*Indian J. Fish.*, 46(1) : 41-47, Jan.-Mar., 1999

# Effects of aeration, U.V. treated seawater and antibiotics on larval growth and spatfall in *Pinctada fucata* (Gould)

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ABSTRACT

The larvae of the Indian pearl oyster *Pinctada fucata* were found to have been affected adversely by aeration during larval phase and consequently the growth, and spatfall were found to be poor. The values obtained in larval growth and spatfall were always less in U.V. treated seawater when compared to unsterilised seawater. This may be due to the destruction of nannoplankters by U.V. irradiation. Among the antibiotics tested, the performance of kanamycin was found to be better at 50 and 100 ppm in respect of growth and spatfall.

## Introduction

The larvae of many bivalves could withstand vigorous mechanical disturbances showing no ill-effects (Loosanoff and Davis, 1963). The same phenomenon was observed by Nayar *et al.* (1984) in the rearing of larvae of *Crassostrea madrasensis,* Appukuttan *et al.* (1988) in *Perna indica* and Narasimham *et al.* (1988) in *Meretrix meretrix*. However, mechanical disturbance in water was not preferred by the larvae of pearl oyster *Pinctada fucata* (Alagar-swami *et al.*, 1987). Aeration causing mechanical disturbance and its effect on the larvae of *P. fucata* was studied.

Waugh (1958) and Walne (1958) have used the ultraviolet treated seawater to control bacteria and fungus. Even under the apparently best conditions occasional mortalities were reported in molluscan larvae and juveniles (Loosanoff and Davis, 1963) and bacterial infection was indicated as the probable cause (Walne, 1958; Loosanoff, 1954; Davis and Chanley, 1956; Guillard 1959). Use of antibiotics and fungicides was suggested to improve survival and larval growth (Davis and Chanley, 1956). During the present studies the effects of U.V. treatment of seawater and the use of some antibiotics on larval growth and spatfall in *P. fucata* were monitored and the results are reported in this communication.

## Material and methods

Experiments were conducted in larval rearing tanks of 5 to 500 l capacity for which the sand-filtered seawater was used. The water was changed once in every two days. The effects of aeration in larval rearing medium was studied by giving aeration through diffuser stone. Continuous aeration was provided throughout the experiment from the day of stocking of larvae till spatfall. A control was kept under identical conditions without aeration. To study the effect of U.V. treatment of seawater, filtered seawater was sterilised by ultraviolet irradiation and used in rearing the larvae. In the control, filtered seawater alone was used. The effect of antibiotics was tested in three experiments. The antibiotics such as kanamycin, crys-4, streptomycin and chloramphenicol were used, each at a concentration of 50 and 100 ppm. The required quantity of antibiotics was added to the larval rearing medium at every water change.

In all the experiments the larval density ws maintained at 2 numbers per ml of water. The growth of larvae in each experiment was recorded once in two days at every water change by measuring a random sample of fifty larvae. Live *Isochrysis galbana* was given to larvae as food at 5,000 cells/larva/day from straight hinge stage to umbo stage, 10,000 cells/larva/day from umbo stage to eyed stage and 15,000 cells/larva/ day till spatfall. The experiments commenced with 2-day old larvae and terminated on the day when the first spatfall was observed. The average water temperature in U.V. treated sea water and in the experiments on aeration varied from 28.2 to 31.2°C, salinity 36.04 to 36.66 ppt, pH 8.12 to 8.33 and dissolved oxygen 3.6 to 4.5 ml/l. In the study on antibiotic treatment the average water temperature fluctuated between 28.4 and 31.7°C, salinity 32.9 and 36.66 ppt, pH 8.11 and 8.12 and dissolved oxygen 3.6 and 5.6 ml/l.

## Results

### *Effects of aeration*

In the first experiment (Table 1, Expt. No. 1A) though the mean size on the day of the first spatfall (20th day) could not be recorded, the effect of aeration could be seen in the percentage of spatfall which was 15.9 % in the control and 0.6 % in the aerated medium. In experiment 1B on the day of spatfall the larvae attained a mean size of 206.5 µm in control and 108.7 µm in the respective treatments. A difference of 97.8 µm in growth and 30.7 % in spatfall was observed between the control and

TABLE 1. *Effect of aeration on larval growth and spatfall in* P. fucata

| Expt. No. | Treatment | Vol. of water (l) | Average growth of larvae in DVM (µm) on day | | | | | | Day of spatfall | Total spatfall | Spatfall (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | 6 | 10 | 14 | 17 | 20 | | | |
| 1A | Non-aerated | 5 | 60.7 | 67.2 | 85.9 | 137.5 | 161.5 | - | 20th | 1,592 | 15.90 |
| | Aerated | 5 | 60.7 | 71.6 | 87.8 | 105.1 | 109.1 | - | 20th | 59 | 0.60 |
| 1B | Non-aerated | 50 | 62.1 | 73.2 | 100.8 | 135.8 | 173.2 | 206.5 | 17th | 40,567 | 40.60 |
| | Aerated | 50 | 62.1 | 71.5 | 80.5 | 91.2 | 99.3 | 108.7 | 22nd | 9,880 | 9.90 |
| 1C | Non-aerated | 50 | 56.9 | 77.4 | - | 118.9 | 153.7 | 278.1 | 18th | 3,649 | 3.65 |
| | Aerated | 50 | 56.9 | 71.0 | - | 93.0 | 128.1 | 138.0 | 18th | 380 | 0.38 |
| 1D | Non-aerated | 500 | - | - | - | - | - | - | | 1,69,943 | 62.31 |
| | Aerated | 500 | - | - | - | - | - | - | | 1,02,777 | 37.69 |

aerated medium. In experiment 1C the larvae attained a mean size of 278.1μm in control and 138.0 μm in aerated medium on 18th day. A similar trend was observed in respect of spatfall which was 3.65 % in control and 0.38 % in the aerated condition. In experiment 1D though the growth data were not recorded, the percentage of spatfall indicated 62.3 % in control and 37.7 % in aerated medium. The pattern of larval growth in control and aerated medium is shown in Fig. 1 and 2 . In all these experiments the growth and percentage of spatfall were higher in non-aerated medium than in the aerated seawater.

### *Effects of ultraviolet treated seawater*

In the first experiment (Table 2, Expt. No. 2A) on the day of the first spatfall (20th day) the larvae showed a mean size of 206.5 μm in control and 196.0 μm in U.V. treated water. The percentage of spatfall was 40.6 and 36.9 in the respective treatments indicating 3.7 % lower settlement of spat in U.V.

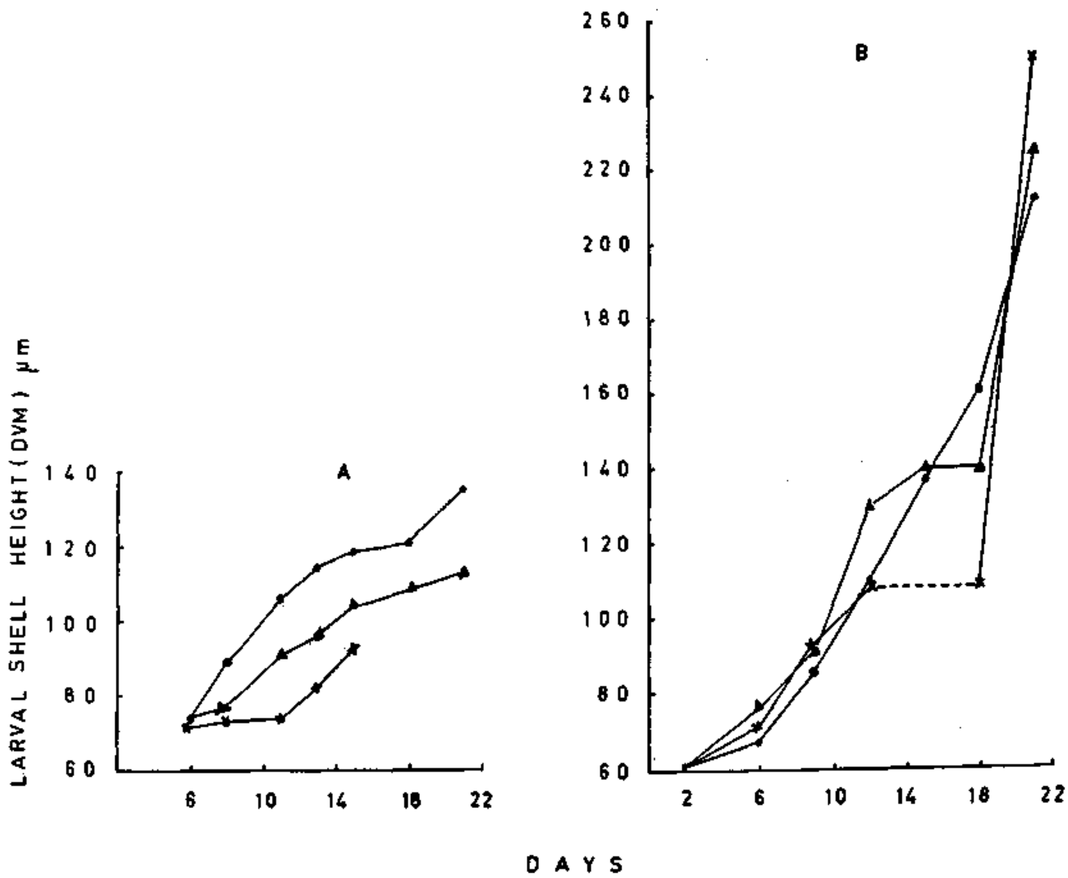


Fig. 1. Trend of growth of *Pinctada fucata* larvae under control (•—•); aerated medium (x—x) and U. V. treated water ( — ).

treated water. In experiment 2B, similar growth pattern and spatfall were obtained. On the day of the first spatfall the larvae in unsterilised seawater attained a mean size of 278.1 μm and in the U.V. treated water it was 164.0 μm. There was only slight difference in spatfall (3.65 % in control and 3.05 % in U.V. water). The results indicate slow larval growth and low percentage of spatfall in U.V. treated water when compared to unsterilised seawater (Figs. 1&2). However, the differences in growth and spatfall were marginal.

***Effects of antibiotics***

In the first experiment kanamycin, streptomycin and crys-4 were tested separately at a concentration of 50 and 100 ppm (Table 3). On the day of the first spatfall the mean growth of larvae was 104.7 μm in control, 115.3 and 139.0 μm in kanamycin, 112.0 and 120.8 μm in streptomycin and 128.0 and 129.7 μm in crys-4 at a concentration of 50 and 100 ppm respectively.

In the second experiment the mean

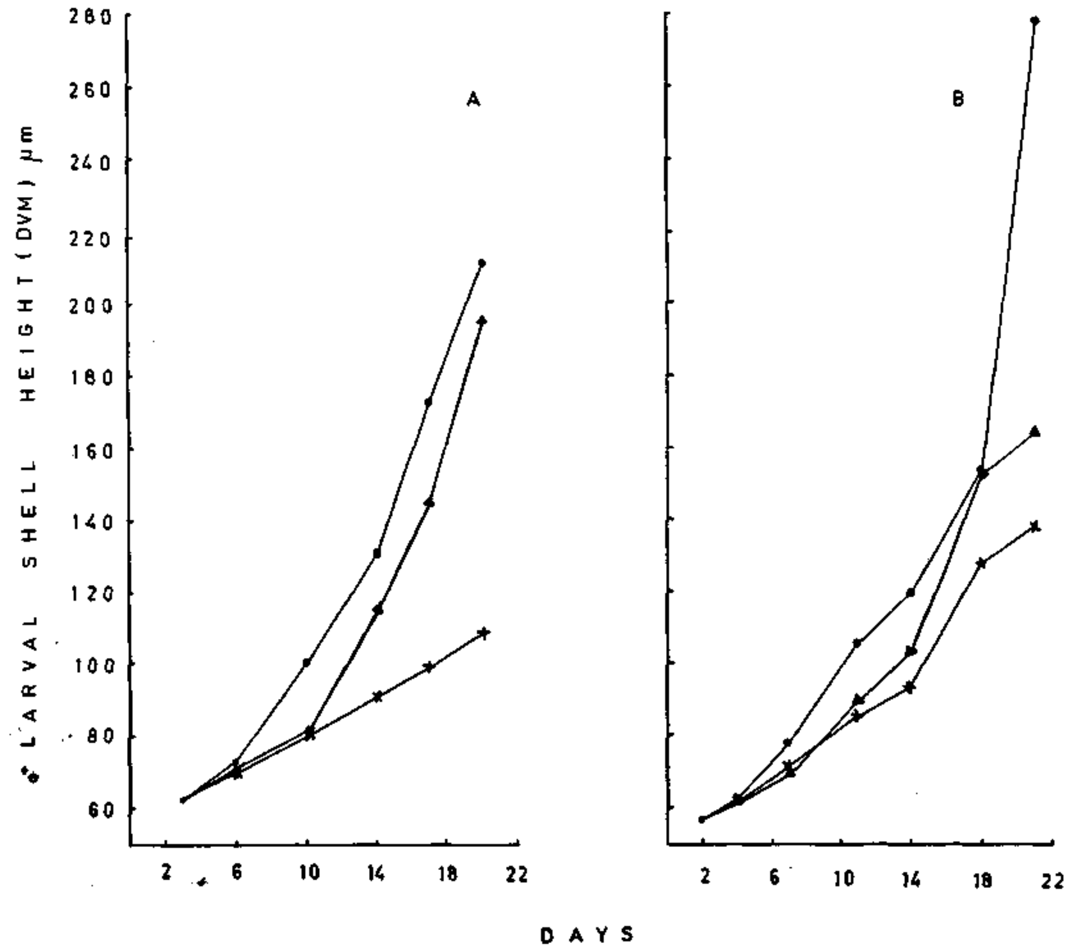


Fig. 2. Growth pattern of *Pinctada fucata* larvae under control (•—•); aerated medium (x—x) and U. V. treated water ( — ).

TABLE 2. *Effect of ultraviolet treated seawater on larval growth and spatfall in* P. fucata

| Expt. No. | Quality of seawater | Average growth of larvae in DVM (µm) on day | | | | | | | Day of spatfall | Total spatfall | Spatfall (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 10 | 12 | 14 | 17 | 20 | (No) | | |
| 2A | Unsterilised | 62.1 | 73.2 | 100.8 | - | 135.8 | 173.2 | 206.5 | 17th | 40,567 | 40.60 |
| | U.V. treated | 62.1 | 71.0 | 81.7 | - | 115.5 | 145.7 | 196.0 | 20th | 36,982 | 36.90 |
| 2B | Unsterilised | 56.9 | 77.4 | - | 105.3 | 118.9 | 153.7 | 278.1 | 20th | 3,649 | 3.65 |
| | U.V. treated | 56.9 | 68.6 | - | 89.7 | 102.8 | 153.1 | 164.0 | 20th | 3,045 | 3.05 |

growth of larvae on the day of the first spatfall was 201.0 µm in control, 197.4 and 201 µm in kanamycin, 204.1 and 192.8 µm in streptomycin, 200.5 and 197.1 µm in crys-4 and 145.2 and 157.8 µm in chloramphenicol at a concentration of 50 and 100 ppm, respectively. The percentage of spatfall was 34.9 % in control, 31.5 and 29.9 % in kanamycin, 42.0 and 28.4 % in streptomycin, 18.3 and 34. 5 % in crys-4 and 28.2 and 1.4 % in chloramphenicol in the respective concentrations (Table 3).

## Discussion

Mortality was common in larval phase even apparently under best conditions. Apart from the chemical factors of seawater, mechanical disturbance in the rearing medium plays a key role in larval development. The necessity for agitation in the rearing medium for a given species of larvae was determined based on their ecological adaptations. Edible oysters, mussels and clams inhabit shallow waters and therefore the larvae of these organisms were able to adjust to rough conditions. This was practically demonstrated in *Crassostrea madrasensis* (Nayar *et al.*, 1984), mussel *Perna indica* (Appukuttan *et al.*, 1988) and great clam *Meretrix meretrix* (Narasimham *et al.*, 1988) where agitation through aeration in the water medium was essential for better growth, survival and spatfall. The pearl oyster *Pinctada fucata* lives in deeper waters (15-20 m depth) on seabed where the disturbance in seawater is minimal when compared to shallow coastal waters. The larvae of this species were found to have adjusted to such conditions. Loosanoff and Davis (1963) demonstrated that the larvae of many bivalves could withstand vigorous mechanical disturbances. Settling of oyster larvae during hurricane indicated that strong water turbulance did not easily destroy larvae or seriously interfere with metamorphosis. In the present study aeration during larval phase adversely affected the growth and spatfall in *P. fucata.*

TABLE 3. *Effect of antibiotics on larval growth and spatfall in* P. fucata

| Expt. No. | Parameters | Control | Kanamycin 50-100ppm | Streptomycin 50-100ppm | Crys - 4 50-100ppm | Chloramphenicol 50-100ppm |
|---|---|---|---|---|---|---|
| 3A | Growth (µm)* | 104.7 | 115.3 - 139.0 | 112.0 - 120.8 | 128.0 - 129.7 | N - N |
| | Spatfall (%) | 0.01 | Nil - 0.034 | Nil - 0.033 | 0.029 - 0.063 | N - N |
| 3B | Growth (µm) | 201.0 | 197.4 - 201.0 | 204.1 - 192.8 | 200.5 - 197.1 | 145.2 - 157.8 |
| | Spatfall (%) | 34.9 | 31.5 - 29.0 | 42.0 - 28.4 | 18.3 - 34.5 | 28.2 - 1.4 |

* : Mean growth on the day of spatfall, N : No experiment conducted.

Sterilisation of seawater by ultraviolet rays was commonly practised. Loosanoff and Davis (1963) invariably used ultraviolet treated seawater in larval rearing at Milford Laboratory. Kelly (1961) demonstrated that U.V. irradiation of seawater was an effective method of destruction of coliform organisms. The U.V. treatment may affect organic material and destroy thiamine which were useful to bivalves (Armstrong *et al.*, 1966; Button, 1968). In the present study the use of U.V. treated seawater resulted in relatively lesser growth and spatfall in *P. fucata* than in unsterilised seawater. It may be mentioned that the sand filtered seawater used in the study contains nannoplankters of size less than 5 µm and the loss of this additional food material to the larvae in the U.V. treated medium would have possibly resulted in lesser growth and spatfall.

The antibiotics are used to control pathogenic bacteria in larval rearing systems. Walne (1958) reported that bacteria may seriously affect the growth of the larvae. Retardation of growth of larvae or mortality was reported in the presence of toxins released into the medium by the bacteria *Vibrio* sp. and *Pseudomonas* sp. (Guillard, 1959). Loosanoff and Davis (1963) recorded rapid growth of clam larvae in cultures containing about 100 ppm of streptomycin (or combistrep), about 33 ppm, of sulfamerazine (sulmet) and 3 ppm of aureomycin. Hidu and Tubiash (1963) reported that the formation of combistrep consisting dihydro streptomycin sulfate, streptomycin sulfate, phenol, sodium citrate, sodium disulphate, water etc., consistently resulted in 25-100 % increase in larval growth. In the present study the growth of larvae was faster in kanamycin at 100 ppm and in crys-4 at 100 ppm in experiment 1 and again in kanamycin at 100 ppm and in streptomycin at 50 ppm in experiment 2. In respect of spatfall, streptomycin at 50 ppm showed high spatfall (42 %) followed by crys-4 (34.5 %). Whenever there is any sign of bacterial contamination in the larval rearing medium, the application of above suggested dosage of antibiotics in the study may be resorted to.

The data of the two experiments indicate that the performance of kanamycin was better at 50 and 100 ppm concentrations in respect of growth and spatfall. Though the application of streptomycin and crys-4 resulted in good growth of larvae and spatfall there was mortality during the study. It is possible that the antibiotics play a role in enhancing the growth and spatfall. Detailed studies are needed to determine the correct dosages of antibiotics into the larval rearing medium to increase the growth and spatfall.

## Acknowledgments

The authors are grateful to Dr. M. Devaraj, Director, Central Marine Fisheries Research Institute for encouragements. Their sincere thanks are due to Dr. K.A. Narasimham, the retired Head of Molluscan Fisheries Division for critically going through the manuscript and suggesting improvements. They are thankful to Dr. K.K. Appukuttan, Head of Molluscan Fisheries Division for his valuable suggestions.

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