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A study on the induced maturation of the Indian pearl oyster *Pinctada fucata* (Gould) at Tuticorin, Tamil Nadu, India

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ABSTRACT

In the induced maturation experiments of the pearl oyster *Pinctada fucata* (Gould), 7.5 ± 3.54%, 6.67% and 15 ± 7.07% fully mature pearl oysters were obtained on day 43, 42 and 36 in oysters fed with mixed algae (T-1), mixed algae and raw corn flour (T-2), mixed algae and raw rice flour (T-3), respectively under laboratory conditions. Among the treatments, it was observed that the (T-3) mixed algae with raw rice flour gave the best results with pearl oysters maturing 62.5 ± 7.08% under laboratory conditions on day 29 itself. Whereas, 35 % ± 7.07 % of fully mature pearl oysters were obtained on the 15th day and 36th day from the farm and laboratory, respectively and none in the non fed. Of the matured animals, 43.33% of stage III animals fed on mixed algae changed to stage IV within 19 days, while gonad of 40 ± 14.14% of pearl oysters without feeding changed to stage IV within 26 days under laboratory conditions. Maturity of 53.12 ± 11.97% pearl oysters did not change when maintained in the farm conditions within 24 days.

Keywords: Feed, Induced maturation, Laboratory, Pearl oyster, *Pinctada fucata*

Introduction

Reproduction in continuously breeding tropical species of bivalve molluscs is not likely to be of the same intensity throughout the year; however, when closely examined, several populations show periods of intense reproduction (Giese and Pearse, 1974). Many tropical species have a bi or semi annual breeding season which is characteristic of areas influenced by monsoons (Raja, 1963). Giese and Pearse (1974) and Sastry (1979) have observed that reproductive cycles of marine bivalves are affected by interactions of endogenous (nutrient reserves, hormonal cycles and genotype) and exogenous factors (temperature, salinity, light and food). The environmental factors responsible for bringing a population to a mature stage so that spawning can be coordinated thereby synchronizing the release of gametes have not received much attention.

Molluscs spawn naturally during certain seasons in a year when the environmental conditions are congenial for this activity. In this context, the concept of induced maturation gains importance as the process can be advantageously controlled for a prolonged period of seed production. In the present study induced maturation refers to the accelerated gonad development using different techniques to achieve sexual maturity, so that they can be used for seed production even when they are comparatively young and out of the spawning season.

The various techniques of hatchery conditioning of broodstock of bivalves have been reviewed by Utting (1993). The technique of maturation and spawning of bivalve molluscs out of season was revolutionized by Loosanoff and Davis (1950) in *Venus mercenaria*. In pearl oysters, it was done in *P. fucata martensii* (Kuwatani and Nishii, 1968) and very recently in *Pinctada mazatlanica* (Saucedo *et al*., 2001). The present work attempts to study the reproductive cycle of the pearl oyster *Pinctada fucata* (Gould), which will be helpful to collect specimens for induced maturation and spawning experiments. The work on larval development was done with the view to developing techniques for the rearing of commercially important pearl oyster *P. fucata* and to elucidate the principles and problems of tropical bivalve larval rearing in general for further investigations.

Materials and methods

All the experiments on induced maturation were conducted in the shellfish hatchery. Treatment 1-8 and Treatment 4 for maturation and 8 were carried out for observing the change of mature gonad at the pearl oyster farm at Tuticorin Research Centre of Central Marine Fisheries Research Institute. Seawater for all the experiments in the laboratory was pre-treated with a series of filtration systems before use. Water samples were collected daily from

the rearing containers (tanks and beakers). Atmospheric and water temperatures were noted with a thermometer. The water quality parameters like salinity, pH, dissolved oxygen, ammonia and hydrogen sulfide were estimated according to Strickland and Parsons (1972).

Induced maturation experiments

Pearl oysters with a size range of 40 ± 2 mm (dorso-ventral - measurement) DVM from the same brood were selected to minimise errors related to age and genetic factors. The epifauna was removed by brushing the surface without damaging the growth process of the oysters. The gonads of the oysters were checked and the stages identified and fixed as per the classification of Chellam (1987). The male and female gonads were classified into different maturity such stages as Stage I: inactive /spent resting (Indeterminate), Stage II: developing /maturing, Stage III: mature and Stage IV: partially spawned (partially spent**).** The respective stages were selected and preserved in neutral formalin and processed for histology by following standard procedures of Humason (1979).

The maturation of gonad in respect to different types of feed was studied in the hatchery where pearl oysters were maintained at reduced temperatures of 23 ± 1 ºC in an air conditioned brood stock conditioning room. Maturation of gonad under natural conditions was also noted. The experimental design using different types of feeding pattern twice in the morning and evening included the following feed combinations: 1. Mixed algae alone (T-1). The pearl oysters were fed 2 litres (cell concentration of 3.4 x 10^6 cells ml^{-1}) of mixed phytoplankton comprising of 90% *Chaetoceros calcitrans*, *Isochrysis galbana* and 10% *Nitzchia* sp, *Pluerosigma* sp. and *Rhizosolenia* sp. 2. Mixed algae + Corn flour (T2) the ordinary raw corn flour@30 mg oyster soaked in filtered seawater for 30 minutes and sieved through 40 µm nylobolt cloth was fed twice a day. 3. Mixed algae + raw rice flour (T3). Same procedure was followed but soaked the raw rice for 30 minutes in filtered seawater and ground to a paste. 4. Oysters maintained in the farm (T4) and 5. without feed control (T5). All the experiments were done in quadruplicates with 50 oysters in each replication. Five animals were sampled from each replicate at weekly intervals and the stages of gonad were noted by sacrificing the animals and examining the gonad smears under the microscope.

Microalgae were locally isolated from the Bay water off Tuticorin and maintained in filtered heat-sterilized seawater as pure algal stock cultures in 5 l Hauffkine flasks maintained at low temperatures (24 ± 1 ºC) under a fluorescent lighting of 12 hour cycle (2000 lux). Cultures were used directly or prepared in 20 l plastic transparent buckets or 20 l glass carboys inoculated with the required exponential stage cultures aseptically. The cultures were harvested in the exponential phase and used after assessing the cell counts using a haemocytometer. The medium used for enriching the sterilized seawater to grow all the algae was the conventional Walne's medium (Walne, 1974). Indoor pure cultures in 20 l tubs/buckets and outdoor 1 tonne white bottomed FRP tanks were also maintained for the use in conditioning and induced maturation experiments. The ordinary white raw rice was weighed in a microbalance and soaked in filtered seawater for 30 minutes and then ground (to make particles less than 40 µm) for 15 minutes to form a paste. Feed for each tank was prepared separately. Locally available corn flour was used for feed preparation.

The oysters were reared in 150-200 l capacity FRP tanks in 100 l of seawater and fed 2 l (cell concentration of 3.4 x 10^6 cells ml^{-1}) of mixed phytoplankton. Both raw rice and corn flour were fed @30 mg $oyster^{-1}$ day^{-1}, in addition to the 2 l mixed algae. Aeration was provided for the suspension of the ingredients in the water column. The ground food was passed through a 50 µm nylobolt cloth to the rearing tanks.

Simultaneously, 200 pearl oysters were used @50 oysters per 40 x 40 x 10 cm size, box type cages made of 6 mm iron rod and netted with 1.5 mm dia nylon thread having 10 mm mesh. The oysters with cages were reared in the floating raft in the farm for observation. The gonads of the oysters were assessed as described previously. Pearl oysters (50 x 4 replicates) were maintained in the laboratory conditions at low temperature of 23 ± 1 ºC in the conditioning room to finding out the regression of the gonad from stage III to stage IV, 200 ripe (Stage III). The regression of the stages of the maturity of gonad under unfed T7 and fed conditions (T6) was assessed as in mixed feed. A similar set of oysters with the same stage was maintained in the farm T8 and further changes in the gonad were noted. One way analysis of variance (ANOVA) using Microsoft Excel programme was also done to find out the best treatment for inducing maturation of *P. fucata* in captivity.

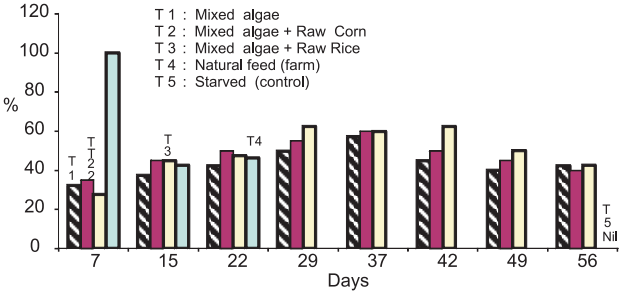

Fig. 1. Effect of different feed on the maturation of pearl oyster *P. fucata;* Percentage of stage II animals

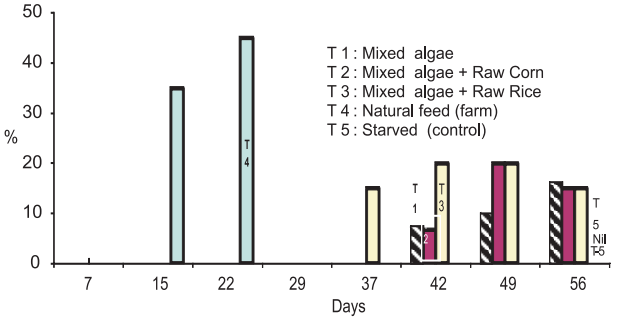

Fig. 2. Effect of different feed on the maturation of *P. fucata:* Percentage of stage II animals and (control without feed)

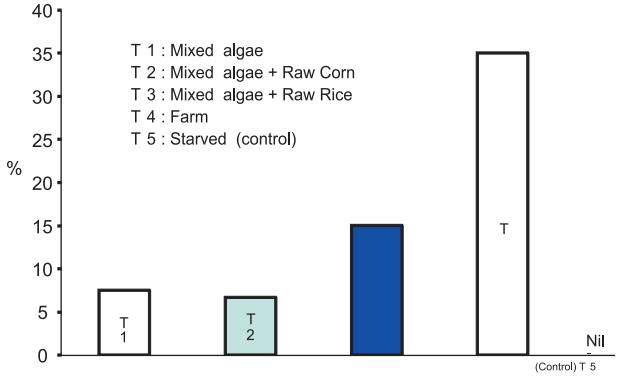

Fig. 3. Induced maturation of the pearl oyster *P. fucata:* Percentage of stage III animals

Results

Under laboratory conditions, 7.5 ± 3.54%, 6.67% and 15 ± 7.07% of fully mature pearl oysters were obtained on day 43, 42 and 36 in oysters fed with mixed algae. Among the treatments, it was observed that the treatment containing mixed algae with raw rice flour gave the best results with pearl oysters maturing 62.5 ± 7.08% in laboratory conditions respectively on day 29 (Fig. 1), whereas 35% ± 7.07% of fully mature pearl oysters was obtained in the farm T4 on the 15th day and none in the non- fed control T5 (Fig. 4). The result was significant between groups ($p<0.1$). Photomicrographs of gonad sections of spent female, mature female and mature male pearl oysters are shown in Fig. 4-5.

The average water quality parameters observed daily from the laboratory during the holding trail was observed as the salinity: 35 ± 1‰ , dissolved oxygen 4.8 ± 1 ml l^{-1} and pH 8.1, ammonia .023 ± .002 ppm. H_2S was absent in the experimental tanks. Ambient water and atmospheric temperatures were 28.6 ºC and 31 ± 1 ºC, respectively.

The environmental parameters in the farm during the experiments were, average salinity 35 ± 1 ‰, dissolved oxygen 4.8 ± 1 ml l^{-1}, pH 8.1, ammonia 0.00136 ± 0.01 ppm. The ambient atmospheric and water temperatures were 30 ± 1 ºC and 31 ± 1 ºC respectively.

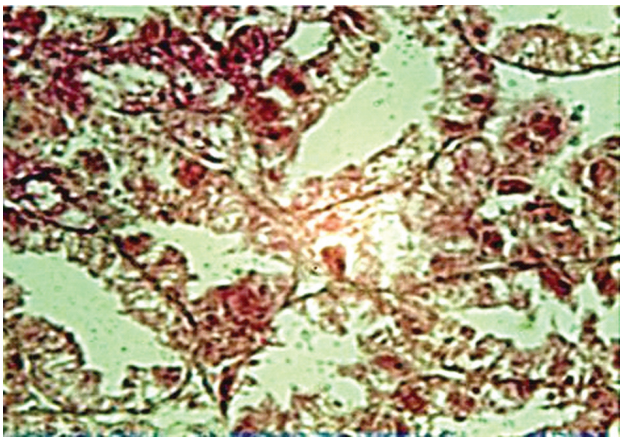

Fig. 4. Histological section of spent female pearl oyster gonad (X 40)

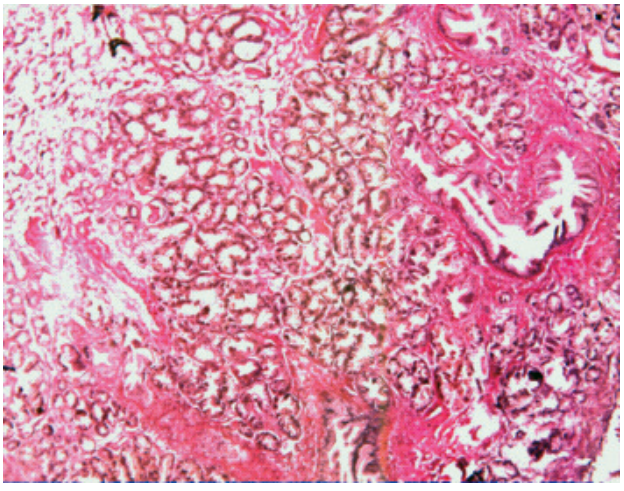

Fig. 5. Histological section of fully matured female pearl oyster gonad (X 10)

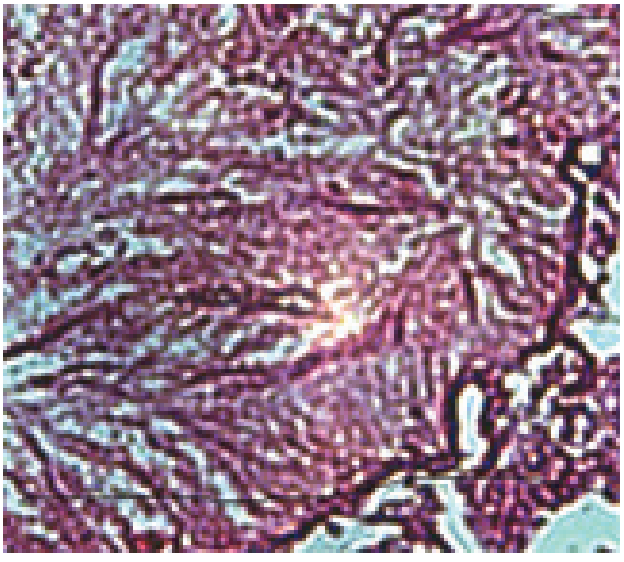

Fig. 6. Histological section of fully matured male pearl oyster gonad (X 10)

About 43.33% pearl oysters of stage III, fed with mixed algae T6, changed to stage IV within 19 days, while 40 ± 14.14% of oysters without feeding T7 changed to stage IV in 26 days. Maturity of 53.12, 11.97% pearl oysters maintained in the farm did not change T 8 within 24 days in the farm conditions, (Table 1).

Table 1. Change of percentage of stage III (mature) to stage IV (spent) in *P. fucata* maintained under different conditions

Treatment	Percentage	Days to attain stage IV
T-6 (Mixed algae)	43.33 ± 11.55	19
T-7 (No feed)	40 ±14.14	26
T-8 (Farm)	53.12 ± 11.97	24

Discussion

The present study on induced maturation showed that the maturation of *P. fucata* was obtained in animals fed with mixed algae and rice flour in 37 days. This agrees with the work of Kuwatani and Nishii (1968) who fed rice powder as a source of diet for maturing adult pearl oyster *P. fucata martensii* and observed maturation in the same number of days. However, Alagarswami *et al*. (1987) reported maturity in *P. fucata* in 45 days when fed with microalgae and corn flour. Haven (1965), working on the maturation of *Crassostrea virginica,* reported that starch primarily influences maturity and meat development and the quantity of enzyme amylase which is capable of hydrolyzing starch occurring in the algae present in estuaries and sea .

The present study indicated that a mixed diet predominated by *Chaetoceros calcitrans* is best for the inducement of maturation in *P. fucata*. Earlier and very few recent works have stressed the role of algae in the conditioning, induced maturation and subsequent successful spawning of temperate and tropical species of bivalves (Bayne *et al*., 1982; Namaguchi, 1997) and also pearl oysters (Saucedo *et al*., 2001).

In this study, the specimens were maintained at a temperature of 23 ± 1 ºC. Conditioning and low temperature for maturation have been successful in *P. mazatlanica* (Saucedo *et al*., 2001) and in many bivalve species (Nayar *et al*., 1988; Nair, 2001; Velayudhan, 2004). However, the study is in contrast to the results of other workers who conditioned bivalves to mature at ambient temperatures (27 – 31 ºC) (AQUACOP, 1979).

In the present study, the maturation of the gonad was obtained on 15 and 37 days in the farm and under laboratory conditions, respectively. According to Gabbot and Walker (1971), two factors which differentiate the hatchery from field conditions are temperature and the amount of food available. According to him, in general, increased temperatures result in higher metabolic rates and this increased energy demand can be met either from food or reserves but, Pouvreau *et al*. (2000) in a tropical environment found particulate organic matter as the main determinant factor for gametogenesis and not temperature. The amount of food they obtain is dependent on the density of the food organisms and the pumping rate (filtration rate) of the bivalve. The bivalve in the hatchery could pump less unfiltered seawater than those in the sea, and the amount of food available was probably dependent on the rate of inflow. Tranter (1959) observed that generally pearl oysters take 7-8 months for gonad development.

The ripe *P. fucata* was held in the farm and lab conditions (in low temperature of 23 ± 1 ºC) for 24 and 19 days respectively. Alagarswami *et al*. (1987) reported the successful maintenance of ripe pearl oysters of the same species of pearl oyster in captivity even though no mention of the maintenance time was reported.

Velayudhan (2004) reported that in induced maturation experiments, 7.5 ± 3.54%, 6.67% and 15 ± 7.07 % of fully mature pearl oysters on day 43, 42 and 36 in fed with mixed algae, T1 mixed algae and corn flour (T2) and mixed algae and rice flour T3 respectively under laboratory conditions.

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