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N. G. Menon and P. P. Pillai
Central Marine Fisheries Research Institute, Cochin

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Studies on the remote setting of two commercially important Indian bivalves

U. Unnikrishnan, V. Kripa, K.S. Mohamed and T. S. Velayudhan
Central Marine Fisheries Research Institute, Kochi-682014

ABSTRACT

The concept of remote setting which involves transporting bivalve larvae, under moist condition and at 5-10°C lower than the atmospheric temperture, to distant areas for settlement was tried for the first time on two species of Indian bivalves. About 68% of the 'pediveliger' larvae of Indian backwater oyster, Crassostrea madrasensis *which were transported at 27±1 °C for 18 hours metamorphosed to settle as spat. The post-set survival rates were 66.2% to 73.4% in 30 ppt and 71.3% to 87.5% in 15 ppt salinity. Higher settlement rate and post-set survival was observed in larvae transported under low temperature than in atmospheric temperature. Transportation of late 'umbo' larvae of Indian pearl oyster* Pinctada fucata *gave high survival rate, but failed to metamorphose as spat.*

Introduction

During the initial stages of commercialisation of edible oyster culture, farmers were dependent exclusively on wild seed. With the expansion of oyster culture the seed requirement could not be met fully from the wild seed stock, which had seasonal and regional limitation. To cop up with demand, technology had developed for hatchery production seeds as early as 1890s. This has also paved the way for the expansion of oyster culture into new cultivable areas where no natural stocks were available. As the setting up of hatcheries and transportation of broodstock to these new areas were not feasible, attempts were made to transport their larvae and juvenile. Initially the set larvae (spat) on cultch were transported from hatchery to culture site. But this procedure which necessitated a large consignment space was uneconomical. Similarly the maintenance of larvae till the spat stage in the hatchery lead to the increase in seed price. Another alternative was to transport the larvae at swimming stages - from "D" to "pediveliger" - in seawater. However this resulted in larval mortalities due to deteioration of water quality. It was at this stage remote setting of spat was tried. Remote setting is the method by which eyed or pediveliger larvae are transported without water, in moist condition to distant places where they are made to set on the cultch material. By this method culturists can store and transport millions of larvae, which require a storage space of a matchbox.

Remote setting, which was first tested in the 1960s got recognition for its importance only in the mid 1970s. The concept of remote setting was first described by Jones and Jones in early '80s and Chew (1984) in his review on recent advances in the cultivation of molluscs, described remote setting as an "exciting technique" successfully employed by several oystermen in the U.S. Significant results were obtained in larval transport and distant setting of Pacific oyster *Crassostrea gigas* (Henderson, 1982), *Crassostrea virginica* (Gibbons, 1988) hard clam, *Mercenaria mercenaria* and the bay scallop, *Argopecten irradians* (Rhodes and Manzi, 1988). Pilot scale experiments with industrial collaboration were also done at the planting site to test the feasibility of supplementing wild seed with hatchery reared remote set spats (Castagna *et al.*, 1989).

To ensure good growth and survival of remote set spats, studies were focused on defining criteria for sighting natural nursery area (Roland *et al.*, 1989). Different aspects of remote setting, like, density (Baud *et al.*, 1991), cultch material (Bitaud and Herve 1991), cost of remote set spat (Holliday *et al.*, 1991, Thomas *et al.*, 1991; Thomas and Burnell, 1992) and economic assessment of setting on natural and artificial cultch (Langan *et al.*, 1997) were studied. In recent years demonstration programmes were scaled up to promote the technology of remote setting among oyster farmers (Meritt, 1998). In India commercial farming of the dominant species *Crassostrea madrasensis* using wild spat started from 1996 in the estuarine systems mainly along the southwest coast. However there are no commercial bivalve hatcheries, and the seed requirement for demonstration purposes and pearl culture are met from the shellfish hatchery of CMFRI. In edible oyster culture, the farmers collect spat by setting rens during the spat fall. The present work is an attempt to study the feasibilty of remote setting in two commercially important bivalves of India, the edible oyster *Crassostrea madrasensis* and the pearl oyster *Pinctada fucata*.

Materials and methods

The pediveliger larvae of *Crassostrea madrasensis* (Preston) and the umbo larvae of *Pinctada fucata* (Gould) produced in the shellfish hatchery at Tuticorin Research Centre of C.M.F.R.I were transported by road to Kochi, about 315 km away from Tuticorin. Transportation was done during the late hours to reduce stress and mortality due to the summer heat. The larvae were filtered through an 80 μ sieve, transferred into a 10 *l* beaker and the number estimated. The seawater with larvae was gently stirred to ensure homogenous distribution and then divided into 10 equal parts of 1 *l* each. For the experiment on remote setting, 1 *l* of larval suspension was filtered through the filter cone made of 20 × 20 cm size fine polyester cloth. After folding once, it was kept over a piece of cotton wetted with seawater. This was placed within a wide mouthed transparent plastic bottle of 250 *ml* capacity which was kept open throughout the transportation period. Eight such moist larval packages were made. Of the remaining larvae in 2 *l* of seawater, 1 *l* each were transferred into the oxygenated seawater in the double layered polythene bags which were placed in transparent buckets and covered. These packets with water kept under atmospheric temperature were treated as "Control" for the experiment .

Out of the eight moist packets for remote setting, four were transported under cool condition within an ice box at 27±1 °C. To maintain uniform temperature and avoid direct contact with the ice pieces, the chamber within the ice box was partitioned into a lower ice chamber and an upper storage/larval chamber by 2 cm thick perforated thermocol. The larval packets were kept above the thermocol. The remaining four moist larval packets for remote setting were kept within a bucket and transported under atmospheric temperature. At Kochi, the edible oyster larvae, transported under moist condition at atmospheric temperature (A) and at low temperature (B) were stocked in 15 and 30 ppt salinities (A-15 ppt, A-30 ppt, B-15 ppt and B-30 ppt) in 15 *l* polythene lined troughs while pearl oyster larvae were maintainted at 25 and 30 ppt (A-25 ppt, A-30 ppt, B-25 ppt and B-30 ppt), with a stocking rate of 4 larvae per *ml*. Water exchange was done daily till the settlement was completed. Controls of both the experiments were maintained at 30 ppt salinity. All the experiments were duplicated.

Standard larval rearing and nursery techniques for *Crassostrea madrasensis* (Nayar *et al.*, 1983; Utting and Spencer, 1991) and *Pinctada fucata* (Alagarswami *et al.*, 1983) were followed during the course of experiment. Up to their settlement the larvae were fed with pure cultures of *Isochrysis galbana* at a rate of 12000 cells per larva per day. During the later stages they were fed with a mixture of *Isochrysis* spp., *Nanochloropsis* spp. and *Chaetoceros* spp. in the equal proportions, at the same feeding rate as mentioned above. The same procedure was followed for Pearl oyster larvae till the closure of experiment.

Experiment 1. Activity of larvae a after transportation

The activity of larvae was observed at hourly intervals during the first 10 hours after transportation. During the subsequent days the activity was monitored once in the morning prior to water exchange and feeding till the completion of settlement. The activity of larvae was categorised into four, active, partially active, dead or empty and spat, based on the following characteristics:-

1. Active larvae: Actively swimming or crawling with well developed velum (eyed stage) or foot (pediveliger stage).
2. Partially active: Though the valves were closed the movements of respiratory apparatus could be seen through the transparent shell valves.Valves were rarely opened and therefore their movements, either by swimming or by crawling were much less.
3. Dead or empty shells: During the initial hours after transportation the dead shells were identified as those with no gill or body movements. Later, while observing the daily activity, most of the dead shells were found to be empty.
4. Spat: These were more flattened in appearance with prominent, irregular growth processes and valve margins. They were sedentary, but were active in feeding. Though the shell valves were more or less translucent, gill and other body movements were visible.

For the activity studies, 4 sub-samples of 1 *ml* each were taken from different points, pooled into an embryo cup and observed through microscope for counting. The sampling was done thrice to minimize the error.

Experiment 2. Settlement rate of larvae transported

Number of edible oyster spat settled per cultch per day was noted to find out settlement rate and also to find out the time required to complete the settlement. 1.2 mm thick P.V.C. pieces of 10 cm length and 6 cm breadth were used as cultch. A hole was drilled at the centre of each cultch to facilitate ren making. The cultch were washed repeatedly in fresh water to remove any noxious substances present on it. They were then aged by keeping them immersed in filtered seawater for at least two to three days. Two pieces of cultch were kept in each trough, which were replaced daily till the completion of settlement. Number of spat attached in each cultch, both inside and outside, were noted to determine the settlement rate. The cultch with spat were then hung on a nylon rope to form a ren which was tagged for identification. The rens were hung into 75 *l* F.R.P. tanks and reared in seawater of required salinity. Water exchange and feeding were done as per the standard procedure (Nayar *et al.*, 1983).

Experiment 3. Growth and survival rate

Once the settlement was over, the total number of spat in each trough were recorded seperately. The initial growth and survival rate were calculated from this data. There after, every 5th day growth and survival were noted.

Results

Remote setting of edible oyster larvae

The pediveliger larvae of edible oyster which were transported in the moist condition were healthy and showed velar movements when stocked in seawater. Details regarding the percentage of active larvae transported under low and atmospheric temperature is given in Table-1. The percentage of active larvae were low, ranging from 24 to 36 % during the 1st hour after stocking in seawater, while 62 % of the larvae in the control showed active velar movements.

Table 1. Percentage of active larvae of edible oyster in 15-30 ppt salinities during the first 10 hrs after transportation.

Hours	**1**	**2**	**3**	**4**	**7**	**8**	**9**	**10**
Control	62	41	42	43	35	28	27	28
A-15	32	14	15	18	62	45	45	48
A-30	32	17	18	20	43	43	45	35
B-15	24	33	43	47	60	66	65	77
B-30	36	44	47	52	66	68	66	67

After the first hour, the percentage of active larvae decreased and reached the lowest value of 28 % in the control. A reverse trend was observed in the larvae transported without water. Their activity gradually increased during the later hours, reaching 35 to 77 %, at the end of the 10th hour. This was higher than the percentage of active larvae in the control. Among the larvae transported under moist condition, those kept in low temperature showed higher activity (67 to 77 %) than the larvae transported under atmospheric temperature (35 to 48 %).

The larvae which were 19 days old at the time of transportation showed settlement on the next day of transportation, ie, on the 20th day. It was seen that the settlement rate in all treatments were very high during the 1st day after transportation. The settlement pattern during the first 10 days is graphically represented in Fig. 1. A-30 had the highest settlement rate, which was followed by B-15, control, B-30 and A-15 respectively. During the subsequent days the settlement rate in all treatments showed a decreasing trend except in A-15 in which high settlement was observed on 7th day.

Variation in percentage survival was shown by the edible oyster larvae after the remote setting. On the first day after transportation 100 % survival was observed in all treatments. In both the treatments A and B high survival rate was found in 30 ppt salinity. Among the larvae transported under moist condition, B-30 showed maximum survival (68

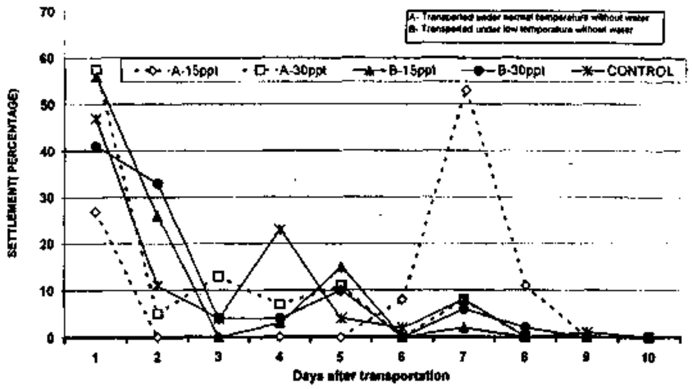

Fig.1. Settlement pattern of *C.madrasensis* larvae

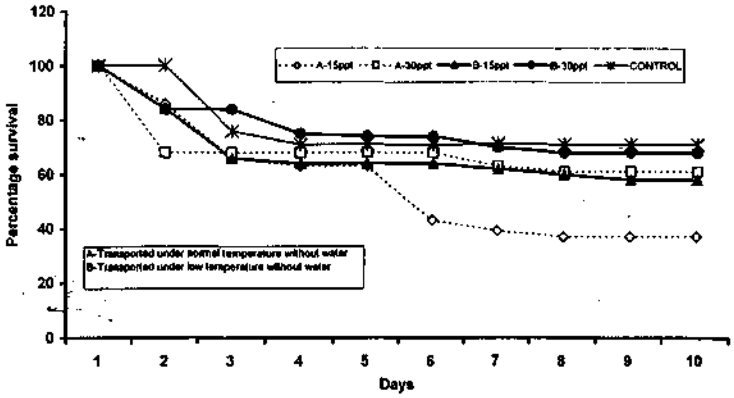

Fig.2. Percentage survival of edible oyster during the first 10 days after transportation

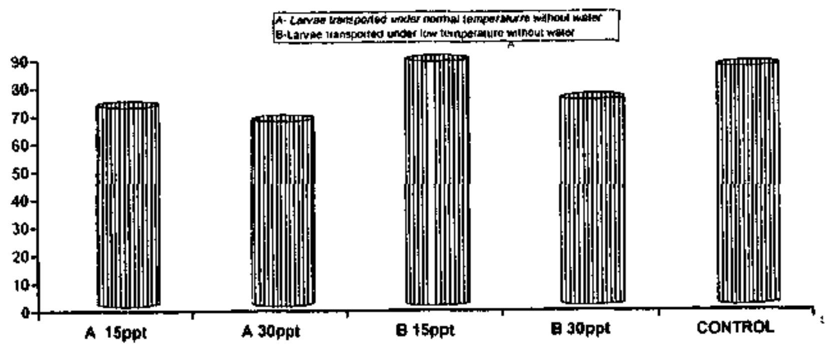

Fig.3. Percentage survival of remote set spat of *C. madrasensis* on the 25th day of rearing

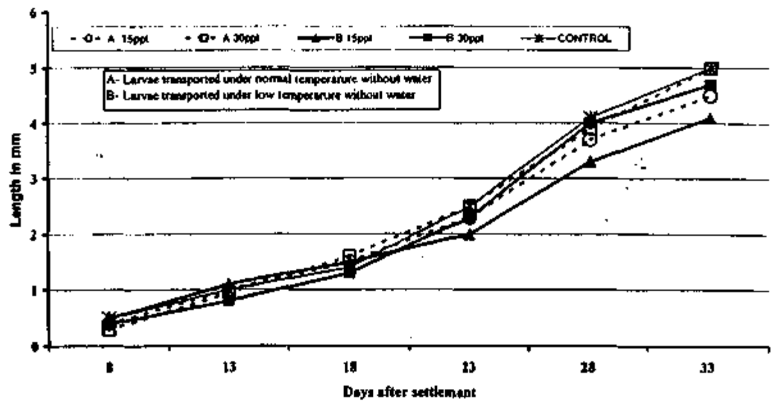

Fig. 4. Growth pattern of remote set spat of *C.madrasensis*

%) but was slightlty lower than that of control (70 %) on 10th day. Details regarding the survival pattern is depicted in Fig. 2. The treatments at 15 ppt showed lower survival rate. Lowest survival (37 %) was observed in larvae transported at atmospheric temperature and reared in 15 ppt.

The spat which had settled were reared in the respective salinities. On 25th day after settlement high survival rate ranging from 66.36 % to 87.59 % were observed in different treatments. The highest survival, 87.59 % was found in B-15, which was higher than the survival in "Control" (85.27 %). The lowest survival, 66.23 % was noted in A-30 (Fig.3).

From the growth rate of the first 33 days after settlement, it is evident that the spat maintained in A-30 treatment showed a growth rate almost equal to that of "Control". Slightly lesser growth rate was shown by B-30 and A-15 while B-15 was the lowest. (Fig. 4).

Remote setting of pearl oyster larvae

The pearl oyster larvae transported under moist condition, under the atmospheric temperature (A) and low temperature (B) were stocked in salinities 25 and 30 ppt after about 18 hours of transportation. The larvae showed more or less similar activity during the first few hours. The percentage of active larvae in different treatments is given in Fig. 5. During the later hours it is seen that greater activity was shown by the larvae transported at cold temperature. The peak activity in both the cases were found at 7th hour. Further, the activity was found to be lowered between 40 to 60 % in the 9th hour. The "Control" treatment showed more or less 100 % activity throughout the period of observation.

Hundred percentage survival was seen during the first 4 days after transportation. The survival of the pearl oyster larvae in different salinities is given in Fig. 6. On 6th day there was a steep decrease in survival in all treatments. Complete mortality was observed in A-25, B-25 and B-30 on 10th day, while 77 % and 40 % survival were observed in "Control" and A-30 respectively.

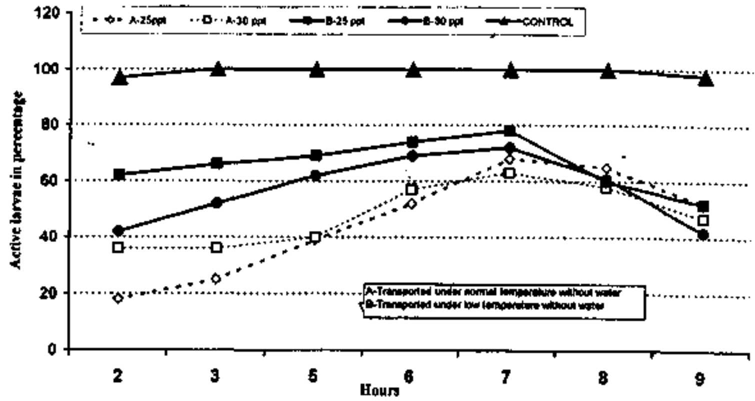

Fig.5. Percentage of active larvae of pearl oyster after transportation

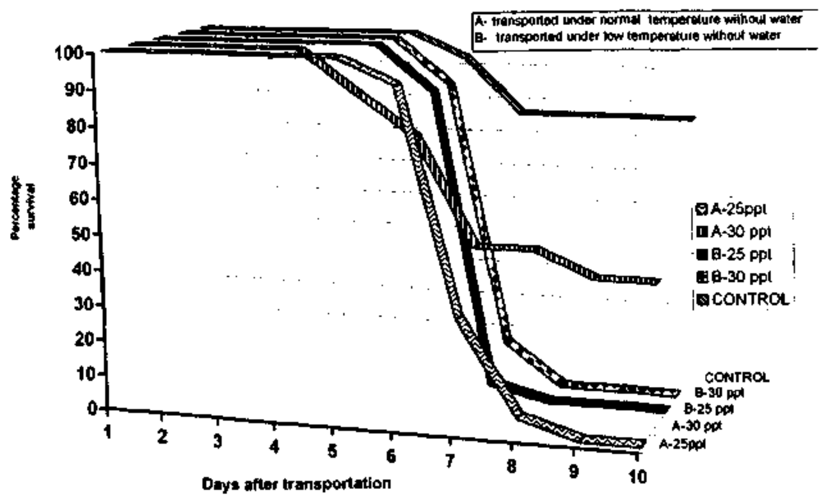

Fig. 6. Percentage survival of *P.fucata* larvae after transportation

Discussion

Remote setting, a recently developed technique to get hatchery-produced setting size pediveliger larvae shipped to near or distant locations for final settlement as seed on cultch materials has been tried successfully in many species of oyster (Chew, 1991). In the present study the concept of remote setting which was tested in the Indian edible oyster *C. madrasensis* gave encouraging results. The larvae transported under moist condition at two storage temperatures of 32±1 °C and 27±1 °C for

18 hours had showed 100 % survival after 24 hours.The settlement rate was 61 to 68 %, which was comparable to the settlement rates observed by Holliday *et al.* (1991) for *C. gigas*. Higher duration of storage up to 6 days in moist condition at 5 °C in a moist wrapping did not produce any depreciation in health, setting or juvenile growth abilities for *C. gigas* (Henderson, 1982).

Commercial settlement rates of 20-30 % were reported for larvae of Pacific oyster during the '80s (Henderson, 1982; Roland *et al*, 1989). For the same species Holliday *et al.* (1991) have reported 68 % set rate after a storage period of 98 hours at 6 °C. The high settlement rate was attributed to the fact that the larvae were fed live algae during the settlement. Low commercial settlement rates were noted when the larvae were unfed or fed on stored algal paste (Roland *et al.*, 1989).

Panggabean *et al.* (1989) investigated the feasibility of straight-hinge larval storage under a variety of test conditions and their subsequent performance. They found that 48 hours storage is feasible and larvae stored at 5 °C exhibited greater survival than those held at room temperature. In the present study also slightly higher settlement rate was observed in larvae transported under low temperatue than those held under room temperature. Holliday *et al.*(1991) observed that percentage of Sydney rock oyster, *Saccostrea commercialis* which set following a cold storage at 11 °C for up to 98 hours was excellent, with 77-85 % survival. These settlement rates are higher than those observed for *C. gigas* and *C. madrasensis*.

The post-set survival rates were high in 15 and 30 ppt salinities indicating that the larvae can be made to set and grow even in low salinities. The survival rates in 15 ppt salinity were as high as 87.50 % which hints the potential for remote setting in the estuarine regions of India. The growth of remote set spat is also comparable to that of larvae transported in water even after 33 days of culture period. This clearly indicates that remote setting does not harm the health and survival of *C. madrasensis* larvae and spat. Roland *et al.*(1989) observed that the proportion of larval setting was affected by circulation rate, temperature, salinity, cultch type and feeding rate. In the present study high settlement was observed when the circulation of water was increased by aeration.

The pearl oyster larvae transported under moist condition showed 100 % survival for 4 days upon exposure to proper rearing conditions.

However their settlement rate was negligible. The pearl oyster larvae were in the "umbo" stage and had not developed into "pediveliger" stage which may be one of the reason for low settlement rate. Survival rate after transportation has been found to depend on age or size of larvae in *C. gigas*.

Holliday *et al.* (1991) studied the use of shell length and eye spot diameter as criteria to decide when the oyster larvae will be ready for the remote settlement. They observed that faster growing Pacific oyster larvae had a significantly larger shell length. They have also indicated the appropriate measurements of shell length of Pacific oyster larvae and Sydney rock oyster larvae which will give high settlement rate. In the present study study the pearl oyster larvae had small shell length. Better results would have been achieved if larger pediveliger were used.

A direct correlation was observed between shipping duration and mortality of seed of hard clam, *Mercenaria mercenaria* and between the seed size and mortality of the seed of bay scallop, *Argopecten irradians* (Rhodes and Manzi, 1988). Greater mortalities occurred in the smaller sized bivalves shipped over the longest period of time. In the present study the duration was limited to 18 hours and this gave good survival rates. However in small size larvae of pearl oyster low settlement rates were noted.

The encouraging settlement rates and survival obtained for *Crassostrea madrasensis* larvae and spat indicate that the remote setting technique can be of considerable potential for this species. The oyster larvae ready to set can be transported to farm sites in distant areas from the hatchery location by means of the simple technique of remote setting and with relatively inexpensive and commercially available materials. The concept of "remote setting" which has revolutionised the oyster industry in temperate countries, can help in the development of oyster farming in India. Remote setting can be recognised as the key to solve the chronic need for the reliable and economical source of oyster seed. With remote setting oyster growers can purchase eyed oyster larvae from a hatchery and set them on the cultch in their own settlement tanks, where they can be fed with cultured algae until they are ready for planting. These techniques can be used in small or large scale undertakings.

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