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Survey about the Effect of Stocking Density and Bacterial Load on the Commercial Production of Pearl Oyster *Pinctada fucata* Larvae in Persian Gulf of Iran

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Abstract: Commercialization of pearl culture requires large scale hatchery production of larvae. The survival of larvae depends on stocking density, environmental factors, diets and diseases. The results of experiments using 4 stocking densities at the rate of 100, 1000, 2000 and 3000 larvae L^{-1} of filtered sea water indicated highest survival of 15.8 and 10.5% in the low stocking densities followed by 1.4 and 0.34%, respectively in other two stocking densities at an ambient temperature of 28.6±0.4°C. Although, the larvae were fed with *Isochrysis galbana* at the recommended cell densities, a positive correlation between increased bacterial load and pearl oyster larval survival was recorded in all the stocking densities. Eventhough, total number of spat produced in 100 and 1000 larvae L^{-1} stocking density was more or less similar and considering the management strategies including man power, cost of production of microalgal culture and infrastructure facilities such as availability of tanks and space in an established hatchery the stocking density of 1000 larvae L^{-1} will be optimum for commercial hatchery production of pearl oyster larvae.

Key words: Bacterial load, pearl oyster, larvae, Persian Gulf, man power, Iran

INTRODUCTION

During the artificial rearing of the bivalve larvae, many different factors affect their growth and survival for example egg quality, food type and quantity, temperature, salinity and water quality (Gosling, 2003). Another important factor is the stocking density. This effect has been little studied eventhough, it is most important in the artificial rearing of mollusc larvae as it determines the production in the hatchery. For pearl oysters, larval rearing densities for *C. virginica* vary from 10 larvae mL^{-1} (Newkirk *et al*., 1977) to 5-8 larvae mL^{-1} (Mallet and Haley, 1983).

For the hard clam, *M. mercenaria*, Hilbish *et al.* (1993) reported initial stocking densities of 10 larvae mL^{-1}. Larval rearing densities for *Pinctada maxima* vary from 1-8 mL^{-1} (Rose and Baker, 1994). The optimal larval density for *Pinctada fucata* has not been determined, however they are generally reared at a density of 3-28 larvae mL^{-1} (Alagarswami *et al*., 1983). A major problem with hatchery culture of *P. fucata* is low survival (generally <5.0%) during larval culture (Subhash *et al*., 2003; Dharmaraj, 2005; Subhash, 2009). Larval mortalities occurring in molluscan hatcheries have often been associated with bacterial contamination and more specifically with vibrios (Tubiash *et al*., 1965; Lipton *et al*., 2003; Leon *et al*., 2005; Hasegawa *et al*., 2008). Sandlund *et al.* (2006) reported that opportunistic bacteria cause almost 100% mortality during larval stages of the bivalve *Pecten maximus*. The findings of Subhash (2009) indicated that hatchery production of *Pinctada fucata* was seriously affected by massive larval mortalities caused by *Vibrio* sp. This indicates that efficient large scale hatchery production of *P. fucata* requires further research to investigate the specific culture requirements of this species. Very little information is available on the effect of stocking density and bacterial load on the survival and settlement of pearl oyster larvae. In this study, researchers consider the combined effect of stocking density and bacterial load on the commercial production of *P. fucata* seed.

MATERIALS AND METHODS

Spawning and larval rearing: The experiments were conducted at the pearl oyster hatchery of Persian Gulf of Central Marine Fisheries Research Institute. Oysters collected from the Gulf of mannar and stocked in dark interior Fiberglass Reinforced Plastic (FRP) tanks were induced to spawn by increasing the water temperature from 27.8-33.6°C. The fertilised eggs were collected in 30 μM sieve and released in to freshly filtered sea water in FRP tanks. About 5 days old larvae were used for the experiments.

Experimental arrangements: Larvae were stocked in FRP tanks of 1.5 ton capacity containing 1000 L of filtered sea water (0.2 μ) at 4 stocking densities: 3000, 2000, 1000 and 100 larvae L^{-1} of filtered seawater with 3 replicates for

each stocking density. Water exchange was done on alternate days. Micro algae, *Isochrysis galbana* was given as feed as per the feeding protocol (Alagarswami *et al*., 1983).

Hydrological parameters and estimation of bacterial load: Hydrological parameters such as temperature and pH were monitored daily. Dissolved oxygen and salinity were monitored on alternate days and once in a week, respectively (APHA *et al*., 1989). The bacterial load of the intake water as well as water from the 4 experimental tanks was determined by Total Plate Count (TPC). Water samples were collected aseptically prior to water exchange on alternate days and plated on nutrient agar plates supplemented with 2.5% NaCl using the Pour Plate Method (Collins *et al*., 2001).

Estimation of larval population: Larval population of *P. fucata* was estimated immediately after exchanging water. The larvae were collected in 100 L FRP tank by using suitable mesh ranging from 20-150 μ (i.e., from D shape Veliger to Plantigrade stage). The water in the beaker was mixed and sub samples (1 mL) were taken in an embryo cup and counted at 100x magnification. The average value obtained by repeated sampling was multiplied with 1,00,000 to get the total larval densities in 100 L (Alagarswami *et al*., 1983). All the statistical analysis was performed by Microsoft Statistica 1.4 software.

RESULTS AND DISCUSSION

Hydrological parameters: No significant (ANOVA, $p>0.001$) difference in hydrological conditions were noted between the different stocking density groups during the experimental period. The temperature (28.6±0.3 to 29.1±0.4°C), pH (8.31±0.02 to 8.32±0.02), dissolved oxygen (4.41±0.14-4.44±0.14 mg L^{-1}) and salinity 29.5±0.05-30.5±1.5 ppt) were within the optimal ranges for larval growth.

Bacterial load and survival of pearl oyster larvae: The bacterial load in the filtered water received in the rearing tank ranged from 1.0-9.0×101 cfu mL^{-1}. High larval mortality of 21.0, 25.0, 26.0 and 10.0% were noted on day 9, 13, 15 and 17, respectively in the stocking density of 3000 larvae L^{-1}. In the stocking densities of 2000 and 1000 larvae L^{-1} larval mortality was noted on day 9, 13 and 15th. In the low stocking density of 100 larvae L^{-1} high larval mortality of 13.6 and 20.6% was noted on day 13 and 15th.

The bacterial load ranged from 1.0×102 to 8.3×103,1.0×102 to 6.1×103, 1.0×102 to 3.0×103 and 1.0×102 to 2.4×103 in the 4 stocking densities of 3000, 2000, 1000 and 100 larvae L^{-1}, respectively. Bacterial load at different stocking densities and mortality of *Pinctada fucata* larvae during the experiment were shown in Table 1.

Survival of larvae and spat production in the four stocking densities: Very low spat production of 0.35 and 1.4% were obtained in the higher stocking density of 3000 and 2000 larvae L^{-1}. In the stocking density of 1000 larvae L^{-1}, 10.5% spat production was obtained and in the low stocking density of 100 larvae L^{-1}, it was 15.8% (Fig. 1). The cumulative mortality and bacterial load during the study period is shown in Table 2.

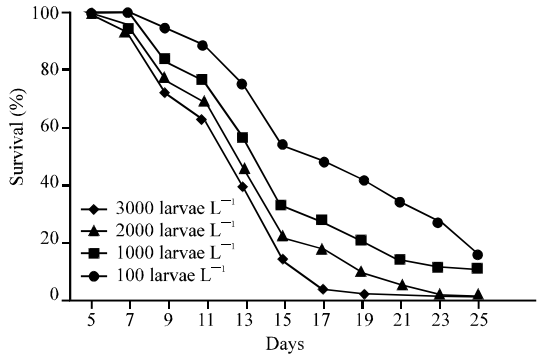

Fig. 1: Survival of lavae and spat production in the four stocking densities

Table 1: Bacterial load and mortality of Pinctada fucata at different stocking densities

Days	Bacterial load	2000 mortality	Bacterial load	1000 mortality	Bacterial load	100 mortality	Bacterial load	3000 mortality
7	2200	4.4	120	0.0	100	0.0	100	6.40
9	2700	20.0	2300	17.8	1100	6.4	140	21.00
11	900	7.3	180	6.8	160	6.8	150	8.30
13	7900	23.5	6100	19.5	3000	13.6	1900	25.00
15	8300	23.0	6000	22.8	270	20.6	2400	26.00
17	1000	4.0	360	6.3	580	6.2	400	10.00
19	250	8.0	700	7.0	500	7.3	400	0.95
21	200	5.0	600	5.9	500	7.6	500	1.00
23	200	28.0	400	2.7	300	8.8	400	0.50
25	100	0.6	100	0.8	100	6.9	300	0.50

Table 2: Cumulative mortality and bacterial load during larval rearing of *Pinctada fucata*

Larvae (1 L^{-1})	Total mortality (%)	Bacterial load (cfu)
3000	99.65	2200
2000	98.60	1700
1000	89.50	890
100	84.20	670

Hatchery production of pearl oysters is relatively new. Reflecting this and the lack of knowledge of culture requirements, a number of studies have reported relatively poor survival of pearl oyster larvae during hatchery culture. Larval mortalities occurring in molluscan hatcheries have often been associated with bacterial contamination favored by rearing conditions such as high temperature, excess food and high larval density. Two types of infestation have been described. The first corresponds due to the specific development of pathogenic bacteria such as Pseudomonas (Brown, 1974), Alteromonas (Garland *et al*., 1983) and Vibrios (Leon *et al*., 2005; Sandlund *et al*., 2006; Hasegawa *et al*., 2008; Subhash, 2009). The second is due to the contamination of sea water by heterotrophic bacteria which induces larval mortalities when their density is high in larval rearing water (Lipton *et al*., 2003; Subhash *et al*., 2003, 2007).

This manifested in to high mortality and poor spat settlement in the present study in which 99.65 and 98.6% of mortality was recorded with a high average bacterial load of 2.2×103 and 1.7×103 cfu mL^{-1} at high stocking density of 3000 and 2000 larvae L^{-1}. On the other hand, a low mean bacterial load of 6.7×102 cfu mL^{-1} with comparatively low mortality of 84.2% was observed in the lowest stocking of 100 larvae L^{-1}. Interestingly a low mean bacterial load of 8.9×102 cfu mL^{-1} with a mortality of 89.5% was noted in the stocking of 1000 larvae L^{-1} (Table 2).

Although, the hydrological parameters such as temperature, pH, salinity and dissolved oxygen content were almost similar in the 4 stocking densities; the bacterial load was higher in the high stocking density. Moreover, high bacterial load was found in the culture tank water during high larval mortalities in all the stocking densities showing a positive correlation between larval mortality and bacterial load.

High larval mortality was found on 9, 13, 15 and 17th day in the stocking density of 3000 larvae L^{-1}. High bacterial load of 2.7×103, 7.9×103, 8.3×103 and 1.0×103 cfu mL^{-1} was noted in the culture tank water when mortality was 21.0, 25.0, 26.0 and 10.0% (Table 2). The results showed a positive correlation between high larval mortality with increasing bacteria load in culture tank water (r = 0.8779). In the stocking of 2000 larvae L^{-1} on 9, 13 and 15th day high mortalities was noted. Here also, the total viable count of bacteria was increasing in the culture tank water during larval mortality (r = 0.9940). High bacterial load of 2.3×103, 6.1×103 and 6.0×103 cfu mL^{-1} was noted when mortality was 20.0, 23.5 and 23.0%. In the density of 1000 larvae L^{-1}, high larval mortality of 17.8, 19.4 and 22.8% was found on 9, 13 and 15th day with a high bacterial load of 1.1×103, 3.0×103 and 2.7×103 showing a positive correlation between bacterial load in culture tank water and larval mortality (r = 0.6403). High larval mortality of 13.6 and 20.6% was noted on 13 and 15th day with a high bacterial load of 1.9×103 and 2.4×103 even in the very low stocking density of 100 larvae L^{-1} confirming the positive correlation of bacterial load and larval mortality (r = 1.0) in the pearl oyster hatchery.

Similar study by Subhash *et al*. (2007) also revealed that the total bacterial load in the culture tank water was found to increase in the hatcheries of *Pinctada fucata* during disease out breaks resulting in heavy larval mortality. A comparable trend of high bacterial load associated with larval mortality was noted 4 times (day 9, 13, 15 and 17th) in the dense stocking system of 3000 larvae L^{-1} during the course of the experiment. The combination of high larval densities, debris from dead larvae and high load of organic matter due to addition of live food could have stimulated the selection and growth of opportunistic bacteria in larval tanks (Skjermo and Vadstein, 1999; Torkildsen *et al*., 2005; Hernandez and Martinez, 2005). Moreover in the low stocking density of 100 larvae L^{-1} also such a trend of decreased larval population associated with high bacterial load was noted 2 times (day 13 and 15th). Thus, bacterial load could be the important (key) factor determining the survival and spat settlement of pearl oyster larvae.

Poor spat setting of 0.35 and 1.4% were observed in higher stocking densities of 3000 and 2000 larvae L^{-1}. Reported studies showed that there is an inverse relationship between larval survival and larval density (Ibarra *et al*., 1997; Doroudi and Southgate, 2000). Krishnan and Alagarswami (2003) reported a low survival of 1.8% in *Pinctada fucata* hatchery at a stocking density of 3000 larvae L^{-1}. Recently, Subhash *et al*. (2007) also reported a low survival of 0.82% in the high stocking density of 5000 larvae L^{-1} in the *Pinctada fucata* hatchery.

CONCLUSION

According to the results at the stocking density of 1000 larvae L^{-1}, the survival was 10.5% and out of 10 lakh larvae stocked an average of 1,05,000 spat were produced. However in the lowest stocking density of 100 larvae L^{-1},

the survival was 15.8% and out of 1 lakh larvae stocked an average of 15,800 spat were produced. Comparing the total number of spat settled in 1000 and 100 larvae L^{-1} stocking densities and considering the management strategies including man power, cost of production of microalgal culture and infrastructure facilities such as availability of tanks and space in an established hatchery the stocking density of 1000 larvae L^{-1} will be optimum for commercial hatchery production of pearl oyster larvae.

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