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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Science 6 (12): 1041-1045, 2003
ISSN 1028-8880

Effects of Egg Density During Incubation on the Quality and Viability of Mussel Larvae, *Mytilus edulis* L.

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Abstract: This study has been conducted to investigate the effect of different egg culture densities during incubation on the development and viability of mussel larvae, *Mytilus edulis* L. The activated eggs were cultured in two different culture densities (less than 5000 eggs l^{-1} and more than 15000 eggs l^{-1}) up to the development of the early shelled veliger or D-larvae stage (ca 72 h). It was found that the percentage yield, quality and viability of mussel larvae (prodissoconch I larvae) were significantly improved if lower culture densities were used ($p<0.05$). The low culture density environment also produced significantly lower percentage of abnormal larvae, particularly the incidence of abnormal shell development ($p<0.05$). This finding is of great importance for viable hatchery production of quality mussel spats. However, further experiments are greatly needed to ascertain the feasible incubation density and the optimal larval concentration especially during the early developmental stage of mussel larvae.

Key words: *Mytilus edulis*, mussel, veliger, egg incubation density, larval culture

Introduction

The mussel, *Mytilus edulis* is a typical benthic invertebrate of many temperate coasts. It has historically not been considered to be as delectable as oysters, scallops, or various species of clams; however, due to intense marketing efforts over the last decade, it is becoming an increasing popular item on seafood restaurant menu throughout Europe and other parts of the world (Mason, 1976).

Since the first successful rearing of this species more than a century ago, mussels have been extensively farmed, not only as major crop, but also as side crop for many marine aquaculture farms. However, though the biology and physiology of *M. edulis* have been frequently described in literature (Bayne, 1976; Booth, 1977; Lutz and Hidu, 1979; Le Pennec, 1980 and Seed and Suchanek, 1992 etc), the aquaculture of this species still have many difficulties to deal with especially in the larval rearing stages. Production of better quality larvae will definitely be beneficial for the aquaculture industry of this commercial species.

Many populations of *M. edulis* spawn all year round, with major peaks of spawning in spring and often a number of further spawning later in the summer, with more restricted spawning periods where adult feeding conditions are poor (Seed and Suchanek, 1992). Fecundity normally varies from year to year. Reproductive output is basically sized and site related and is also influenced by temperature, food supply and tidal exposure (Bayne *et al*., 1983) among other factors. Mussels, like the majority of shallow water bivalves, produces large numbers of pelagic larvae of up to 8×10^6 eggs/individual or more and spend several weeks in the plankton before reach the settlement phase (Bayne *et al*., 1978). Their characteristic of reproductive strategy includes: high fecundity (Thompson, 1979; Bayne *et al*., 1983 and Sprung, 1983), high larval mortality (Thorson, 1946 and Yap, 1977) and high dispersal (Crisp, 1974; 1975).

The lecithotrophic stage of mussel larvae is indeed the most important phase that allows them to select a suitable substrate for settlement before metamorphosing and start its dominant mode of life as sessile organism (Widdows, 1991). The growth, survival and the success for mussel larvae to complete their cycle depend on several factors, which include adequate food supply, suitable environmental condition, predation and contact with areas suitable for settlement (Widdows, 1991) and only fractions of the larvae will subsequently settle and reach adult stage.

There are only few studies focused on the early development of mussel larvae i.e. when fertilized egg develop through the embryonic development, gastrulation and development of trochophore to early veliger or prodissoconch I larvae stages. Most of hatchery culture practices concerned more on the culture environment and successful feeding of larvae until they metamorphose. Therefore the main objective of this study is to investigate the effect of different egg incubation densities

on the quality and viability of early veliger produced. When other factors are remained consistent, it was hypothesized that low egg incubation density will produce better larval quality and viability compared with high culture density. Productions of better veliger quality will guarantee a more successful feeding and development of the succeeding stages.

Materials And Methods

Experiments with embryos and larvae generally require considerable care and attention in handling. At all stages, larval cultures are susceptible to pathogenic bacterial infections, which generally result in the death of all larvae within a few days. In this study all glassware and other materials were cleaned and sterilized using dilute chloros, a commercial Sodium Hypochlorite solution.

About 30 ripe mussels of more than 25 mm lengths were selected and cleaned by scraping off barnacles and other fouling organisms attached. The mussels were then placed in a shallow tray of filtered seawater at room temperature (>15°C) and were induced to spawn by injecting 2 ml of 0.5 M KCl into the mantle cavity, according to the method of Bayne *et al.* (1975). After injection, the mussels were left out of water for 1 h at room temperature, which is no higher than 20°C. After emersion, the mussels were placed individually in 1 l glass beaker in filtered seawater at temperature between 15 to 20°C. Mussels were allowed to remain in individual beakers until spawning commences. Male spawn produces a milky appearance while female spawn tends to lie on the bottom of the beaker and is very pale orange/brown, sometimes almost white in appearance. After spawning the eggs and sperms were promptly cooled to 15°C, the temperature in which all further operations were performed.

The eggs were collected and placed in a 1 l cylinder and their numbers were estimated using a Haemocytometer. The number of spermatozoa on the other hand was not counted but only estimated: for a strong milky sperm suspension, *circa* 10 ml of sperm suspension was added directly to 1 l of egg suspension. The mixture was however further inspected under microscope to indicate whether sufficient sperm have been added, to a ratio of sperm to egg of at least 10:1. In mussels a ratio of 100 to 1000 sperms per egg is generally considered adequate to successfully fertilize the eggs. A stirrer was used to gently keep the activated eggs in suspension in the cylinder.

Around 30 to 40 minutes after activation the eggs were again inspected to check whether polar bodies have been produced, which indicates that egg activation and probably fertilization, has been successful. After the formations of polar bodies are confirmed, the eggs were promptly but gently transferred into two 1 l dishes to get the effects of low and high egg incubation densities. For each trial, the treatments are summarised as follows:

Treatment 1 (low culture density): 50 ml of activated eggs, topped up to 700 ml with filtered seawater.

Treatment 2 (high culture density): 450 ml of activated eggs, topped up to 700 ml with filtered seawater.

Both dishes were covered with Perspex sheet and incubation took place in a 14°C temperature controlled room. The developing eggs/embryos were then left undisturbed for 3 days (72 h) to allow for gastrulation and development through trochophore larval stages to early shelled veliger or 'D' larval stage. After this period the larvae were analysed for abnormalities, which includes: abnormal velum, abnormal 'bunch of grapes', abnormal rudimentary shell and abnormal unshelled larvae.

Results

The result of this experiment is presented in Table 1. The low culture density treatment range between 2857.14±412.39 to 4523.24±629.94 eggs l^{-1} whilst the high culture density treatment range between 19285.71±2592.51 to 24285.71±1889.71 eggs l^{-1}. The developing larvae (prodissoconch I larvae) were divided into percentage of normal larvae; percentage of larvae with abnormal velum; percentage of abnormal shell and percentage of undeveloped eggs. In this experiment the percentage yield range from 13.67±0.88 to 26.67±2.19 and 9.33±1.42 to 11.00±1.53; the percentage abnormal velum range from 7.10±1.53 to 13.33±2.40 and 11.33±2.91 to 12.00±2.65; and the percentage abnormal shell range from 56.67±1.67 to 67.57±2.08 and 70.33±3.93 to 73.00±3.06; for low and high

Table 1: The effect of different culture densities on the condition of veliger larvae stage of *M. edulis* (after 72 h incubation periods). N = larvae with normal velum and normal shell formation; AbV = larvae with abnormal velum formation; AbS = larvae with crippled or abnormal shell formation; UND = undeveloped egg

	Low Culture Density					High Culture Density				
Trial	%N	%AbV	%AbS	%UND	No. eggs l^{-1}	%N	%AbV	%AbS	%UND	No. eggs l^{-1}
1	22.00±1.53	7.10±1.53	67.57±2.08	3.33±0.88	2857.14±412.39	10.00±0.58	11.33±2.91	73.00±3.06	5.67±0.33	16285.71±2592.51
2	26.67±2.19	10.13±2.52	56.87±4.50	6.33±0.88	3095.24±1325.66	11.00±1.53	11.67±1.20	70.33±3.93	7.00±1.53	20476.19±3122.59
3	23.33±4.26	13.33±2.40	56.67±1.67	6.67±1.45	4523.81±629.94	9.33±1.42	12.00±2.65	71.83±0.44	6.83±2.09	24285.71±1889.82

Table 2: Tukey's pairwise comparisons on percentage of normal veliger larvae developed from the three trials under low and high culture density treatments. Values are intervals of column level mean – row level mean. The (*) sign denotes a significant different ($p<0.05$). Critical value = 4.75, family error rate = 0.05 and individual error rate = 0.00569. %N = percentage of normal veliger larvae; LD = low culture density; HD = high culture density

	%N LD-1	%N LD-2	%N LD-3	%N HD-1	%N HD-2
%N LD-2	-15.273				
	5.940				
%N LD-3	-11.940	9.273			
	-7.273	13.940			
%N HD-1	1.393	6.060	2.727		
	22.607(*)	27.273(*)	23.940(*)		
%N HD-2	0.393	5.060	1.727	-11.607	
	21.607(*)	26.273(*)	22.940(*)	9.607	
%N HD-3	2.060	6.727	3.393	-9.940	-8940
	23.273(*)	27.940(*)	24.607(*)	11.273	12.273

Table 3: Tukey's pairwise comparisons on percentage of larvae with abnormal shell development from the three trials under low and high culture density treatments. Values are intervals of column level mean – row level mean. The (*) sign denotes a significant different ($p<0.05$). Critical value = 4.75, family error rate = 0.05 and individual error rate = 0.00569. ABS = percentage of larvae with abnormal shell; LD = low culture density; HD = high culture density

	ABS LD-1	ABS LD-2	ABS LD-3	ABS HD-1	ABS HD-2
ABS	-3.31				
LD-2	24.731				
ABS	-3.131	13.831			
LD-3	24.931	14.231			
ABS	-19.465	-30.165	-30.365		
HD-1	8.598	-2.102(*)	-2.302(*)		
ABS	-16.798	-27.498	-27.698	-11.365	
HD-2	11.265	0.565	0.365(*)	16.698	
ABS	-18.298	-28.998	-29.198	-12.865	-15.531
HD-3	9.765	-0.935(*)	-1.135(*)	15.198	12.531

culture density treatments respectively. The percentage of undeveloped eggs was found to be consistently very low where more than 90% of the egg suspension were successfully fertilized and produced larvae from all trials. Since all data were normally distributed (Anderson-Darling test, low a^2 and $p>0.05$) and showed significant heterogeneity of variances (Bartlett's test) therefore comparisons between trials using ANOVA (oneway) were performed. There is at least one mean differs from at least one another in terms of percentage yield and percentage of abnormal shell development ($p<0.05$), but no significant differences was detected between the percentage of larvae with abnormal velum and percentage of undeveloped eggs ($p>0.05$). Further analysis using Tukey's pairwise comparisons were then performed and presented in Table 2 and Table 3. It was found that all trials from low culture density showed significantly higher percentage of normal larvae compared with the high culture density treatments (Table 2). Furthermore the low culture density treatments also showed significantly lower incidence of larvae with abnormal shell where trials 2 and 3 were significantly inferior compared with all trials from the high culture density treatments (Table 3). The present study clearly showed that lower egg incubation densities produced significantly better larval performance. The whole experiment in general showed very high percentages of abnormalities, with most of the prodissoconch I larvae were found to have abnormal shell development while the others showed abnormal velum formation.

Discussion

The present study found that the low culture density treatment showed better larval performance where they produced significantly higher percentage of normal larvae. However, the percentage yields data were low as only less than 30% of normal larvae were recorded. The population density used in this experiment might have probably been too dense when compared with natural larval density. Nevertheless it is actually hardly feasible to conduct a laboratory experiment that comparable with the natural larval density. Attempts on natural populations have previously been tried and some authors have reported a maximum values range only between 3 to 40 larvae l^{-1} (Rees, 1954; Schram, 1970; Hernroth and Ackefors, 1979); which is far below the density used in this experiment.

The result was in agreement with previous experiments where growth rate were shown to decline with increasing larval density (Loosanoff, 1954 and Loosanoff and Davies, 1963). Furthermore Sprung (1984) reported only minor effects of density on larval growth observed when cultured below 1000 larvae l^{-1} culture density. However most of the data reported earlier were based on the whole pelagic stages whilst this study only concerning the stage I growth, i.e. up to D-larvae or straight hinge larvae only (prodissoconch I larvae).

The present study also showed a significantly higher percentage of larvae with abnormal shell development when reared in high culture density environment. This finding also in agreement with previous study where Field (1922) reported many incomplete forms or crippled D-shell larvae were encountered in overcrowded cultures. In addition Sprung (1984) also found that *M. edulis* larval development was significantly depressed and took longer time to reach settlement stage if cultured in dense environment.

Generally the early stage of bivalve larvae development up to prodissoconch I stage represents a period of intense morphological activity when there is complete dependence on the stored energy resources acquired from the adults during vitellogenesis (Bayne *et al*., 1975). During this vital stage of development, the endogenous factors such as genetic variation provides a very significant contribution to the succeeding larval

performance. Instead of the external factors, viability and larval performance could have been affected by variations in the quality and quantity of nutrient resources invested by adults in the gametes, particularly in the eggs (Widdows, 1991). Poor egg viability and thus poor performance of succeeding larval stage might have resulted from the direct effect of environmental stress on the adult mussels (Bayne, 1972 and Bayne *et al*., 1975). Increased in the number of abnormal cleavage stages and a smaller number of healthy larvae are often encountered from stressed adults (Bayne *et al*., 1975). However, in the present study the variability associated with adult mussels can be excluded as they were exposed to the same culture conditions and after all eggs from different broods were mixed together before assigned to the different treatments.

Most of hatchery culture practices focus more on the successful feeding of cultured larvae. However this study interestingly indicate that the period before the onset of feeding are also important. After fertilization, normal embryonic development during cleavage, gastrulation and development to the first shelled larval stage are important for the success and viability of the succeeding stages. This will certainly involves the quality of the eggs produced and more importantly the culture environment that permits favourable condition for the subsequent development right after fertilization.

The biomineralization of developing larvae for example is one of the factors that might have significantly contribute to the successful development of mussel larvae. It is a complex of biochemical and physiological processes, depending on the endogenous activity of an organism as well as influences from surrounding environment. The process actually continuous during the entire life cycle, but the one during the early stages of development is the most important. Those influencing the type and structure of biominerals are includes: saturation of medium with calcium ions, environmental temperature, salinity, pH, etc (Wilbur 1964; Taylor *et al*., 1969; Lowenstam 1981; Watabe 1983 and Wilbur and Saleuddin 1983). These processes take place in tissues and shells and manifest themselves in calcification, decalcification and in polymorphic transitions of calcium carbonate in particular shell layers. The significantly high percentage of D-larvae with abnormal shell observed in high culture density treatment might have been the direct effect of stress during this early larval development. However no solid conclusion can be made on this matter and therefore further studies, which focused on the activity attributed to the biomineralization and other process during the early larval development are greatly needed to explain this phenomenon.

As a conclusion, this study showed that the percentage yield, quality and viability of mussel larvae were significantly improved if lower culture density was used. The low culture density environment also produce significantly lower incidence of abnormal larvae, particularly the abnormal shell development. Further experiments are greatly needed to ascertain the feasible larval concentration, which of course will be of great importance for viable hatchery production of mussel spats. Future studies should also be focused on the activities attributed to the complex biochemical and physiological processes during the early stage of mussel larval development.

Acknowledgements

Many thanks to the School of Ocean Science, Menai Bridge, United Kingdom for providing the facilities for this experiment. First author would also like to thanks the National University of Malaysia for the scholarship award that make this study possible.

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