatment T_4, T_5 and Control. No significant difference was observed between treatment T_4, T_5 and Control. Highest CF was observed in treatment T_5 (62.80 ± 1.51) followed by Control (57.55 ± 0.93) whereas lowest CF was found in treatment T_1 (29.40 ± 2.03).

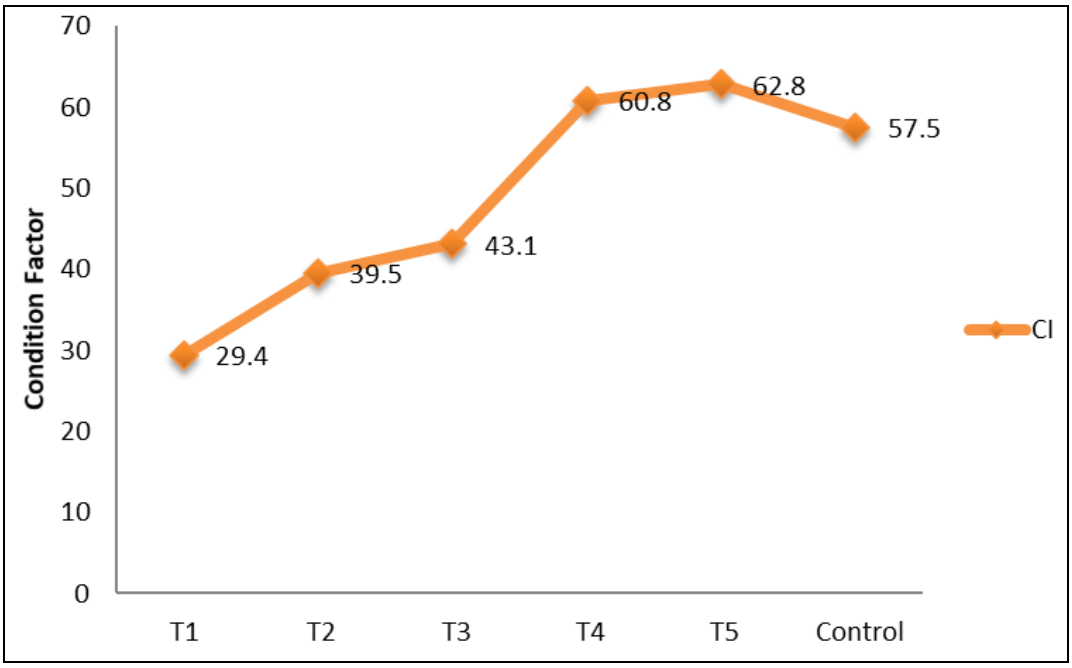

Fig 4: Effect of salinity on Condition Factor of *P. erosa*

4. Discussion

In organism, reproductive behaviour is the most important stage and this behaviour is chiefly control by both, the external and internal agents [19] . The external factors decide the gametogenesis processes in marine organism [9]. Amongst external factor, temperature is one of the vital agents, controlling the reproductive phases including all metabolic activities [10] . In the oyster, *Crassostrea angulata* (gigas) spawning behaviour is induced by temperature [16]. According to Ruiz *et al*. [15], the development in sex cells and spawning behaviour amongst various clams are controlled by temperature and salinity. In the present study, both male and female clam showed the maturation of gonads as the temperature 32 °C and the descending trend was observed in 30 °C, 28 °C and 26 °C, respectively. Similarly the results showed that the oocyte maturation was faster at salinity 35 psu and the best conditioning temperature was 32 °C. The Condition Factor (CF) was higher at 32 °C and at 30 psu salinity, whereas, no significant difference was observed in CF at 30 and 35 psu. These results suggest that the efficient conditioning temperature and salinity is related to the natural spawning season. Therefore, the normal spermatogenesis and oogenesis process in gonads were faster
at higher temperature and optimum salinity. In case of male gonads, at extreme temperatures (low and high) and salinities the primary germ cell i.e., spermatogonia and spermatids were condensed, hence their further development was stopped [20]. The follicular membrane integrity was also collapsed, so complete structural architecture of follicle in male gonad was destroyed [20].

In earlier studies, few workers like Lannan *et al*. [7] and Ruiz *et al*. [15] have proved that the development of sex cells is temperature dependent. While on the other hand, female gonad showed significant alterations like decline of stroma and follicular rupture with further, arresting of oogonia, complete autolysis in oocytes and such alterations were observed particularly at low and high salinities and temperature ranges.

A study by Suja and Muthiah [18] has observed significant effects of different temperatures on oocyte diameter. The observations revealed comparatively more decreased in oocyte diameter at 23 °C range than 28 °C. Honkoop and Van der Meer [4] also observed that, the temperature and immersion affects the egg size in Cerastoderma. However, in Macoma no significant change was observed, whereas the egg number was constantly affected. Paulet and Boucher [14] has stated that water temperature regulate all important phases of gamete development.

According to Laing *et al*. [6], in adverse climatic conditions clams suppressed their oxygen requirement in spite of conserving the energy and maintenance for growth. Lubet *et al*. [14] has observed that successively increasing temperatures cease gametogenesis process in the gonad of *M. galloprovincialis*. Generally, in an organism at increasing temperature, gonad become transparent, it depicted that gonad tissue was utilized for maintenance. Therefore, it affects both developing spermatocytes and oocytes and their number was reduced. Relatively, temperature effect was more prominent on male gonads than female gonads at extreme low temperatures i.e., 14 and 19 °C and high temperature ranging above 34 °C. From these changes
in both the clam species, it is evident that both gonads undergo cytological alterations when exposed to temperature, whereas at extreme temperature the gametogenesis (spermatogenesis and oogenesis) process has been completely affected.

5. Conclusion

This study depicts changes in gonadal tissues of both male and female gonads of mangrove clam *P. erosa* when exposed to temperature ranging from 26 to 32 °C and salinity between 10 to 35 psu. This information will forecast the necessity of

optimum water temperature (extreme low and high) for maturation of clams during the conditioning. In culture practices and for conservation measures this information will be needed for propagation of this mangrove clam *P. erosa* particularly in the context of climate change

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