STUDIES ON THE ECO BIOLOGY OF THE INDIAN PEARL OYSTER, *Pinctada fucata* (GOULD)

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By

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DEDICATED TO THE ALMIGHTY AND MY BELOVED PARENTS

CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON THE ECO - BIOLOGY OF THE INDIAN PEARL OYSTER, *Pinctada fucata* (GOULD)**" is an authentic record of research work carried out by **Shri. T. S. Velayudhan** under our supervision and guidance in the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfillment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology, and that no part there of has been presented before for the award of any other degree, diploma or associateship in any University

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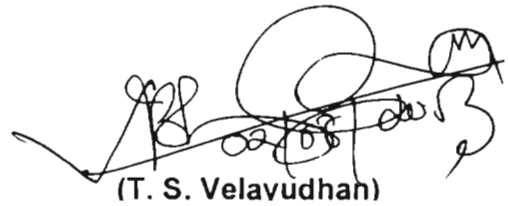
I, **Shri. T.S. Velayudhan**, do hereby declare that this thesis entitled **"Studies on the Eco - biology of the Indian Pearl Oyster, *Pinctada fucata* (GOULD)"** is a genuine record of research work done by me under the supervision and guidance of **Prof. (Dr.) N. R. Menon**, Director (Retired), School of Marine Sciences, Cochin University of Science and Technology, Cochin - 16 and has not been previously formed the basis of the award of any degree, diploma or associateship in any University.



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CHAPTER 1
GENERAL INTRODUCTION

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GENERAL INTRODUCTION

Aquaculture can be defined as culturing aquatic organisms and harvesting them for human benefits. An important branch of aquaculture is mariculture. Mariculture, the organized culture of marine organisms in the sea has a very long history. It is believed that the first attempted mariculture was that of molluscs. Japanese farmed oysters on intertidal stretches of the shore around 2000 B.C. Aristotle mentions the cultivation of oysters in Greece while Pliny gave details of Roman oyster farming from 100 B.C.

The total world Fish and Shellfish aquaculture production in 1999 was provisionally put at 32.9 million metric tonnes (mmt). In 1998 fish and shellfish through maricultute accounted for 35 % of the total aquaculture production with the molluscs contributing 9143 thousand tonnes, (47 %) valuing US $ 8479 million (FAO, 2000). Total food fish supply is growing at a rate of 3 – 6% per annum since 1961 and protein derived from fish, crustaceans and molluscs account for between 13.5 – 16.5 % of animal protein intake of the human population.

Pearl is very fascinating subject associated with nature's mystery. The pearl is formed in a living animal, it is counted as one among the nine gems and it has got a significant potential for economic development of the country. The Vedas, the bible and the Koran speak on pearls, giving them one of the highest places for it, associating with purity, virtue, chastity and wealth. The pearls are the ideal export commodity; they are non-perishable, shipping costs are negligible and lucrative markets are already established by the producers.

The important species of marine pearl oysters sea are *Pinctada maxima, P. margaritifera, P. m. galtsofei, P. mazatlanica, Pinctada fucata, Pinctada fucata martensii* and *P .radiata*. These oysters produce finest pearls. There are several other species such as *P. schemnitzii, P. sugillata, P. atropurpurea* and *P. anomioides* distributed and have only limited commercial

value with respect to pearl and pearl production. Winged pearl oyster *Pteria penguin, P. formosa, P. sterna, P. colymbus* produce pearls very rarely. Windowpane oyster *Placuna placenta* produce pearls of very small size. The sea mussel *Mytilus edulis*, green mussel, *Perna viridis* and *Perna indica* produce small seed pearls occasionally but these are not considered as gems. Several species of 'Giant clam *Tridacna* contain pearl formations of occasionally larger size, as big as a football, but they are porecellaneous without mother-of-pearl .The non nacreous but beautiful pearls are obtained from the Venerid clam *Merenaria mercenaria*. The abalone, *H. discus, H. gigantia, H. seiboldii, H. Kamtschatkana, H. rufescens* produce multi coloured irregular pearl with nacreous layers some times with strong iridescence especially red and green colour. Several other genera such as *Patella* (Rainbow limpet), *Trochus* (top shells). *Turbo* (turban shell) belonging to the nacreous secreting primitive order *Archeogastropoda, Littorina* (periwinkle), *Strombus* (conch), *Cassis* (helmet shell) belonging to the non-nacreous order Mesogastropoda; and *Murex* (murex), *Xancus* (sacred chank shell), *Melo* (bailor shell) belonging to the highest order Neogastropoda do produce pearls. Rare non-nacreous whitish cream pearl have also been reported from one species of Cephalopod - the *Nautilus pompilius*.

Black pearls produced from baick-lip pearl oyster *Pinctada margaritifera* are costlier and have colour ranges from black to gray. The white lip or silver lip pearl oyster *Pinctada maxima* produces larger pearls of size up to 22 mm in diameter. The golden, yellow, ash, pink, ivory and peacock colour pearls are produced by *Pinctada fucata* .The blue or green tinged pearls are produced by *Haliotis rufescens* and the *Strombus* sp. produces reddish or purplish coloured pearls. The fresh water pearls are generally called white pearls but pink, ivory ash coloured pearls are also produced by fresh water mussels. The golden tinged pearls produced by the pearl oysters in the Gulf of Mannar are the famous "oriental pearls". The white pearls produced by the fresh water mussels in European rivers are the "Occidental pearls". Now these pearls are named according to the area where it is produced such as Tahitian, French Polynesian, Caribbean, South Sea pearls, Chinese, Indian and Japanese. According to Tisdell and Bernard

(2000) from French Polynesia the Australian pearl now commands a much higher price, about US$ 180-200 per gram, compared to US$ 25-30 per gram for the Tahitian pearls.

At present world pearl trade accounts for US $ 479.0 million (Anon., 2000). The import of pearl from India was 0.17 US $ million in 1996 (Anon., 1996). By transfer of technology village level marine pearl production through direct involvement of small-scale fisheries has resulted in the production of pearls worth US $ 2178 during 1993 (Pillai *et al.*, 2000).

Indian waters have seven important species of pearl oysters among them *Pinctada fucata* (Gould) is the most important one. Pearl oysters have been exploited for their natural pearls from time immemorial. The Gulf of Mannar and Gulf of Kutch are the well-known haunts of this resource and pearl fisheries had been organized in the `paars' of Gulf of Mannar. Over a span of 300 years commencing from 1663, only 38 natural pearl fisheries have been conducted in the Gulf of Mannar. The last continuous fishery was held at Tuticorin from 1955-1961 and over 81 million oysters were fished which fetched revenue of 2.2 million rupees. In the Gulf of Kutch the last fishery was conducted in the year 1966-67.The fishery for natural pearls has been discontinued in the Gulf of Mannar due to scarcity of fishable pearl bearing adult pearl oysters.

Jameson (1901) stated on the identity and distribution of pearl oyster species of *Pinctada*.He opined that it is very difficult to separate one another owing to the absence of well marked diagnostic characters. For achieving satisfactory results in the production of cultured pearls, it is absolutely essential to have a precise knowledge of the morphology, and the anatomy of the animal. The growth and survival of the pearl oysters either in the natural beds or in the culture farms are strongly influenced by the environmental conditions and the productivity of the area. Hence a detailed understanding of the environmental conditions is a prerequisite to take necessary measures for the farming of pearl oysters. In the normal condition the pearl oysters reared in the farm are subjected to fouling and boring by

different organisms, which can affect the growth, survival and in turn indirectly affect the quality of pearl production.

Giese and Pearse (1974) have reviewed some of the factors that regulate the course of gonad development to maturation in bivalve molluscs. Seasonal gonad developments of molluscs restrict the spawning to a particular seasons of the year. Molluscs spawn naturally during certain seasons in a year when the environmental conditions are congenial for this activity. This physiological characteristic generally limits the process of seed production and availability, which is highly disadvantageous for the commercial culture of molluscan species. In this context, the concept of induced maturation gains importance as the process can be advantageously controlled for a prolonged period of seed production. For any successful hatchery technology, the most important aspect is the complete control of the life cycle of the candidate species under captivity. The most important step for this is the successful induction of maturity in captivity. In the present study induced maturation refers to the accelerated gonadal 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. Induction of maturation of pearl oysters out of the spawning season will allow the hatchery to function and produce spat throughout the year. Larval rearing protocols and subsequent growth of oysters help us to design a foolproof hatchery technology for *P. fucata*.

The present work aims to study induced maturation of the pearl oyster for induced spawning experiments. The work on larval development was done with a view to developing techniques for the artificial rearing of commercially important pearl oyster *P.fucata*, and also to elucidate the principles and problems of tropical bivalve larvae in general for detailed investigations in the future.

Larval rearing is also very important for the success of any hatchery. As the number of larvae that ultimately settle as spat is very crucial for financial viability of ant oyster culture. For optimization of the conditions

for healthy larval growth, it is necessary to know the various factors, which influence larval life under controlled hatchery conditions.

The present study is designed to probe into the details of the basic aspects of the biology related to the hatchery technology of *Pinctada fucata* and the understanding of the factors which influence induction of maturation, spawning, larval rearing and spat settlement. This would go a long way in the upgradation of hatchery technology of the Indian Pearl oyster *Pinctada fucata for* a commercial level seed production.

Studying morphology and anatomy gives us a better understanding of the species. We can predict the spawning seasons and plan our hatchery work according to that. To overcome the postoperative mortality rejection of inserted nucleus, malformed pearls etc; a study of the morphology and anatomy of pearl oyster is very important. The pearl-sac, which is responsible for the formation of cultured pearl, is derived from the mantle tissue. Hence, some basic studies were carried out on the structure of mantle, its histology, growth of the grafted mantle and formation of pearl- sac in the Indian pearl oyster, *Pinctada fucata*.

The information gathered on the above aspects is presented under four chapters. Each chapter contains an introduction, review of literature, materials and methods, results and discussion.

CHAPTER 2

TAXONOMIC STATUS AND ECOLOGY

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TAXONOMIC STATUS AND ECOLOGY

2.1 INTRODUCTION

Pearl oysters that produce pearls comes under the genus *Pinctada* and the most important species under this genus are *Pinctada maxima, P. margaritifera, P. m. galtsofei, P. mazatlanica, P. fucata* and *P .radiata*. These oysters produce finest pearls and there are several other species such as *P. chemnitzii, P. sugillata, P. atropurpurea* and *P. anomioides* which are not available in fishable quantity with less commercial value in respect of pearl and pearl production. Winged pearl oyster *Pteria penguin, P. formosa, P. sterna* and *P. colymbus* produce pearls very rarely while window pane oyster *Placuna placenta* produce seed pearls of very small size (Shirai, 1994).

Since the pearl oysters produce pearls of different colours and shape. The study on the taxonomy, distribution, abundance and dimensional variations among species are most important.

This chapter deals with, Taxonomic status and distribution of *Pinctada fucata* (Gould); ecological conditions of the environment inhabited by the oyster and information on the important foulers.

2.1.1 Taxonomic Status of Pinctada *fucata* (Gould)

Out of the twenty eight species of pearl oysters that are known from the different parts of the world, seven species inhabit the Indian waters.They are *Pinctada fucata* (Gould), *Pinctada margaritifera* (Linnaeus), *P.chemnitzii* (Philippi), *P. sugillata* (Reeve), *P. anomioides* (Reeve) and *P. atropurpurea* (Dunker), *Pteria penguin* (Jameson) (Plates 2.1 – 2.5). The important works on the taxonomy of Indian pearl oysters are those of Prashad (1932), Hynd (1955), Rao (1970), Rao and Rao (1974) and Velayudhan and Gandhi (1987). Recently Gervis & Sims (1992) reviewed the taxonomic status of the various species of *Pinctada*.

Pearl oysters are cosmopolitan in distribution Hynd (1955), Alagarswami (1991), Gervis and Sims (1992) and Shirai (1994).

A few studies have been made on the physico chemical parameters of the pearl oyster beds and the pearl farms from India (Kelaart, 1859; Herdman, 1903-1906; Hornell, 1905, 1913,1916, 1922) Sewell, 1927; Malpas, 1929; Chidambaram *et al.*, 1951; Chacko *et al.*, 1954; Jayaraman, 1954; Ganapathy and Murthy, 1955; Prashad, 1957; Varma, 1960; Malupillay, 1962 a, b; Freda Chandrasekharan *et al.*, 1967 a, b; Mahadevan and Nayar, 1967, 1973,1974, 1976; Alagarswami, 1970; Easterson and Mahadevan, 1980; Victor, 1983; Victor and Velayudhan, 1987).

In the normal condition the pearl oysters, which are reared in the farm, are subjected to fouling and boring by different organisms. Fouling is a menace to the oysters as reported by Herdman (1903, 1905 a, b), Hornell (1916), Kurian (1950), Daniel (1956), Antony Raja (1959), Ananthanarayanan (1967), Alagarswami and Chellam (1976), Renganathan *et al.* (1982), Chellam *et al.* (1983), Dharmaraj and Chellam (1983), Velayudhan (1983, 1988), Appukuttan (1987), Dharmaraj *et al.* (1987), Victor and Velayudhan (1987) Dev and Muthuraman (1988).

Studies have also been made in other pearl farming countries on the different aspects of fouling of cultured pearl oysters and cages etc (Shipley and Hornell, 1906; Takemura and Takashi, 1955; Nishii, 1961; Nishii *et al.*, 1961; Mizumoto, 1964; Yamamura *et al.*, 1969; Wada, 1973 b; Arakawa, 1980; Haws *et al.*, 1995; Doroudi, 1996; Taylor *et al.*, 1997).

2.2 MATERIALS AND METHODS

2.2.1 Study and Sampling Areas

Studies on the taxonomy, ecology and fouling pearl oysters were carried out at **Tuticorin** waters (TRC) and the farm site at the Tuticorin harbour area (Figure 2.1, 2.2) (8 ° 45 ' N latitude and 78 ° 12 ' E longitude) located in Tamil Nadu on the south east coast of India. Materials for taxonomic studies were collected from **Okha** in Gujarat (22 ° 28 ' N latitude and 69 ° 05 ' E longitude), **Vizhinjam** (8 ° 22 'N latitude and 76 ° 59 ' E longitude) in Kerala and **Mandapam** (9 ° 16 ' N latitude and 79 ° 12 ' E longitude) in Tamil Nadu (Plate 2.6, Figure 2.1).

2.2.2 Taxonomy

Taxonomic status of the species was studied as per the procedures of Hynd (1955), Rao and Rao (1974) and Velayudhan and Gandhi (1987). Samples collected from the study areas were cleaned and examined live or preserved in 5 % formalin. Thirty pearl oysters each from Gujarat, Vizhinjam, Tuticorin and Mandapam were examined to study the taxonomic status of *Pinctada fucata* from Indian waters. Though the oysters were sampled irrespective of their sizes, only pearl oysters of the size 70 mm length (DVM) were selected from each of the localities for the comparative study of the shell characters. The specimens from Gujarat were the largest sampled from India. Dimensional relationship of farm grown oysters in Tuticorin and natural and hatchery produced spat were also studied to find out whether any differences in the shell characters and other dimensional parameters existed (Figure 2.3 & 2.4). Anatomical features such as structure of the anal papilla, folds of the digestive system and the structure of the nervous system were studied in detail. The hardness of the shells was graded as hard, medium hard and brittle according to the breaking capacity of the shell and oysters. The periostracum colour was given as normal if the periostracum was reddish brown on a pale yellow background.

2.2.3 Ecology of Pearl Oyster Farm

Monthly water samples (August 1998 – July 1999) from the Tuticorin Harbour Pearl Oyster farm was taken and the water quality parameters like temperature, salinity, dissolved oxygen, pH, transparency, silt, SiO_3-S, PO_4 -P, calcium, magnesium, chlorophyll and primary productivity were estimated following procedures of Strickland and Parsons (1972) and APHA (1985). The results are tabulated and given in graphs and tables.

2.2.4 Fouling in the Pearl Oyster Farm

The period of the field experiments was for 12 months extending from August 1998 to July 1999.

In this study four sets each containing five ground glass panels (Plate 2.10A) of size 20 cm x 20 cm x 5 mm (surface area –800cm²) with 5 mm dia, holes at 4 corners tied with 4 mm synthetic rope were fixed at depths of 1, 2, 3, 4 and 5 m. A total of 20 panels were suspended horizontally. Of this 10 panels (2 replications for each depth) were suspended in the shaded area of the raft with bamboo mat and the remaining 10 numbers (2 replications for each depth) were suspended in the lighted area of the same raft moored at 5 - 7 m depth in the harbour basin. The half a portion of the raft was covered with bamboo mat to avoid sunlight falling on the area with a view to studying the effect of illumination on the settlement of barnacle and other related organisms in the pearl oyster farm. The partition of the raft was done in east west direction to get equal amount of sun light on both the shaded and non-shaded portion of the raft. A total of 240 panels (120 numbers each for shaded and non-shaded treatments) were suspended during the study period.

For counting the fouling organisms a metal frame of grid size 20 x 20 cm partitioned into 4 cm x 5 cm rectangular sub areas was used. This frame was placed over the panel and the total count of barnacles and other foulers in each randomly selected sub area were noted by using the random tables. From the weight of foulers the intensity of their occurrence on the panels was determined. The total weight and numbers of barnacles as well as the total weight of other fouling organisms for each depth in each month (from August

1998- July 1999) were noted separately and calculated for surface area growth. In this study as the majority and the bulk of fouling organisms were barnacles and all the other fouling organisms were insignificant in numbers and weight, these were pooled together and taken as the total other fouling organisms and calculated for each depth and month.

To study the effect of fouling on pearl oysters simultaneously at different depths, 5 frame nets in duplicates of size 65 x 45 cm made of 5 mm diameter iron rods each with 5 compartments were used (Plate 2.10B). A total of 50 pearl oysters of size 45-50 mm in DVM (dorso ventral measurement) were arranged in 5 rows in the each frame net and suspended vertically from the raft at one metre intervals upto 5 m depth. These oysters were observed for the intensity of fouling on them. The mortality of oysters if any was also recorded during the study.

2.2.5 Statistical Analyses

One way Analysis of Variance (ANOVA) using Microsoft Excel computer software was also done for finding out whether there is any significant ($p < 0.05$) morphometric measurements variations between populations of P. *fucata* from different regions of India.

A randomized complete block design three way factorial Analysis of Variance (ANOVA) using MSTAT computer software was done for finding out whether there is any difference ($p < 0.05$) between the effect of shading and no shading, depth 1-5 m and seasonal variation for barnacle fouling.

If the F value any of the treatments were significant then of the treatments were significantly different, then the best treatment was found out through pair wise (Students t – test ($p < 0.05$)) comparison of treatment means using Critical Difference (CD).

Correlation was also done and correlation coefficient (r^2) was calculated to find out whether there is any correlation between the water quality, barnacle fouling, barnacle weight, and other fouling organisms of

oysters. A similar correlation was also conducted to find out the relationships between depth and mortality of oysters, duration and fouling intensity.

2.3 RESULTS

2.3.1 Taxonomic Status of *Pinctada fucata* (Gould)

Pearl oysters of the family Pteriidae are commercially utilized throughout the world. The two recognized genera *Pinctada* and *Pteria* occupy a taxonomic position within this family (Gervis and Sims, 1992).

Phylum:	Mollusca
Class:	Bivalvia
Subclass:	Pterimorphia (Suzuki, 1985)
Order:	Pterioida (Suzuki, 1985) Mytiloida (Richard, 1985)
Sub-order:	Pteriaceae
Family:	Pteriidae
Genus:	*Pinctada*
Species:	*fucata* (Gould) 1850

***Pinctada fucata* (Gould) 1850**

"Die perlen mutter muschell", Chemnitz, 1785,"Neues Systematisches Conchylien-Cabinet."8. (Nurenberg.) p. 126, pl. 80, fig. 717

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Avicula fucata Gould, 1850, Proc. Boston Soc. Nat. Hist.3: 309-12, p. 309; 1852, United States Exploring Expedition -1838- 48."12, p.441, figs. 551, 551 a.

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A. occa Reeve, 1857, Conchologia Iconica." 10.Monograph of the

B. genus Avicula (London), pl. 8, fig. 24.

A. lacunata Reeve, 1857, Conchologia Iconica." 10.Monograph of the genus *Avicula* (London), pl. 10, fig. 29.

A. aerata Reeve, 1857, Conchologia Iconica." 10.Monograph of the genus *Avicula* (London), pl. 10, fig. 32.

A. fucata Reeve, 1857, Conchologia Iconica." 10.Monograph of the genus *Avicula* (London), pl. 17, fig. 74.

Meleagrina aerata Paetel, 1890,"Catalog der Conchylien –Sammlung ".3.(Paetel Bros . : Berlin.) p. 204.

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2.3.1.1 Description

Shell

The shell features of animals observed from different regions of the Indian coast are given in Table 1. The hinge line is fairly long, its ratio to the broadest region of the body (APM) of the shell is about 0.88 and its ratio to the longest dorso-ventral-measurement (DVM) is about 0.80. In both the valves there are hinge teeth, one each at the anterior and posterior end of the ligament. The shell has at its edge 6-8 radial bands of reddish brown colour on a pale yellow background .The shell grows by producing imbricating lamellae at the distal border the gaps getting filled up gradually. The non-nacreous border on the inner face of the valves possesses brownish or reddish patches coinciding with the external rays. The nacreous areas of the ears relative to the rest of the shell decrease with age slightly.

In very rare cases the pearl oysters collected from Mandapam, Tuticorin, Vizhinjam and Gujarat a single growth process was found arising as a band extending from umbo to the margins of both valves, which was of green, black, white, cream, red and yellow in colour (Plate 2.8) In stray occurrences pearl oysters collected from the natural beds and farms had completely green coloured periostracum.

The spat collected from the natural beds and those produced in the hatchery and grown in the farm had prominent growth processes till they attained 6-9 months of age animal are more than One year or more old, the animals had growth processes eroded from the umbo region towards the margin of the shell and in due course the growth processes get recessed and are seen only at the shell margin (Plate 2.7).

Immediately below the hinge line, a sinus indents the posterior border. This is known as the posterior ear. The sinus is deep in the posterior ear. The posterior ear is relatively large. The byssal groove is short and forwardly curved depression on the external surface of the right valve, running from the umbo to the byssal notch.

The large adductor impression is placed sub centrally occupying 1/3 to ½ of the diameter of the shell. The pallial line and scars caused by the insertion of the pallial muscles are fan shaped bundles, formed of outwardly radiating fibres. Usually there are 12-15 insertion scars between the umbonal regions. Besides these distinct scars, there is a narrow continuous insertion band confluent with the posterior and ventral edges of the adductor scars. This scar merges with that of the adductor scar. The hinge line is a narrow edge and runs along the greater part of the straight dorsal edge. Elongated narrow ridge like lateral teeth are present as paired deviation of the nacre of the hinge line, posterior to the ligament.

In the older specimens it was observed along hinge line where the shell edges meet. The gap between the two shells (outer edges) increases as a function of age. This phenomenon was not observed in young oysters.

Observations in this study showed that specimens from Gujarat had maximum dimension (Plate 2.9). The measurements were as follows DVM 92.11 mm, APM (84.24 mm), hinge length (HL) of 75.68 mm, thickness (depth) of 25.94 mm and weight (45.2 g) than other centres. The colouration of the shell was different. The oysters of Gujarat had thicker nacre / periostracum/ shell thickness than that of other places.

The data on the morphometric shell characters of *P. fucata* from the different regions of India are given in Table 2.1; colour pattern of the shell is given in Table 2.2.

Statistical analysis of the data using One Way ANOVA ($p < 0.05$) showed the following results between the shell characters of *P. fucata* from different regions of India.

There was no significant difference between the treatments in the characters of adductor muscle width, lower shell thickness "A" and "B" (Table 2.7, 2.12, 2.13).

On examination of the thickness (depth) of the shell (Table 2.4), There was no significant difference ($p < 0.05$) between the thickness of pearl oysters from Gujarat, Tuticorin and Vizhinjam, but thickens of Mandapam oysters was significantly less ($p < 0.05$) than that of oysters from other areas (Table 2.4).

However, the thickness of the upper shell was significantly different in the case of oysters from different regions ($p < 0.05$) (Table 10). In the upper shell thickness "A" and "B" Gujarat oysters differed significantly ($p < 0.05$) from all the others. Oysters from Vizhinjam and Mandapam areas did not register any difference in the upper shell thickness "A" and "B" (Table 2.10).

Both in the case of hinge length and APM, though significant difference ($p < 0.05$) was noted between the oysters from the different centres (Table 2.3, 2.5). In the case of the adductor muscle length, only oysters from Mandapam differed with that of the other areas (Table2.6).

Studies on the digestive system and nervous system (detailed information given in Chapter IV) showed that the anal papillae of *P. fucata* are trifold and the renogenital aperture not prominent only during spawning. In the case of the nervous system, there were four nerves, which enervate from the pedal ganglion.

2.3.1.2 Distribution

Pearl oysters enjoy cosmopolitan distribution occurring in all the areas of tropical and subtropical belt from the Tropic of Cancer to the Tropic of Capricorn. Pearl oysters are truly marine forms and do not occur in estuaries or backwaters. The number of species decreases across the Pacific. However in certain areas the distribution extends outside this limit as in the case of Japan oyster* where Pinctada *fucata* var *martensii*. The northern limits are determined by the warm currents ' Kuroshio' and 'Tsushima ' where the coastal water temperature is between 25 - 30 °C.

***Pinctada fucata* (Gould)** has a wide distribution from Western Pacific Oceania (Korea and southern China), Australia, Indian Ocean to the Red sea and the Persian Gulf, with Lessepian (migrants through the Suez Canal) into the Mediterranean. The subspecies *Pinctada fucata martensii* is a temperate variety and is found only in Japan (Alagarswami, 1991; Gervis and Sims, 1992).

In the Indian region *Pinctada fucata* occurs in the Gulf of Mannar (off the coasts of Tamil Nadu in India and Sri Lanka) Andaman & Nicobar Islands, Gulf of Kutch (coast of Gujarat), south west coast of India (Kerala) and Lakshadweep Islands (Figure 2.1).

2.3.1.3 Ecology of Pearl Oyster Farm

The ecological condition of the pearl oyster farms situated in the Gulf of Mannar and the Tuticorin bays were studied. The general hydrological condition observed and the distributions of some essential elements are presented in this section.

2.3.1.3.1 Transparency

The average clarity /transparency of the pearl oyster farm was upto a depth of 1.406 m during the whole period. The seasonal pattern of clarity /transparency in the pearl oyster farm for both the surface and bottom waters is given in the Figure 2.5. Maximum precipitation observed upto 2.45 m during the month of October 1998 with a minimum 1.033 m in February 1999 (Figure 2.5). At a depth range of 6.00 -6.75 m the light penetration was up to 2. 45 m (Figure 2.5). Silt showed a peak (46.19 g) at 3 m in September1998. The seasonal and depth wise silt deposition in both non shaded and shaded panels is given in the Figures.2.25, 2.26.

2.3.1.3.2 Temperature

The temperature of the waters of the oyster farm was sampled for the period from August 1998 to July 1999. The temperature range between 26.15° C to 31.0° C. marginal differences were seen between the surface and bottom temperatures. There was difference between temperature of the bay and the oyster farm (Figure 2.6).

2.3.1.3.3 Salinity

The yearly mean surface salinity from August 1998 to July 1999 was 33.67 ‰ and at the bottom 33.65 ‰. In the Harbour pearl culture farm, it varied from a minimum of 29.97 ‰ (March 1999) to a maximum of 36.25 ‰ (September, 1998) for the surface water while, the corresponding minimum and maximum values for the bottom water was 29.97 ‰ (March 1999) and 36.5‰ (September 1998) respectively. The seasonal pattern of salinity in the pearl oyster farm for both the surface and bottom waters is given in the Figure 2.7.

2.3.1.3.4 Dissolved Oxygen

The surface waters of Tuticorin harbour were rich in dissolved oxygen throughout the study period. The seasonal pattern of dissolved oxygen in the pearl oyster farm for both the surface and bottom waters is given in the Figure 2.9. The average volume of dissolved oxygen content in

the surface water of pearl oyster farm was 4.827 ml l^{-1} and 4.989 ml l^{-1} for the bottom water respectively during the study period. The minimum mean dissolved oxygen in the surface water of the farm was 3.96 ml l^{-1} (April 1999) to a maximum of 5.57 ml l^{-1} in August 1998, while, the corresponding minimum and maximum values for the bottom water was 4.06 ml l^{-1} (April 1999) and 6.25 ml l^{-1} (February 1999) (Figure 2.9).

2.3.1.3.5 pH

The average pH of the surface water was 8.17 and for bottom water it was 8. 16. The average surface water pH of the pearl culture farm showed a minimum of 8.1 (December 1998) and a maximum of 8.26 (July 1999) (Figure 2.8). The same trend was shown by the bottom water with a minimum 8.1 in December (1998) and a maximum of 8.24 in October 1998 (Figure 2.8).

2.3.1.3.6 Chlorophyll

The chlorophyll values indicate a high seasonal variability. The average chlorophyll value for the surface water was 2.060 g /m^3 while it was 0.80 g /m^3 for the bottom water respectively during the study period (Figure 2.10).

2.3.1.3.7 Primary Productivity

The mean monthly values of gross productivity of the surface water showed a minimum of 0.220 g C /m^3 /12 hours day (April 1999) and maximum 2.244 g C /m^3/ 12 hours day (November 1998). The corresponding minimum and maximum values for the bottom water was of 0.176 g C /m^3 /12 hours day (July 1999) and 2.153 g C /m^3/ 12 hours day (November 1998) respectively (Figure 2.3.8). The average productivity of the surface water was 1.043 g C/m^3/12 hours day and 0.570 gC/m^3/12 hour's day for the bottom water (Figure 2.11) during the study period at Tuticorin harbour farm (Figure 2.11).

2.3.1.3.8 Phosphate

The phosphate content did not show any seasonal variation. The monthly mean phosphate content in the surface water recorded a minimum of

0.480 µg at PO_4^- P l^{-1} (April 1999) and a maximum of 2.4 µg at $PO4^-$ P l^{-1} (December 1998) where as it was 0.4 µg at $PO4^-$ P l^{-1} during April 1999 and 3.0 µg at $PO4^-$ P l^{-1} during December 1998 at the bottom (Figure 2.12).

2.3.1.3.9 Silicate

The monthly mean silicate content in the surface water had a minimum of 1.00 µg at SiO_3-S l^{-1} (October 1998) with a maximum of 15.255 µg at SiO_3-S l^{-1} (August 1998). The bottom water showed a minimum of 1.083 µg at Si SiO_3-S l^{-1} (October 1998) and maximum of 12.613 µg at SiO3-S l^{-1} (August 1998) (Figure 2.13).

2.3.1.3.10 Calcium

The monthly mean calcium content in the surface water recorded a minimum of 0.350 g l^{-1} (June 1999) with a maximum of 0.446 g l^{-1} (July 1999). The bottom water showed a minimum of 0.357 g l^{-1} (June 1999) and maximum of 0.451 g l^{-1} (October 1998) (Figure 2.14).

2.3.1.3.11 Magnesium

The monthly mean magnesium content in the surface water had a minimum of 1.224 g l^{-1} (June 1999) with a maximum of 1.336 g l^{-1} (August 1998). The bottom water showed a minimum of 1.221 g l^{-1} (September 1998) and maximum of 1.562 g l^{-1} (July 1999) (Figure 2.15).

Analysis of correlation coefficient between water quality parameters revealed a strong direct correlation between primary productivity, dissolved oxygen, chlorophyll, silicate and period (Table 2.15).

2.4 Studies on the Fouling Community of the Farm

Fouling of oysters, cages, and materials used for the settlement of spat, different types of cultches used in pearl oyster farming normally get heavily settled by an aberrant group of marine organisms normally referred to as fouling animals.

Various groups of invertebrates are found in the fouling community although hydrozoans, polychaetes, bryozoans, cirripids and

mollusks are the major offenders. Relative settlement pattern indicated that these organisms are found on the non shaded part of the panel and that the intensity of settlement decrease with depth (Plate 2.11A, Figure 2.17 & 2.18).

Among the groups of animals settled, barnacle was the most dominant one. A list of species, which were recorded from the fouling community, is given in the Table 2.14.

Silting of panels also recorded seasonal trend. The details are given in the Figures 2.25 & 2.26

The rate of mortality of pearl oyster was found to be controlled by the intensity of fouling. The release of live oysters kept in cages for fouling studies resulted in settlement of foulers on the oyster shell, which eventually lead to high mortality rates (Figure 2.27 &2.28). Curiously enough shallow depths in the farm recorded heavy fouling. The intensity of fouling was less at 5 m (Figure 2.27 & 2.28).

Analysis of variance showed that there were both temporal and spatial variations in barnacle settlement. Similarly there were significant correlation in barnacle fouling with reference to number, weight and shades (Table 2.16 & 2.17). Three factor analysis of variance revealed that there is significant difference ($p < 0.01$) in total weight of barnacles set in different months and also at different depths. At two different shades also, the total weight of barnacles set were found to be significantly different ($p < 0.05$). All the interactions between, seasons, depth and shading were also significant ($p < 0.01$) (Table 2.16 &2.17).

Heavy silting of panels reduced fouling. It is likely that smothering of fresh settled larvae and lack of suitable and stable foot hold during the process of metamorphosis and settlement of the majority of positively geotropic larvae of the major fouling communities prevents the pattern of settlement on silted surfaces.

2.5 DISCUSSION

The taxonomy of *Pinctada* was not properly understood until Hynd (1955) described the species. Pearl oysters originally ascribed to the sub genus *Margaritifera* under the genus *Pteria* (Plate 2.6 B) are referable to the genus *Pinctada* (Prashad (1932), Iredale (1939), Hynd (1955), Rao (1970), Rao and Rao (1974), Velayudhan and Gandhi (1987) and Gervis and Sims (1992)). Indian pearl oyster *Pinctada fucata* differs from the other species in the following features: The hinge is long and straight, the long axis of the shell is at right angle to the hinge, the left valve is a little deeper than the right; there is a byssal notch on each valve at the base of the anterior lobe, the coloration of periostracum varies and is predominantly brownish red with radial markings in a yellow background (Rao, 1970).

The Indian pearl oyster was formerly known as *P. vulgaris* and Japanese pearl oyster as *P. martensii*. Both these species have now been synomymised under *P. fucata,* although Japanese workers prefer to call their species as *P. fucata martensii* (Shirai, 1994).

A comparison of the shells showed that the shells of oysters growing in the east coast are decisively larger than those of the west coast, notwithstanding the fact that the oysters of Gujarat coast was singularly bigger than those oysters living in any other part of the west or east coast of India. The shell characters on which the taxonomy of the pearl-shell is based are subject to wide variation (Hynd 1955). The variation can be attributed to a number of causes, of which geographical separation and ecological differentiation are important. In addition all species show marked changes in appearance with age. The population structure of an area with reference to ecological conditions is highly variable within the same species of different localities. These variations may be attributed to non-genetic. These types of variations are noted in the pearl oysters of the species *Pinctada fucata* (Gould) collected from Gujarat, Vizhinjam in the west coast and Tuticorin and Mandapam in the east coasts of India. The total shell thickness of the Gujarat oysters were highest than that from all other areas, this probably as an adaptation to the long duration of desiccation as a result of exposure during

low tides. This adaptation was to withstand the scorching sunrays falling on the shells of the exposed oysters during low tides and the more thickens to the shells to avoid heating up of the internal organs of the pearl oysters.

Both environmental and genetic factors influence shell characteristics (Hynd, 1960; Wada, 1984). Colour, shape, thickness and nacre quality of *P. margaritifera* vary between localities in the Red Sea (Crossland 1957; Reed 1966) and the French Polynesia (Ranson 1957; Domard 1962; Service de la Peche 1970). Shell size and shape are inheritable in *P. fucata martensii* (Wada 1984, 1986 a, b, 1987). Nacre and pearl coloration are also largely genetically controlled (Wada 1983, 1986 b), however trace elements and minerals in the ambient medium can influence the coloration of nacre of pearl (Mizurnoto 1976; Wada and Suga 1977)*.

Hynd (1960) used morphometric ratios and shell colour to separate the two Australian subspecies of *P. albina*. Shell color patterns and growth rates showed marked geographical discontinuity, but variability in shell shape due to environmental influences will decide the classification and related aspects of the species from a space specific stand point

The generic name used in this study was given by Prashad, (1932) and Iredale, (1939), as *Pinctada roding* (Hynd, 1955). Jameson (1901) made a broad subdivision of the genus based on the presence or absence of hinge teeth. Those with hinge teeth are called *Margaritifera maxima* and those without *Margaritifera margaritifera.* These species are at present *Pinctada maxima* (Jameson) and *Pinctada margaritifera* (Linnaeus). The result of this study is in agreement with the earlier reports of Prashad and Bhaduri (1933), Hynd (1955). Rao (1974) explained the taxonomy of pearl with description and identification of different species of pearl oysters with diagrams. The anal papilla of M. vulgaris has five folds (Herdman 1904) while Shiino (1952) had shown only three in that of *P. martensii* (Dunker). In *P. fucata* (Gould) the anal papillae is trifold. In *M. vulgaris* there are three nerves inervating to the anal papilla from the pedal ganglion, however in *P. fucata* (Gould) in the present case four nerve inervations were seen. Herdman

(1904), Shiino (1952) noticed the renogenital aperture respectively from *M. vulgaris* and *P. martensii.*

In *P. fucata* (Gould) it is very difficult to distinguish the renogenital aperture but during spawning it is noticed at the anterior part of the inner gill attachment, sperms and eggs are released from a vertical slit situated at the anterior part of the gill filament. Kuwatani (1965 *a*) differentiated the stomach of *P. martensii* from that of *P. vulgaris.* In *P. vulgaris* the left intestinal groove arise from the pouch leading to the origin of the groove.

In recent years, electrophoretic methods have been used to differentiate species and identify distinct groups among geographically isolated populations (Wada 1982; Blanc 1983; Blanc *et al.*, 1985; Li *et al.*, 1985) and between successive generations (Wada 1986a, 1986c). Differences between *P. margaritifera* from adjacent atolls in the Tuamotu Archipelago, French Polynesia, reflect restricted exchange of larvae between lagoons (Blanc 1983; Blanc *et al.*, 1985). Populations of P. *fucata martensii* from different locations of Japan also showed genetic differences (Wada 1982, 1984). The studies on the population of pearl oysters in India reveals that enzymatic profile studies are necessary for the proper identification of species (Sapna, 1999) although she could delineate any variations between the populations of *P.fucata* from different regions of India.

Proteins in pearl oyster hinge ligaments have shown higher order of affinity between *P.margaritifera* and *P. maxima* (Kikuchi and Tamiya 1987). Although karyotypes can indicate relationships between higher taxa, no such differences occur among *Pinctada* species (Wada 1976 a, 1978; Komaru and Wada 1985; Wada and Komaru 1985). Although Subtle differences between karyotypes are found between *Pinctada* spp. and *Pteria penguin* (Wada and Komaru 1985).

Victor (1983) and Victor and Velayudhan (1983) studied the hydrographic conditions of the culture farms at Veppalodai, Gulf of Mannar and Tuticorin.

The present study clearly indicates that farms nearer to the shore and those in shallow areas have more turbid water and this could affect rate of spat settlement. The South West and Northeast monsoons are active in the southeast coast of India and this could account for the high turbidity in the farm during this period.

Victor and Velayudhan (1987) observed that high silt deposition coincided with low salinity and temperature conditions, while the present study showed pearl settlement and silt during the pre monsoon months On the contrary in this study peak settlement of silt was in the pre monsoon months.

Not much work has been done on the turbidity of pearl oyster areas during the different seasons of the year. The pearl oyster larvae are positively photo tactic in the veliger stage and the process metamorphosis proceeds normally under favourable lighted conditions. The flood water discharged from rivers of East Coast during the northeast monsoon carry with it considerable silt which causes great turbidity of the waters of the pearl banks over the pearl banks, particularly those situated towards the shore.

Temperature plays a vital role in the physiology of marine invertebrates. Victor (1983), Victor and Velayudhan (1987) have reported double oscillation of temperature with a peak in summer months and a low coinciding with the Northeast monsoon months. According to Ganapathy and Murthy (1955) the gradual increase in the surface temperature is due to the reversal in the direction of currents. The second oscillation, extend from June to December when the temperature begins to fall in June reaching a minimum in July - August coinciding with the south-west monsoon, The third one extend from November - December period when the temperature decreases in which the temperature begins to fall in November until the minimum is reached in February. The steep fall in the surface temperature was primarily due to the onset of the northeast monsoon and the influx of fresh water into the sea from the numerous rivers and rivulets. The air and sea temperature appear to be correlated with rainfall. The air temperature is as low as that of sea surface temperature during the peak rainfall period. During non -rainy or least rainy

periods in June -August the air temperature is higher than that of the sea temperature in the east coast.

Temperature limits between species are the main factor that influences distribution. Presence of *P. margaritifera* in the Eastern Australian Coast line is clearly temperature related (Hynd, 1955; George, 1978). *P. margaritifera* from the Southern Great Barrier Reef is "deformed or stunted" (Hynd, 1955). The temperature tolerance range of the Australian *P. maxima* fishery is 19-32°C, *P. margaritifera* also have a similar range. *P. fucata martensii,* being a temperate variety on the other hand has a temperature range of 10-25 °C (Alagarswami, 1970) with hibernation taking place below 13 ° C (Kafutu and Ikenoue, 1983). Yamashita (1986) reported heavy mortalities of stressed *P. maxima* during winter. Dybdahl and Pass (1985) and Pass et al. (1987) found heaviest mortalities in cultured *P. maxima* during the cold months. *P. fucata martensii* hibernates in temperature less than 13 °C and suffers from heat stress in temperatures greater than 28 °C.

The optimum temperature for the growth of pearl oyster in Japan is found to be 20-25 °C. Here above 28 °C the pearl oysters show signs of exhaustion. Spawning is affected by heating (Mizumoto, 1979). Temperature also determines the rate of deposition of nacre both on shells (Cahn, 1949) and on nuclei (Watanabe, 1952; Matsuii, 1958; Alagarswami, 1975; Velayudhan *et al.*, 1996) and therefore limits pearl culture sites to areas within the optimum temperature ranges. However, even though the growth of pearls is reduced with lower temperature, the quality or lustre is improved due to the thinner layers of nacre and so most of the harvesting of pearl is carried out during winter (Gervis and Sims, 1992).

In the Gulf of Kutch *P. fucata* grew vigorously in winter months when the temperature varied from 23° C- 27° C (Gokhale *et al.*, 1954). In the Gulf of Mannar a slight decrease in temperature triggered spawning in the farm oysters and during higher temperature, gonad development was observed. Growth–temperature relationship is presumably valued only upto a maximum temperature for optimum growth.

Similar was the case of salinity where two distinct peaks were recognizable influenced by the North East monsoon. According to Victor (1983) high salinity during southwest monsoon (June onwards) and low salinity in northeast monsoon (November onwards) and high silt deposition in December are some of the features observed at Veppalodai pearl culture farm. The same was observed by Victor and Velayudhan (1987) in the pearl culture farm at Tuticorin. Sewell (1927) stated that the salinity in the Bay of Bengal is high in the southwest monsoon period and low during the northeast monsoon months. In the Gulf of Mannar, according to Malupillai (1962 b), the salinity reached 35.19 ‰ in September 1998. Freda Chandrasekaran *et al.* (1967) found low salinity in January dropping in July. Jayaraman (1954) noted a salinity range of 28:35 ‰ in January to 36.4 ‰ in May. Alagarswami and Victor (1976) found the average values of salinity in the oyster farm at Veppalodai (near Tuticorin) ranging between 32.15 ‰ - 33.50 ‰ during 1974-1976. The lowest value recorded was 31.26 ‰ in January 1974. This indicates the pattern of salinity variations that can be normally encountered in the pearl oyster beds. Although the pearl oyster lives within this range, seasonal fluctuations in conjunction with other environmental factors are known to exert influence on the physiological functions and reproductive activity of the oyster. By and large the pearl oyster being truly marine in its entire life cycle is not known to tolerate great variations in salinity. However, some pearl oyster farms in Japan are known to be located in areas, which are subject to the influence of freshwater discharge from rivers.

Malpas (1929) stated that changes in temperature and salinity induce the pearl oyster to spawn. He contended that low salinity in December-January and high salinity in July-August acts as breeding stimulus. This can be considered only as one of the probable factors influencing the spawning. Hornell (1910), Moses (1928) and Rao (1951) correlated spawning maxima with changes (dilution) in salinity for *Crassostrea madrasensis*. Detailed assessment of salinity effects and tolerances is difficult in natural conditions because the effects may be increased, decreased or masked by other simultaneously effective environmental factors like light, temperature, water movement and interactions between co-existing organisms etc.

PLATE 2.1

A. *Pinctada fucata* (Gould, 1850)

B. *Pinctada fucata* (Gould, 1850)

1. Right shell inner view 2. Left shell outer view

PLATE 2.2

A. *Pinctada margaritifera* (Linnaeus, 1758)

B. *Pinctada margaritifera* (Linnaeus, 1758)
Right shell inner view

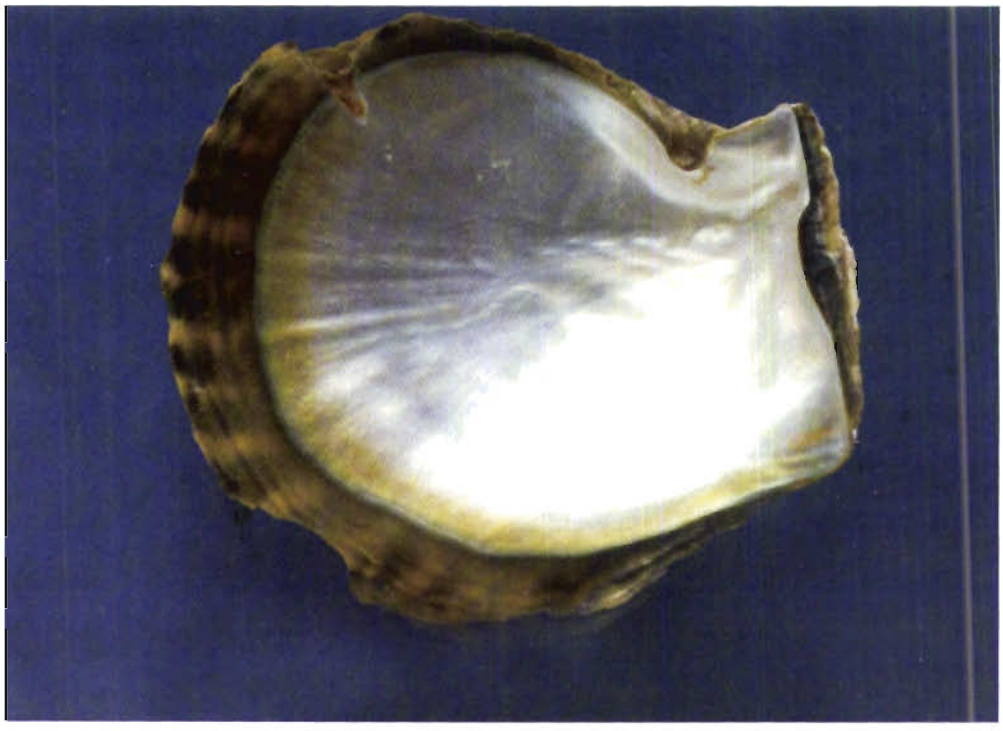
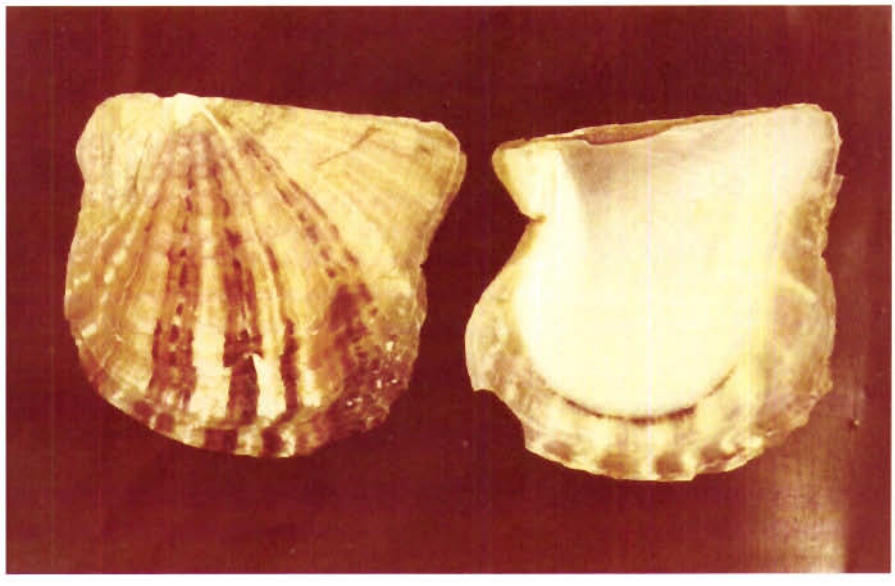


PLATE 2.3

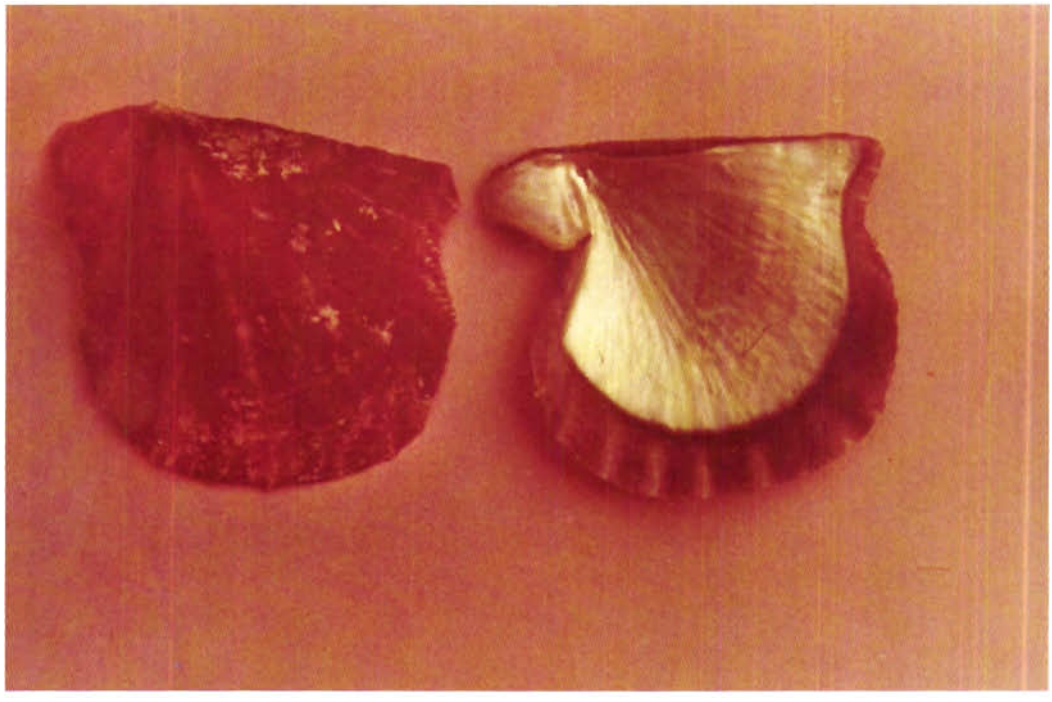
A. *Pinctada sugillata* (Reeve, 1857)



B. *Pinctada chemnitzii* (Phillippi, 1785)

PLATE 2.4

A. *Pinctada atropurpurea* (Dunker, 1852)



B. *Pinctada anomioides* (Reeve, 1857)

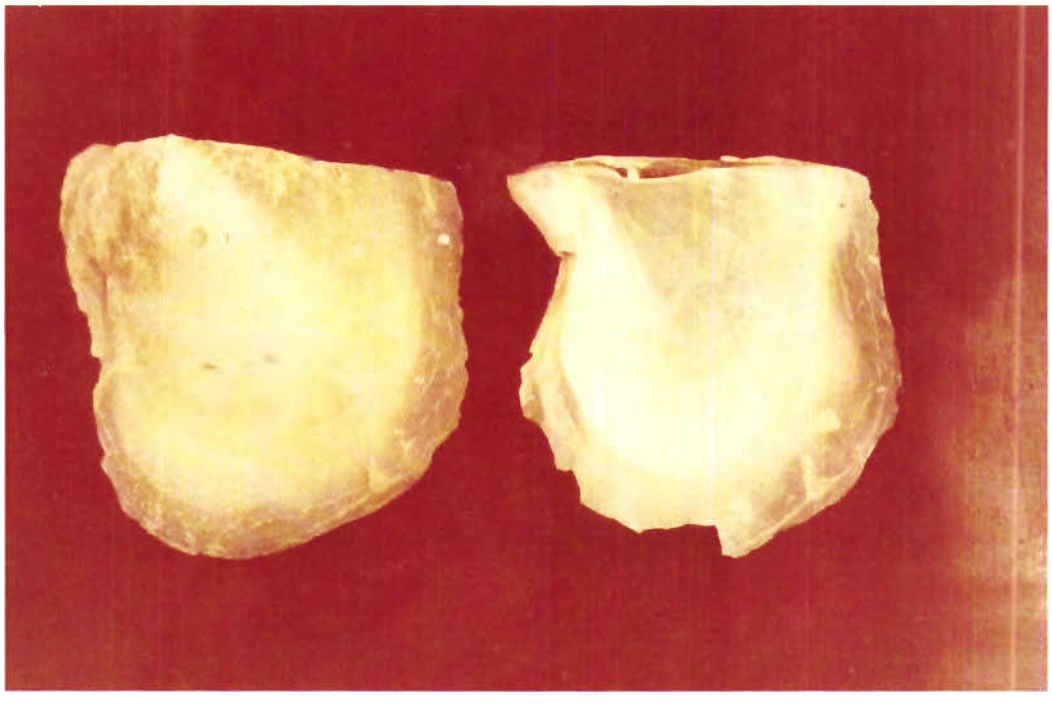
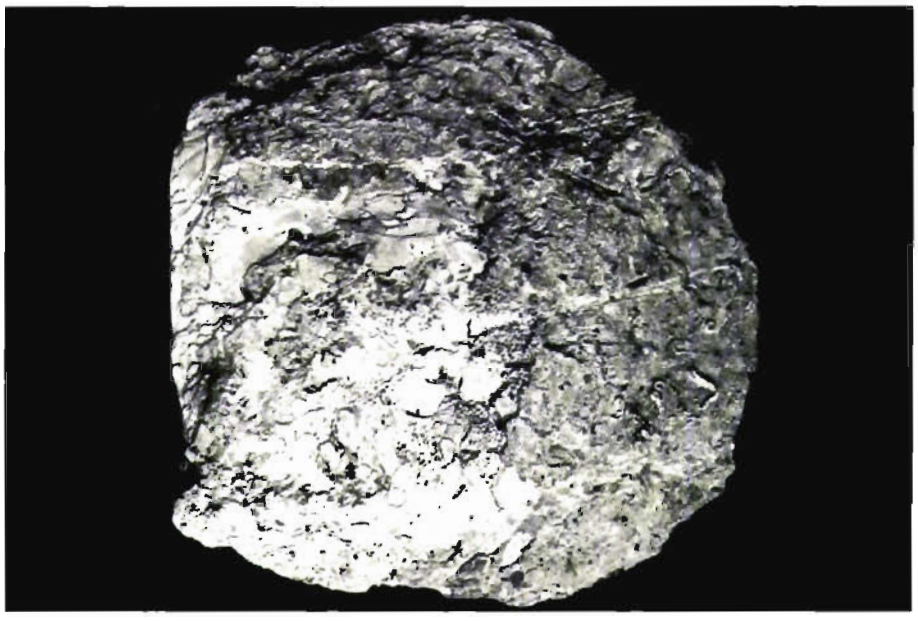


PLATE 2.5

A. *Pinctada maxima* (Jameson, 1901)



B. *Pteria penguin* (Roding, 1798)

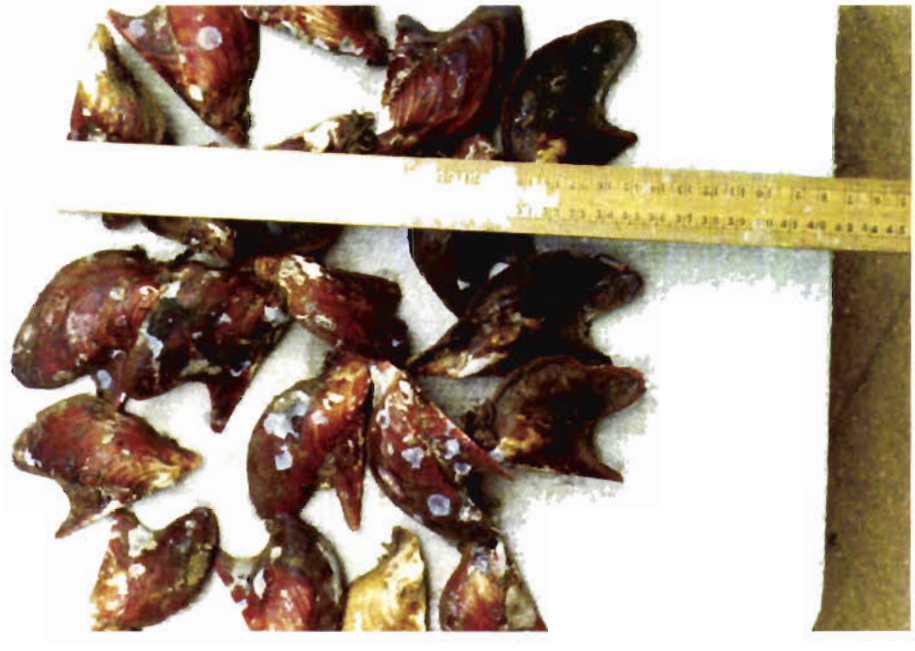


PLATE 2.6

A. Tuticorin Shellfish Hatchery of C.M.F.R.I

B. Pearl culture farm at Tuticorin Major Harbour

PLATE 2.7

A. Spat with well developed growth process
B. Green coloured spat
C. Spat with red, plane coloured growth process
D. Adult oysters farm reared without prominent growth process

PLATE 2.8

A. Pearl oysters from the natural bed with different colours on the shell
B. Healthy pearl oysters from the natural bed settled on the pinna shell
C. Pearl oysters settled on the anchor with white, red and plane growth process

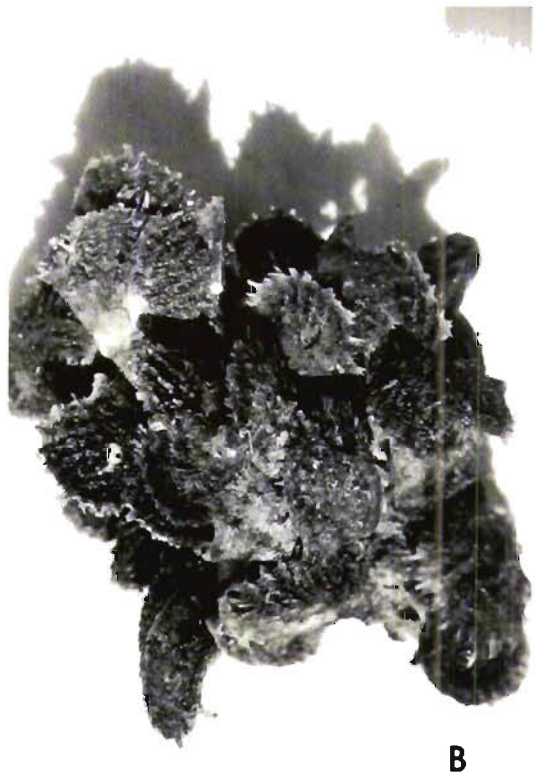


PLATE 2.9

Specimens of *Pinctada fucata* collected from A. Gujarat ; B : Tuticorin ; C : Vizhinjam and D : Mandapam

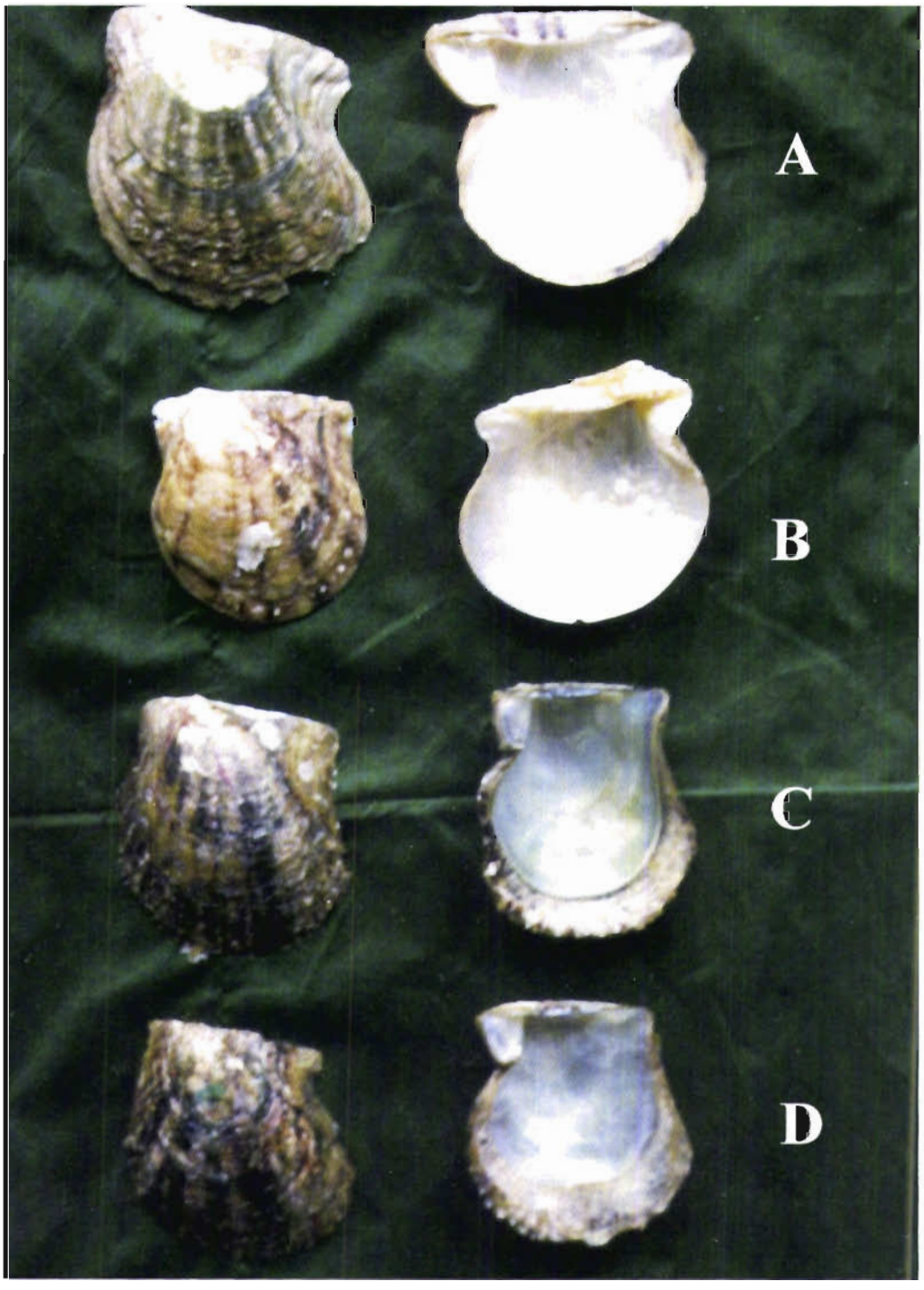


PLATE 2.10

A. Ground glass panels fouled by tubiculous polychaetes

B. Frame net with live pearl oysters to study the effect of fouling in the farmed oyesters

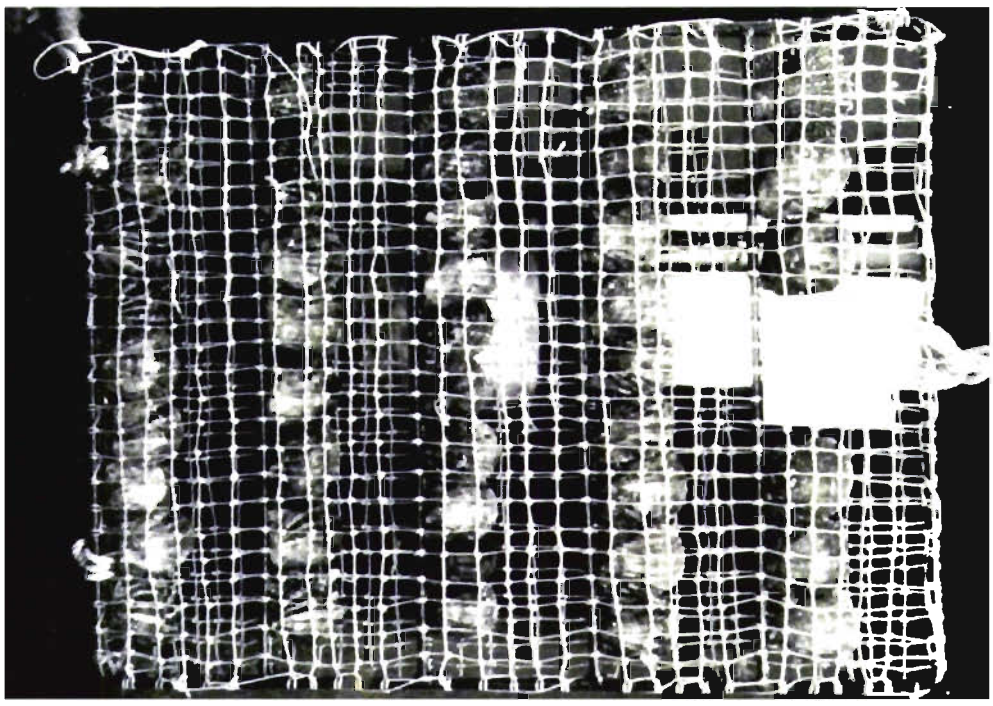
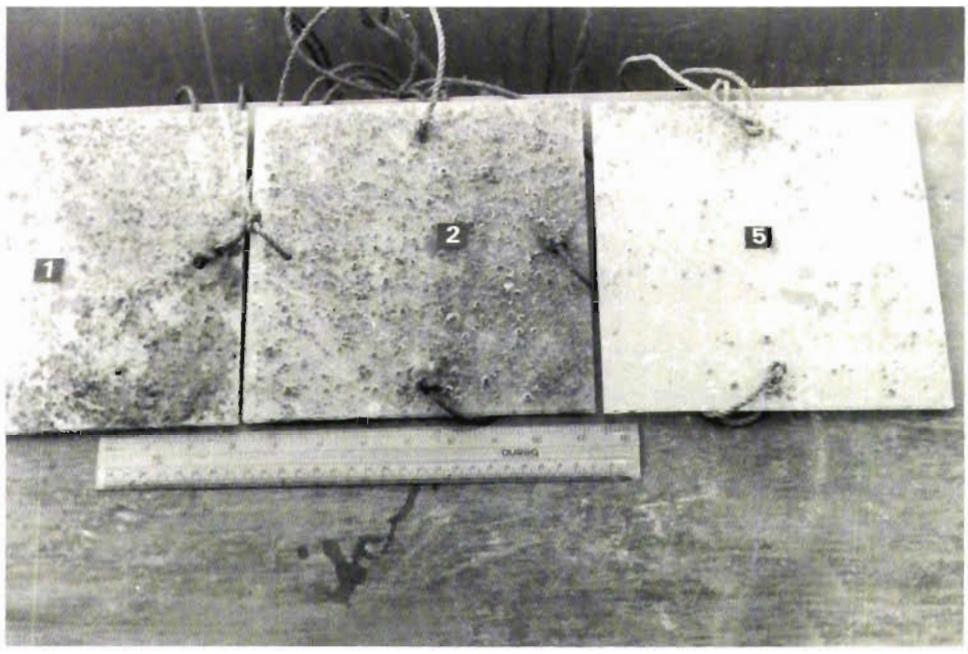


PLATE 2.11

A. Ground glass panels suspended from shaded raft at different depths showing less settlement of barnacles at 5 m depth.



B. Ground glass panels suspended from shaded raft at 3m depth showing barnacle settlement

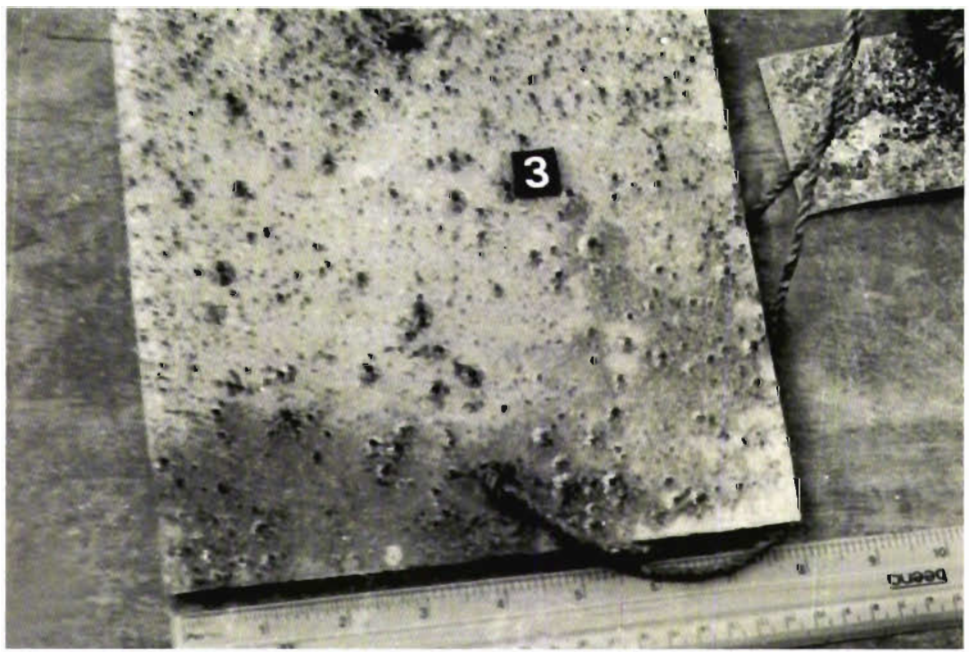
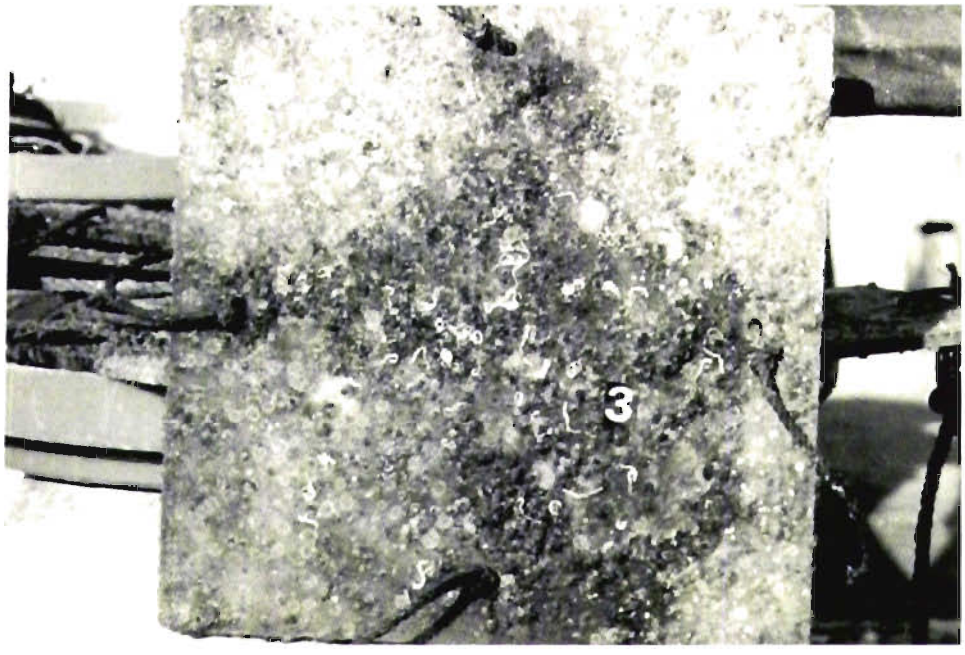


PLATE 2.12

A. Ground glass panels suspended at 3 m depth from non shaded raft showing settlement of barnacles and tubiculous polychaetes.



B. Ground glass panels suspended at 3 m depth from shaded raft showing less number of barnacles

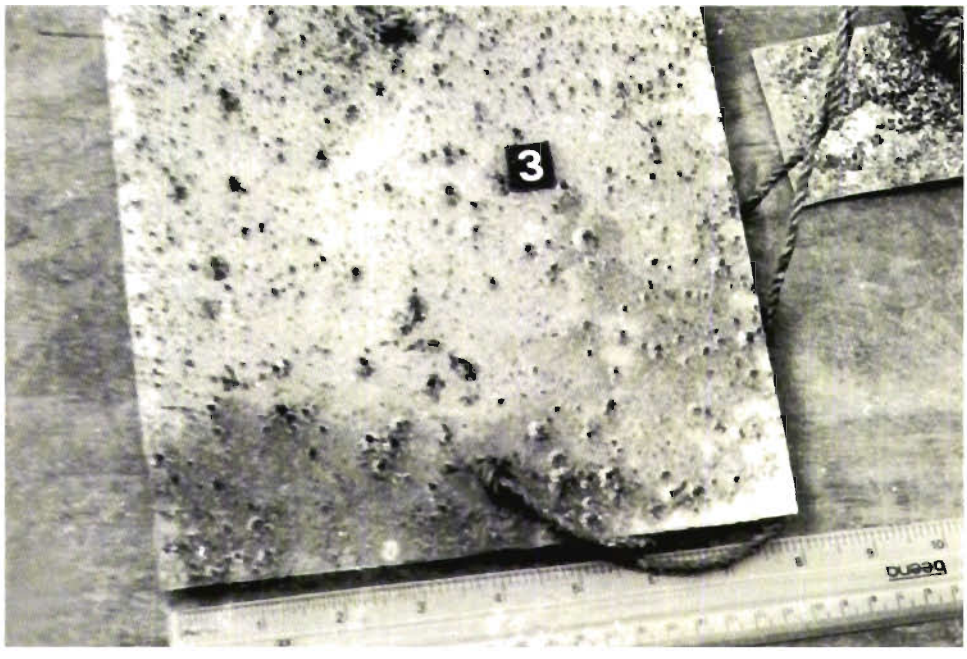
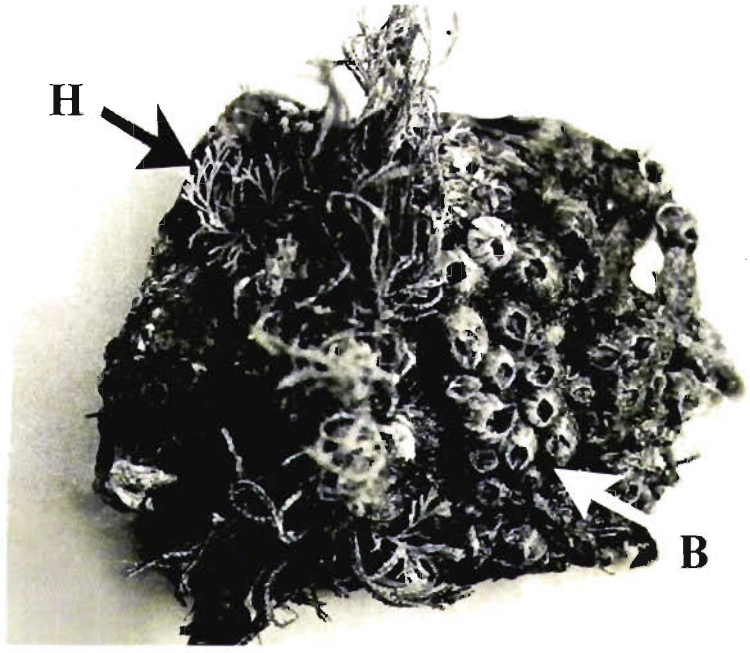


PLATE 2.13

A. Pearl oyster fouled by Hydrozoan (H) *Thuiaria palans* and Barnacle (B) *Balanus amphitrite variegatus*



B. Pearl oyster fouled by Sponge (C) *Callyspongia fibrosa* on both valves and Barnacle (B) settlement below the umbo

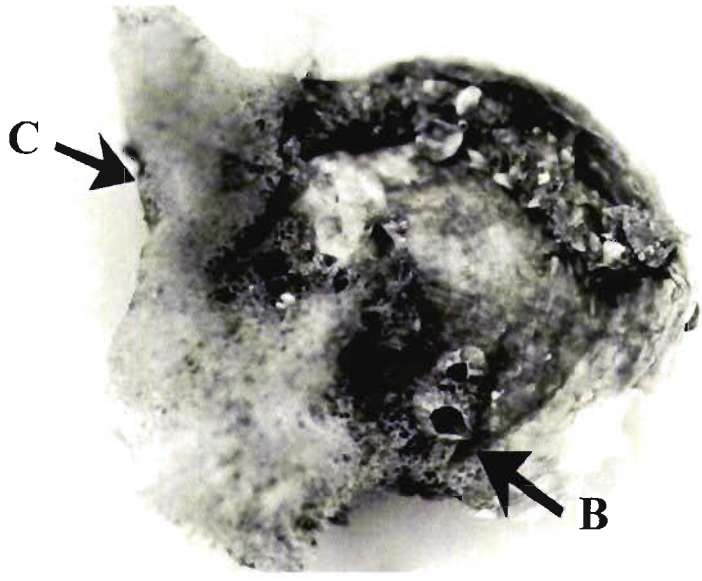
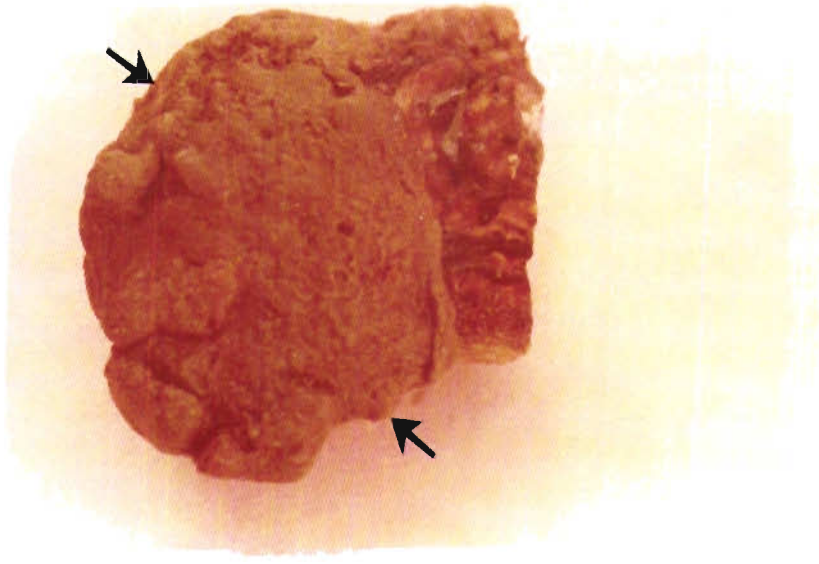


PLATE 2.14

A. Compound Ascidian *Botrilloides* sp. (Black arrows) fouling on pearl oyster *Pinctada fucata*



B. Pearl oyster rearing cage meshes fouled by " False Spat" *Avicula vexillum* (arrows)

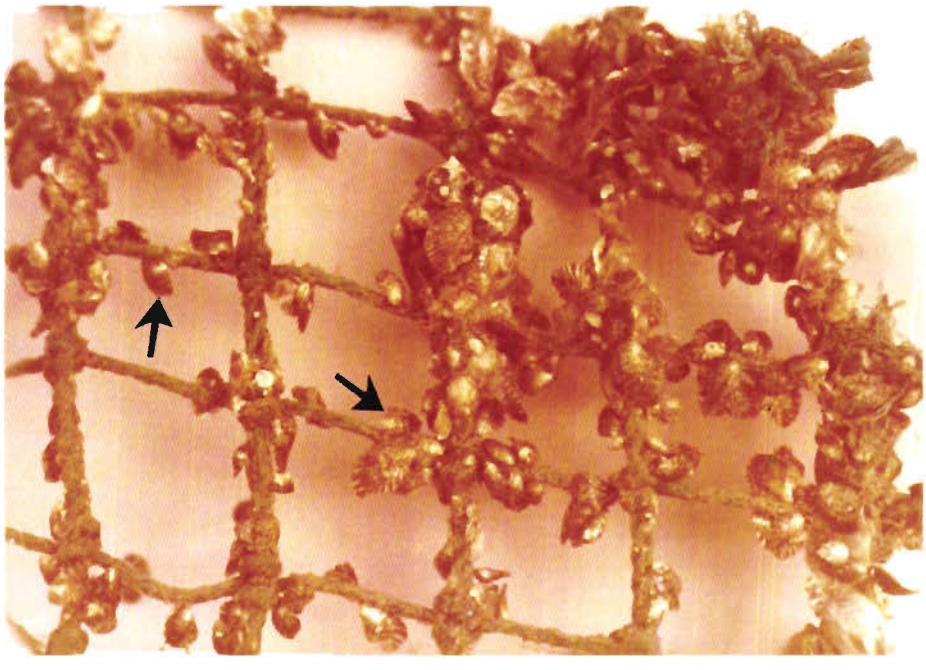
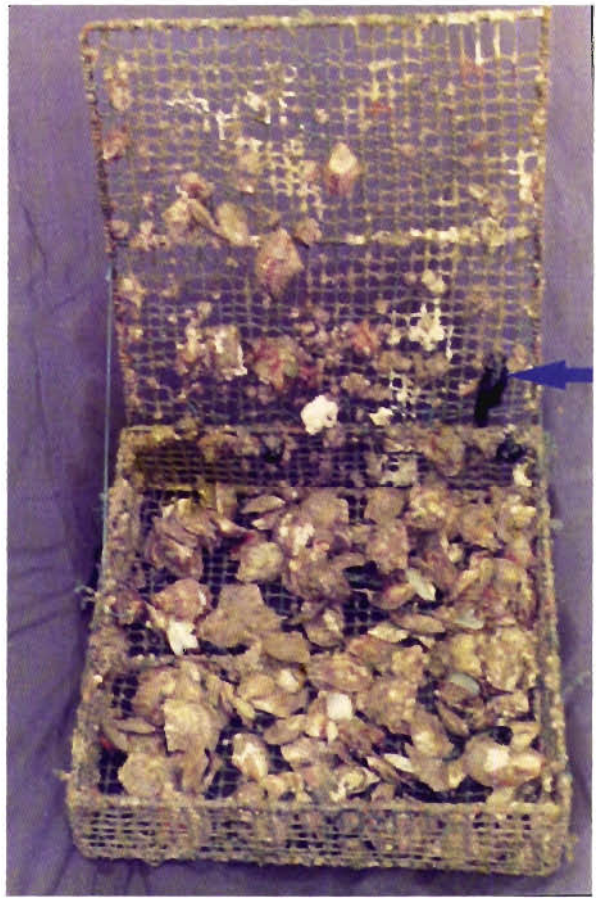


PLATE 2.15

A. Simple Ascidian (arrow) fouling on pearl oyster cage



B. *Blennius steindanchneri* fish sheltered in the dead pearl oyster shell

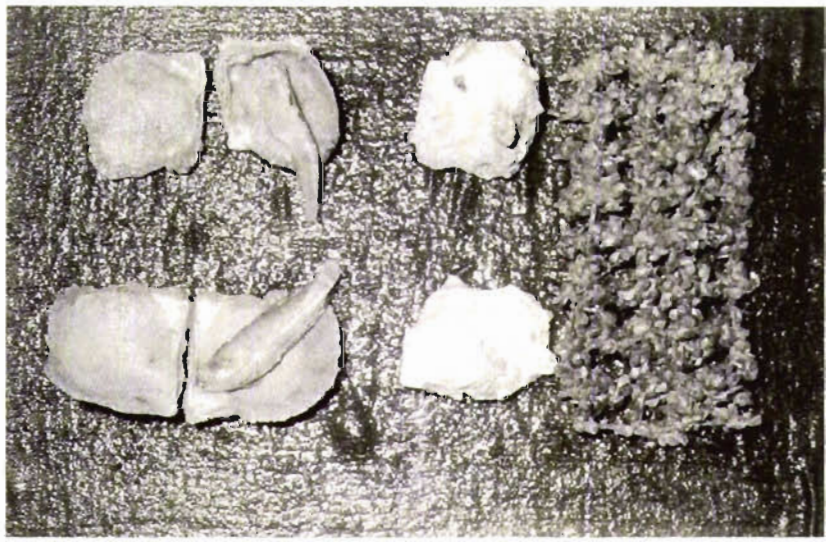


Figure : 2.1 Map of India showing distribution of pearl oysters ; a-f : species of pearl oysters ; O : represents samples collected for taxonomic studies

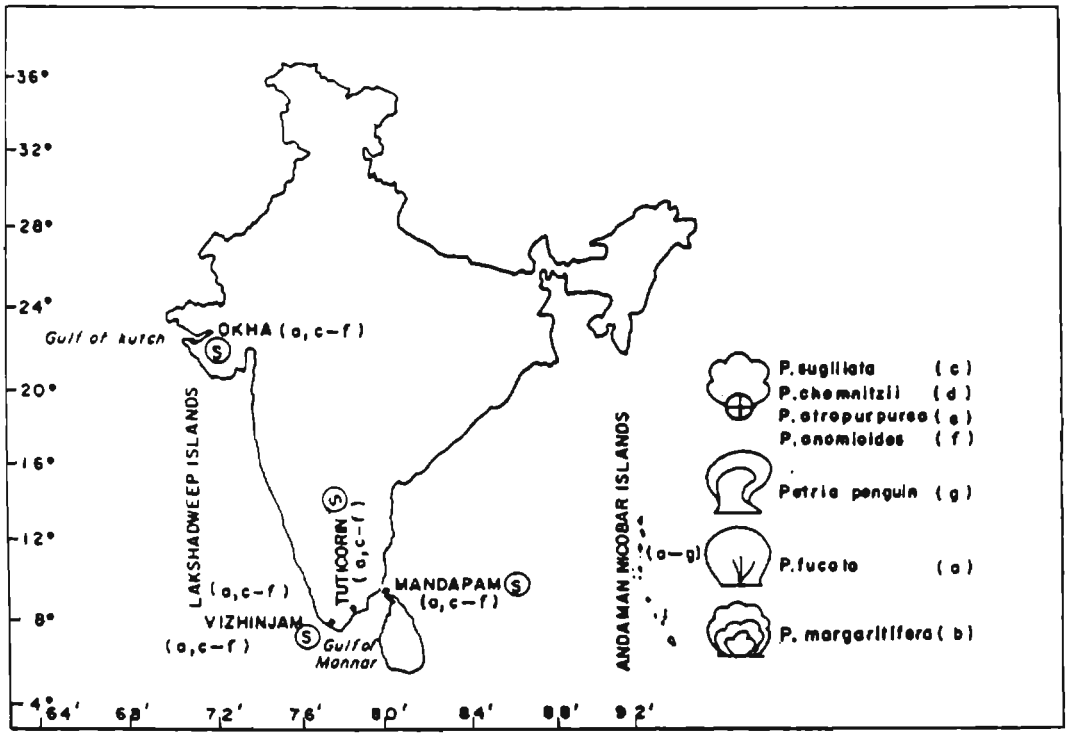


Figure : 2.2 Map of Tuticorin Harbour basin another study site. NW : North breakwater ; SW : South breakwater ; R :▦: Rafts in Harbour basin ; PST : Passenger terminal and berths ; HB : Harbour mouth

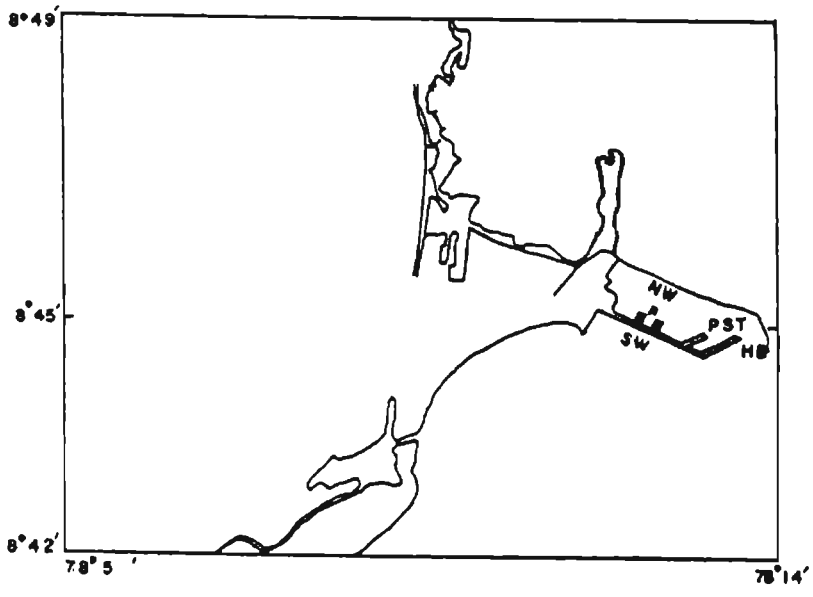


Figure : 2.3 Shell dimensions of pearl oysters, as used in growth measurements

HL=Hinge length ; APM=Antero-Posterior Measurement ;
DVM=Dorso-Ventral Measurement ;
AW=Adductor muscle width and AL=Adductor muscle Length

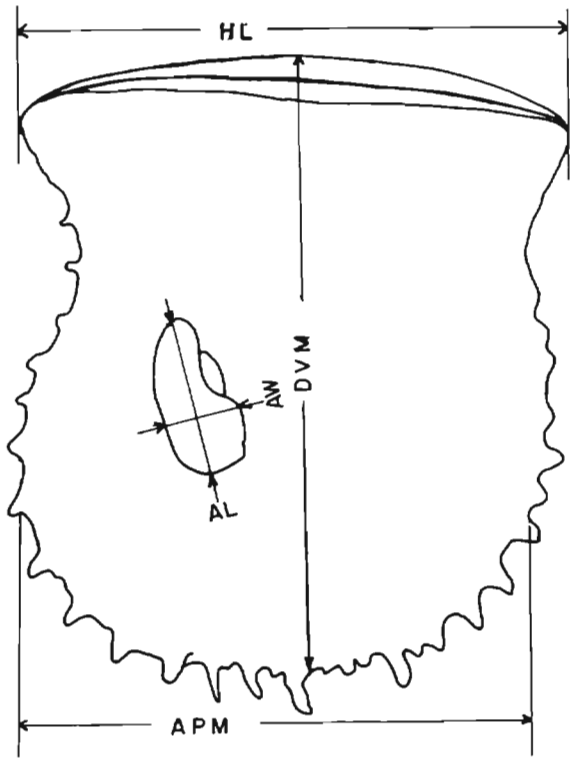


Figure : 2.4 Shell morphometric characters measured

A&B=Thickness of Left (Upper Shell) US and Right (Lower Shell);
T(Depth)=Total thickness of animal; HD=Heel Depth
(Hinge Depth) and HW=Hinge Width

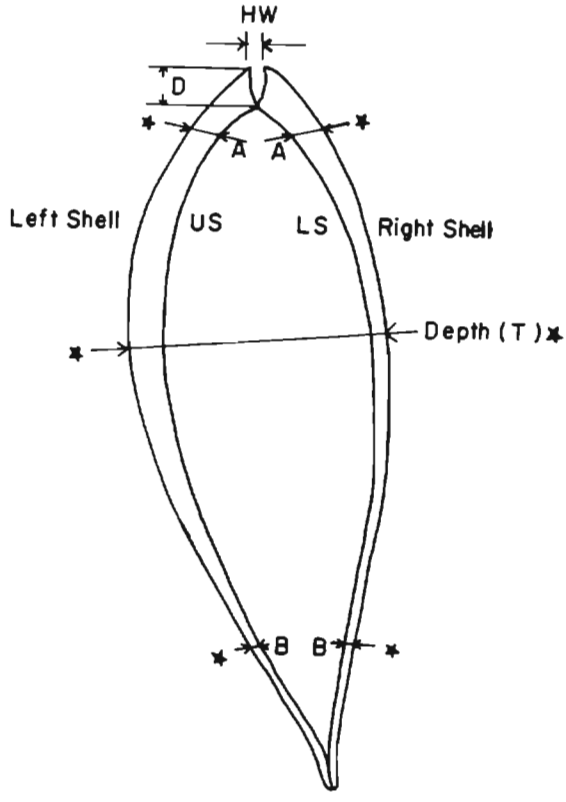


Figure 2.5: Seasonal variations of transparency in the pearl oyster farm at Tuticorin

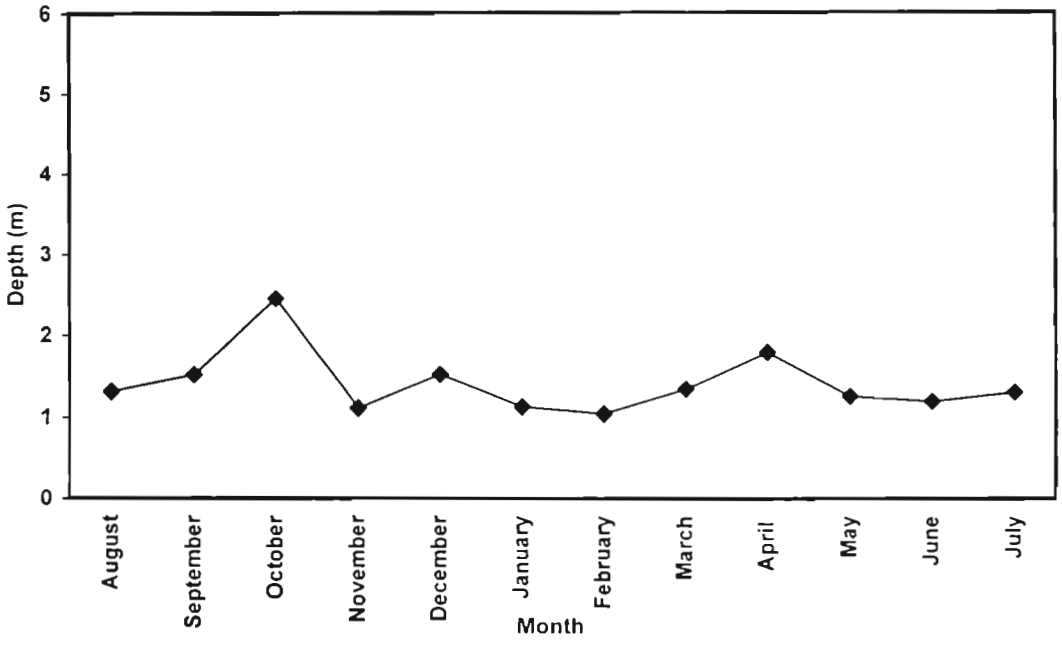


Figure 2.6: Seasonal variations of temperature in the pearl oyster farm at Tuticorin

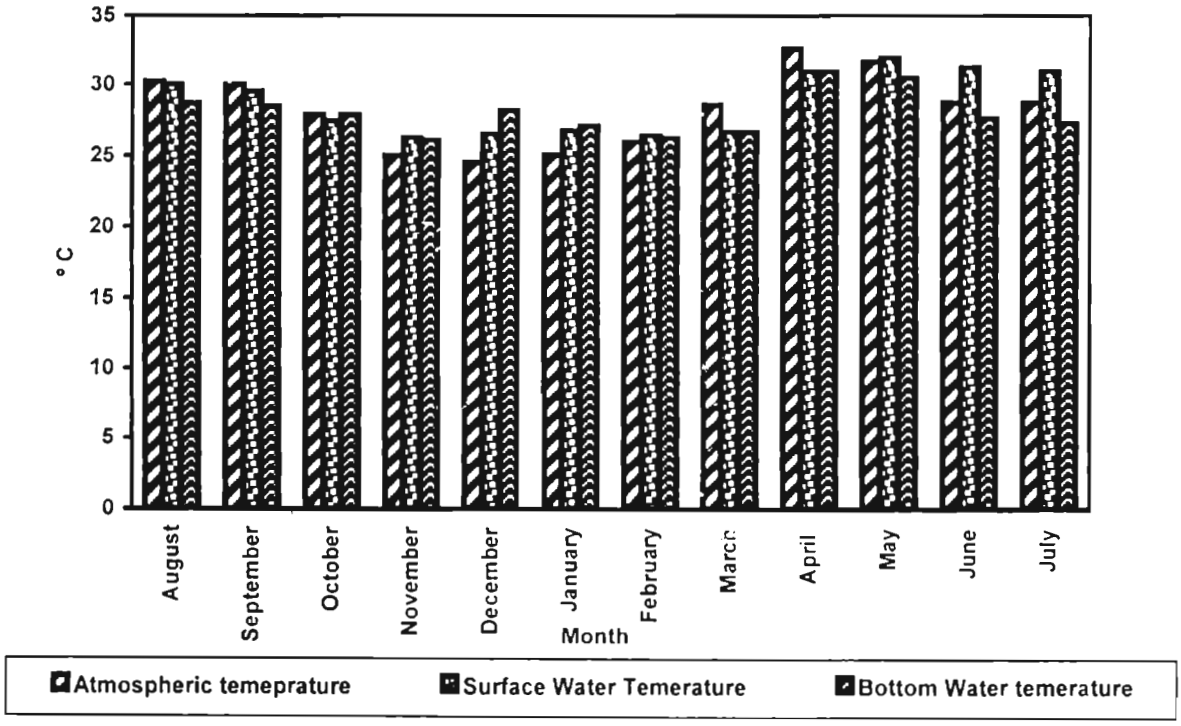


Figure 2.7: Seasonal variations of salinity in the pearl oyster farm at Tuticorin

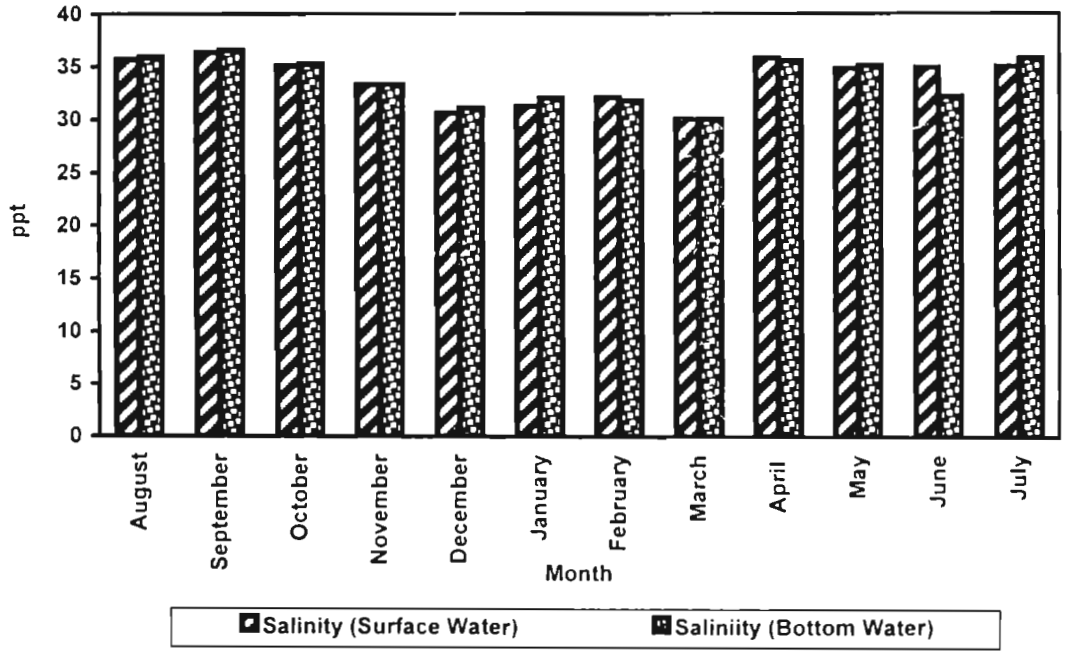


Figure 2.8: Seasonal variations of pH in the pearl oyster farm at Tuticorin

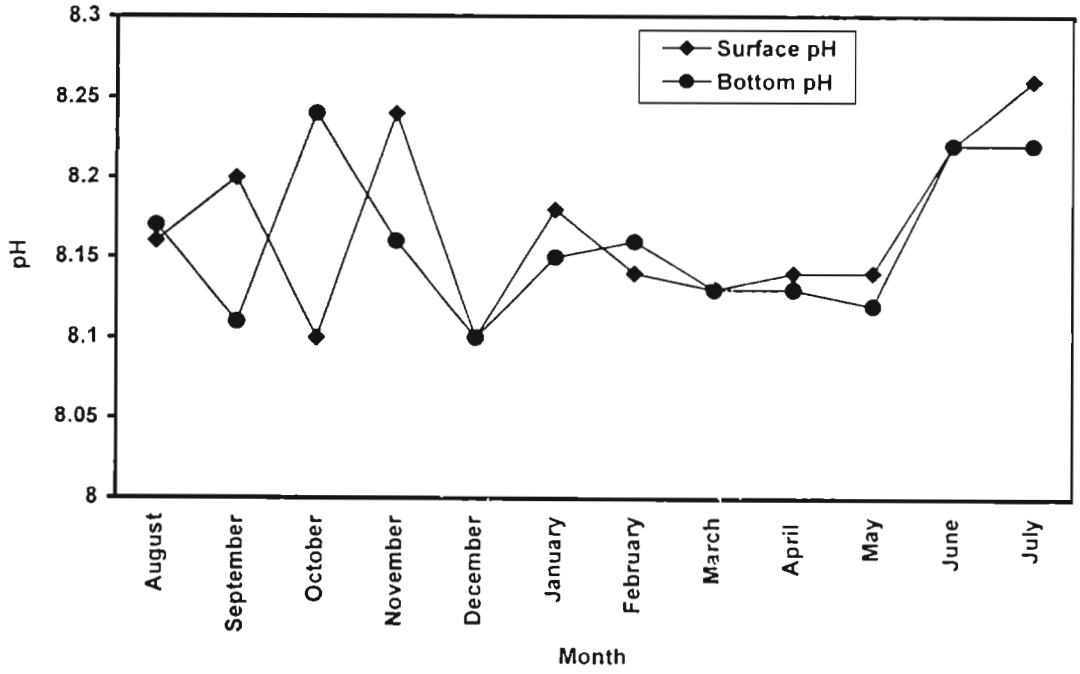


Figure 2.9: Seasonal variations of dissolved oxygen in the pearl oyster farm at Tuticorin

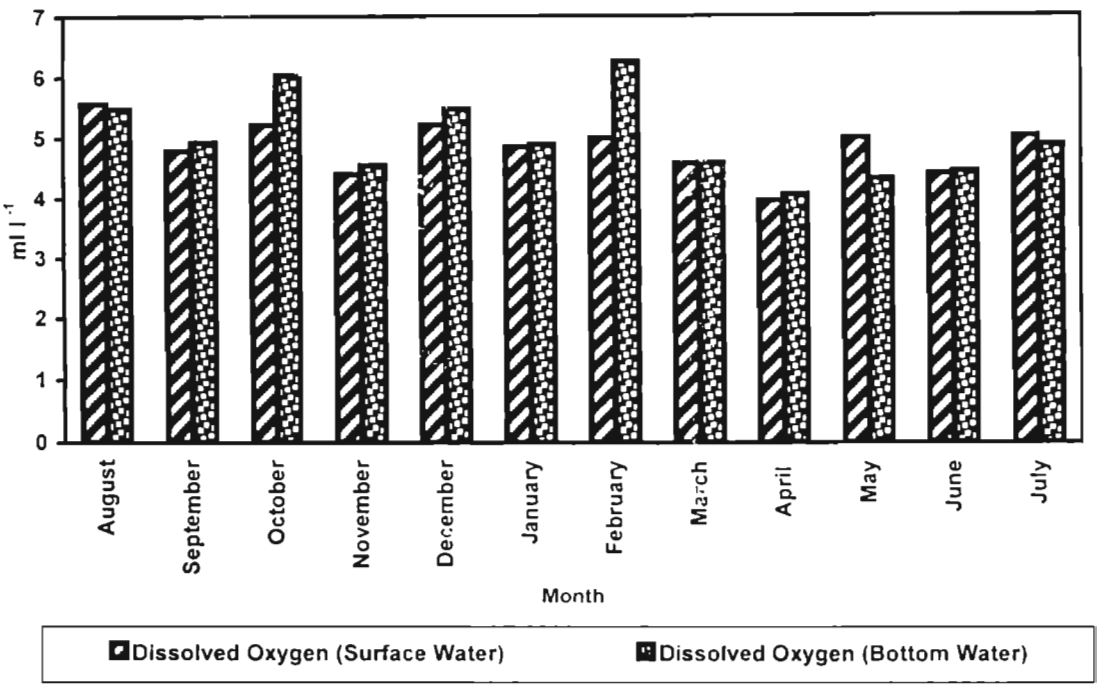


Fig 2.10: Seasonal variations of chlorophyll in the pearl oyster farm at Tuticorin

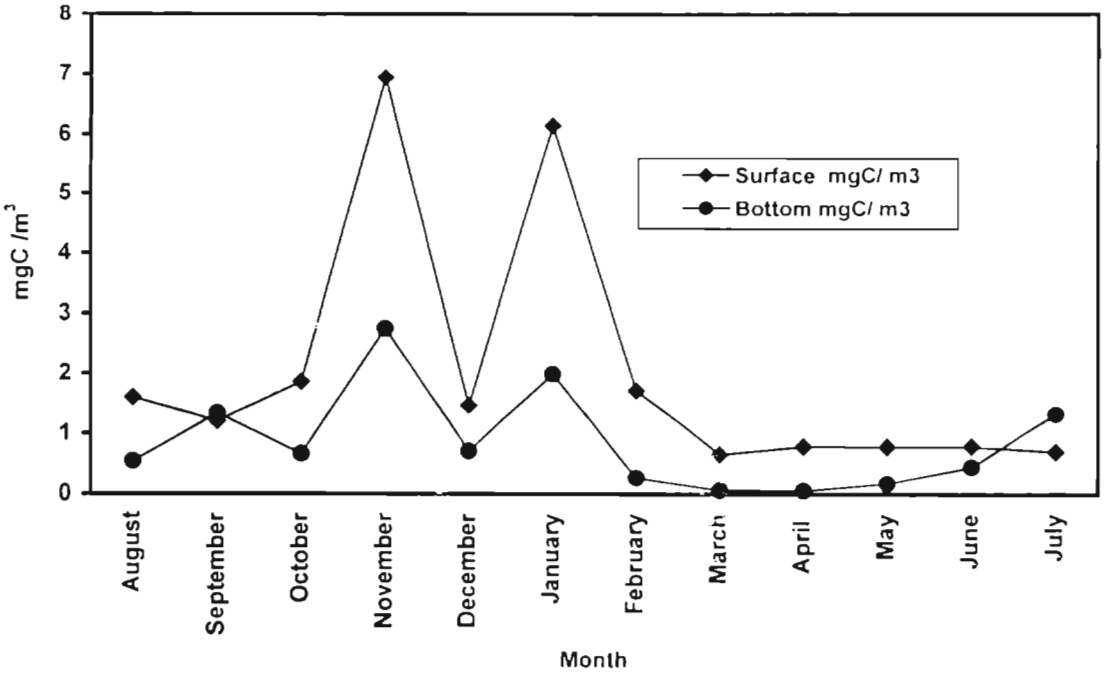


Figure 2.11: Seasonal variations of primary productivity in the pearl oyster farm at Tuticorin

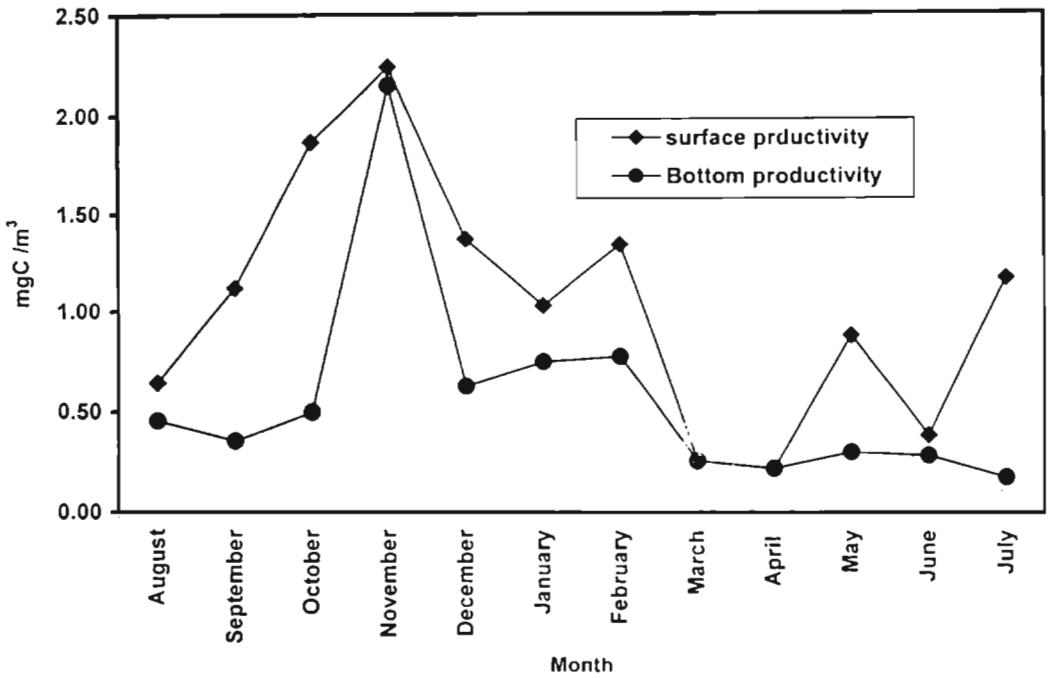


Figure 2.12: Seasonal variations of phosphate in the pearl oyster farm at Tuticorin

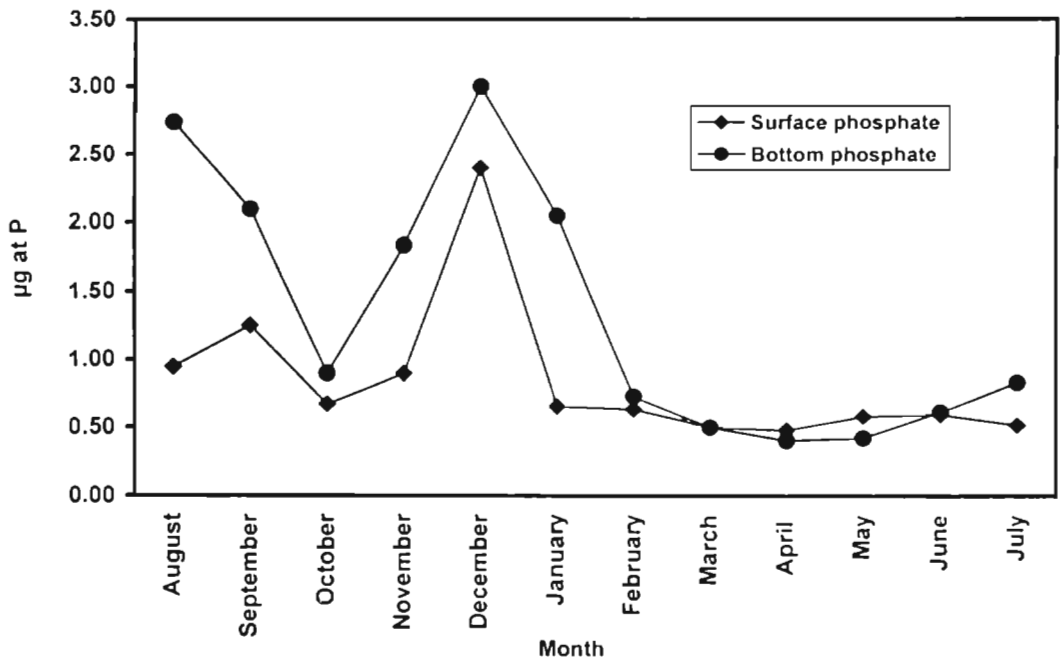


Figure 2.13: Seasonal variations of silicate in the pearl oyster farm at Tuticorin

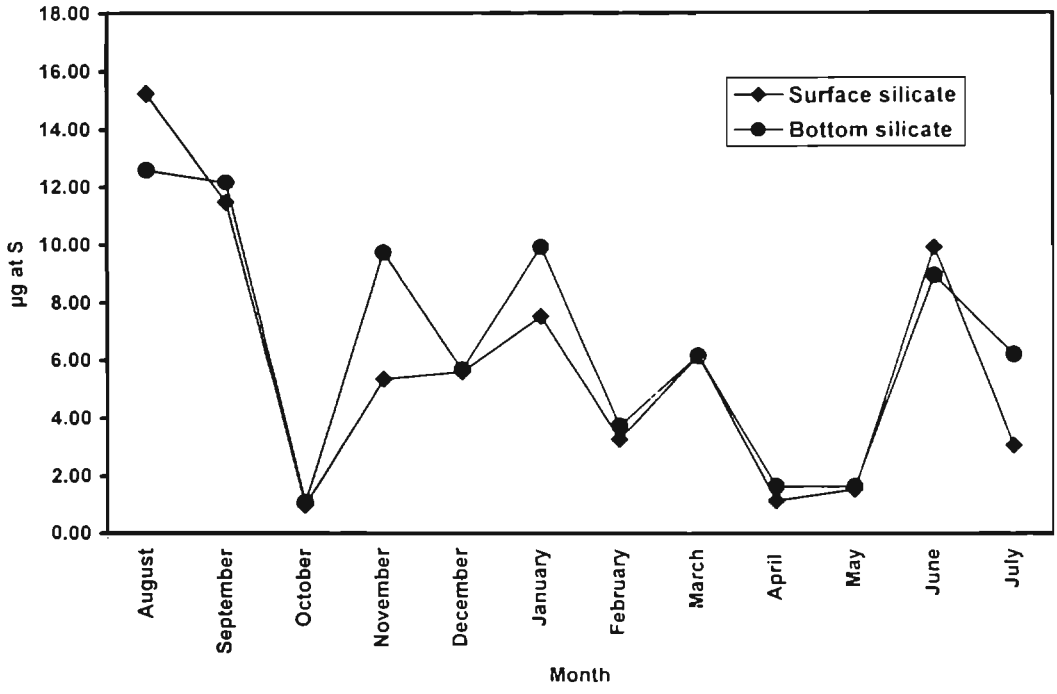


Figure 2.14: Seasonal variations of calcium in the pearl oyster farm at Tuticorin

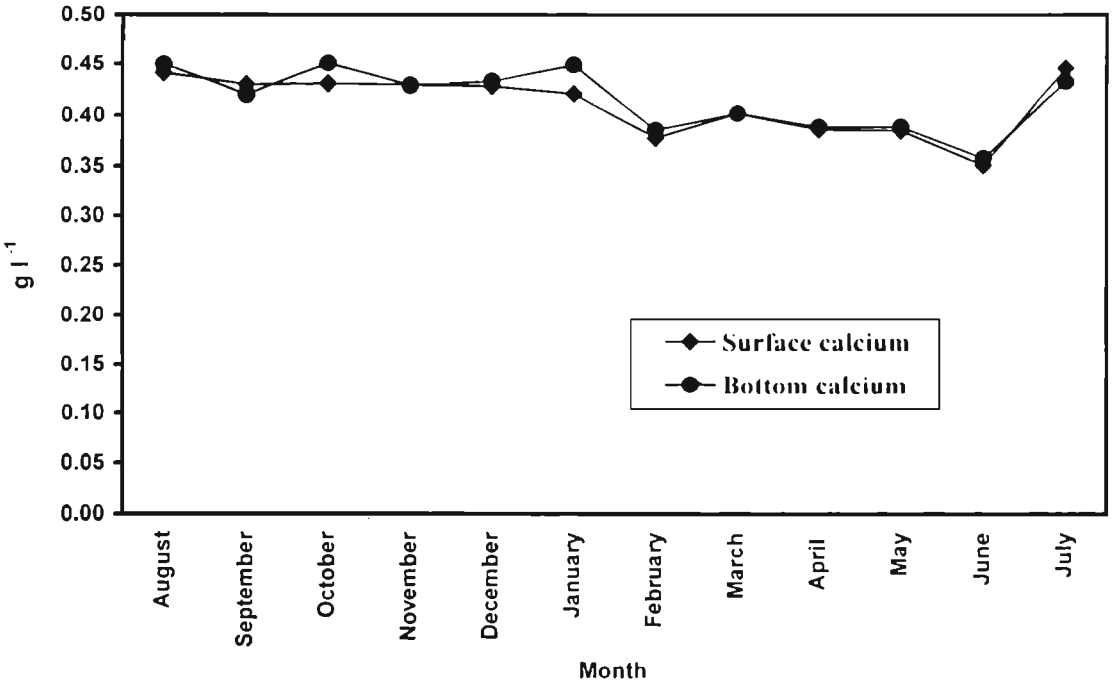


Figure 2.15: Seasonal variations of magnesium in the pearl oyster farm at Tuticorin

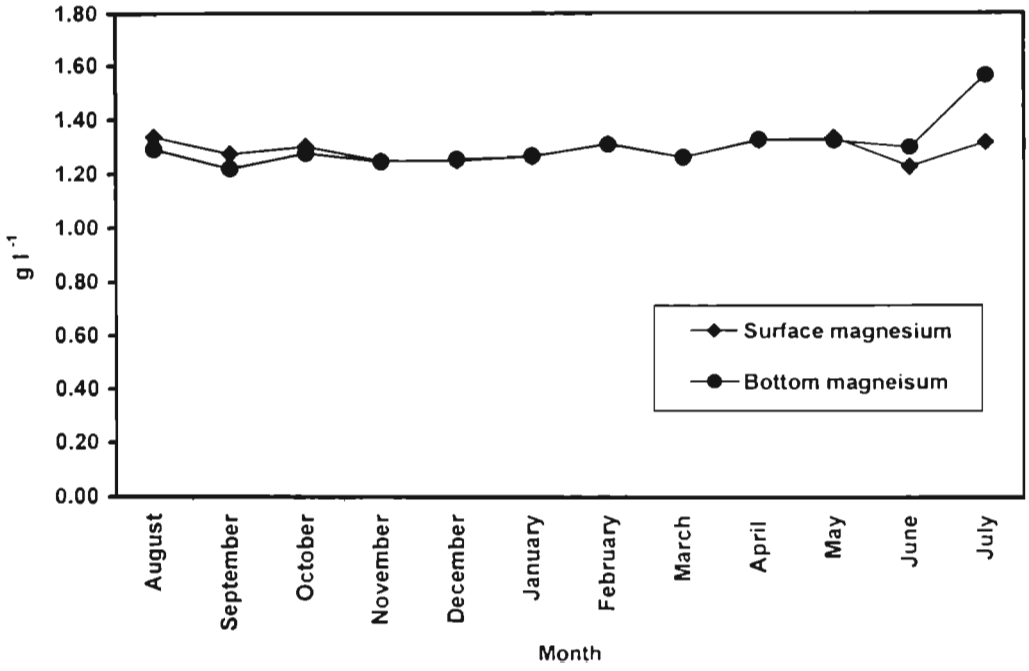


Figure 2.16 (A). Depth wise pattern of fouling organisms on non shaded panels hung in the pearl oyster farm at Tuticorin

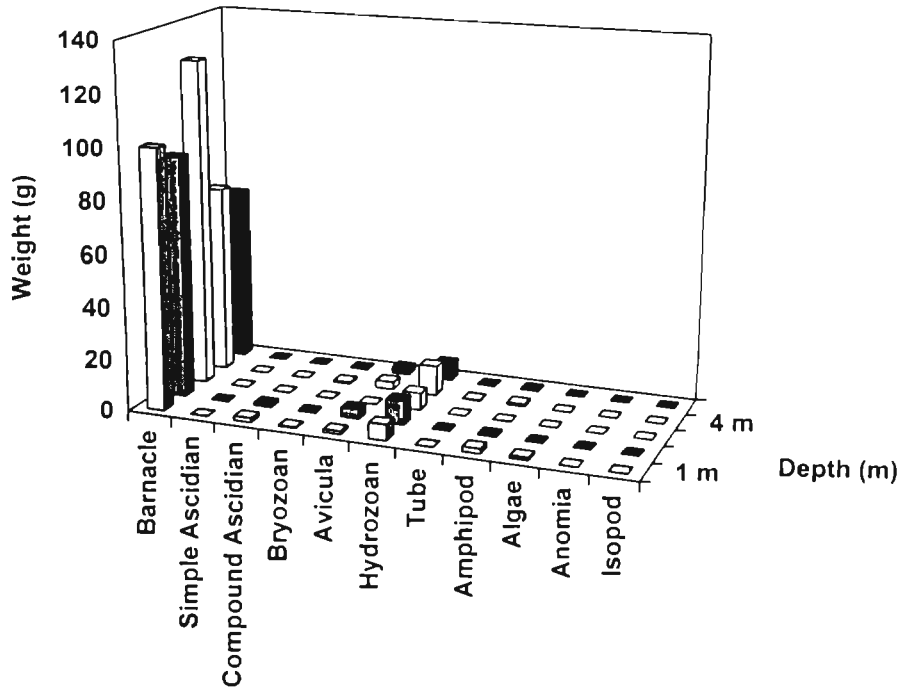


Figure 2.16 (B). Depth wise pattern of fouling organisms on shaded panels hung in the pearl oyster farm at Tuticorin

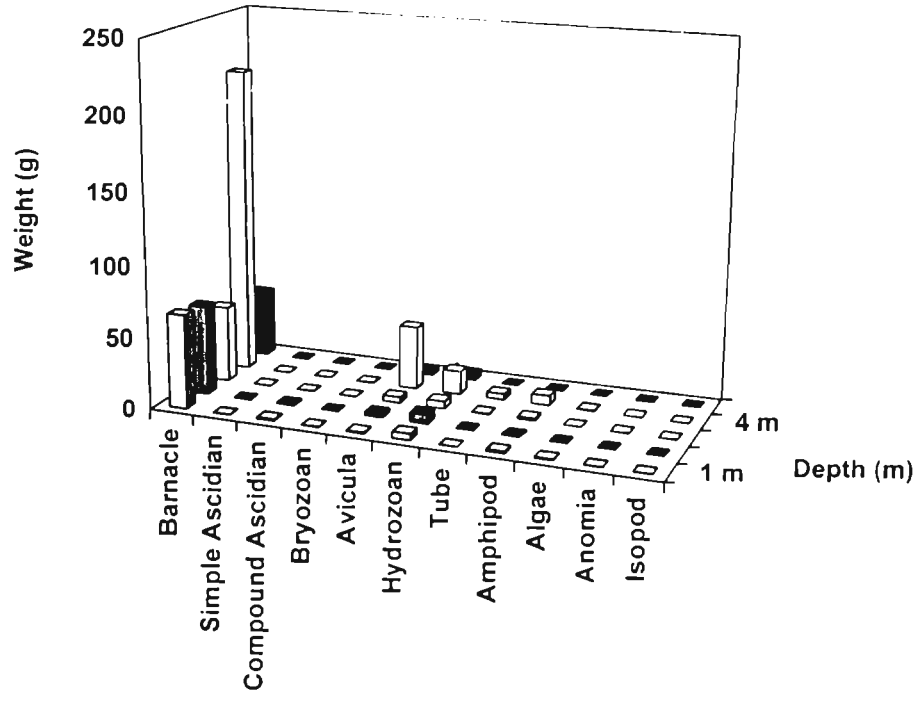


Figure 2. 17. Seasonal variation of total weight of fouling at different depths in pearl oyster farms in non shaded panels

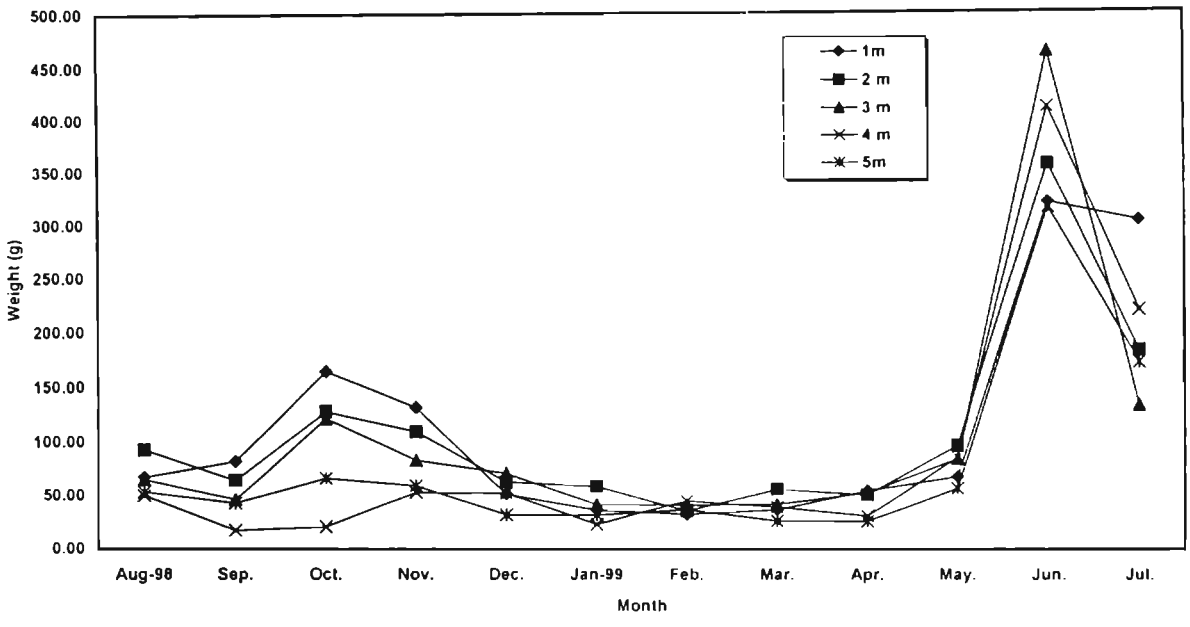


Figure 2. 18. Seasonal variation of total weight of fouling (including barnacles) at different depths in pearl oyster farms in shaded panels

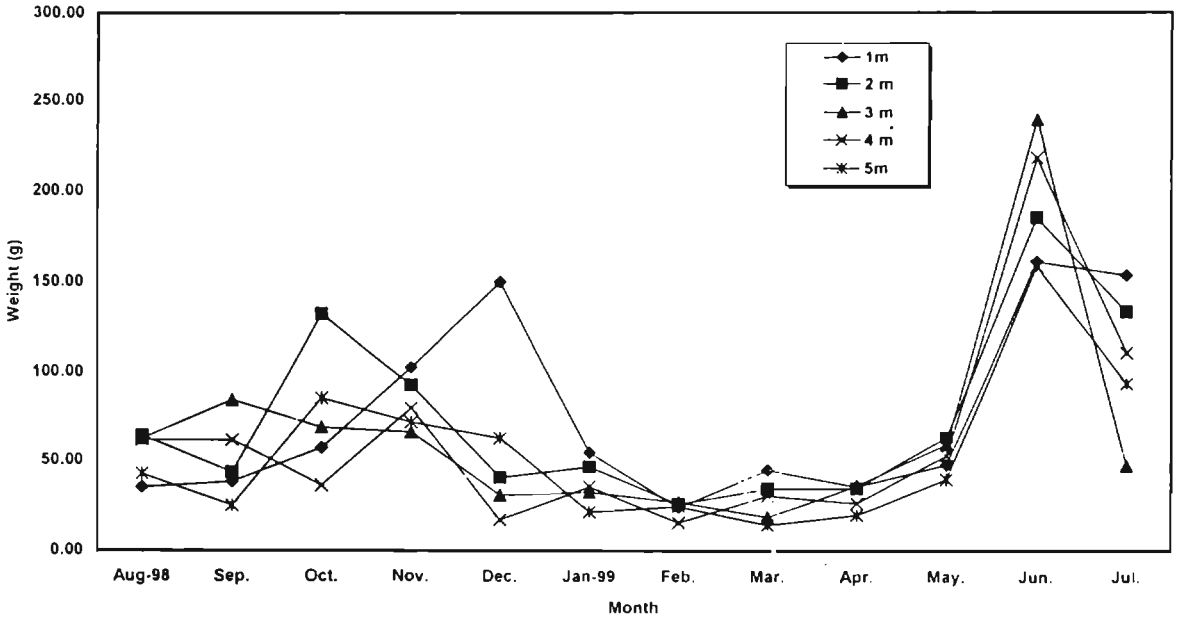


Figure 2.19. Seasonal variation of total numbers of barnacles at different depths in pearl oyster farms in shaded panels

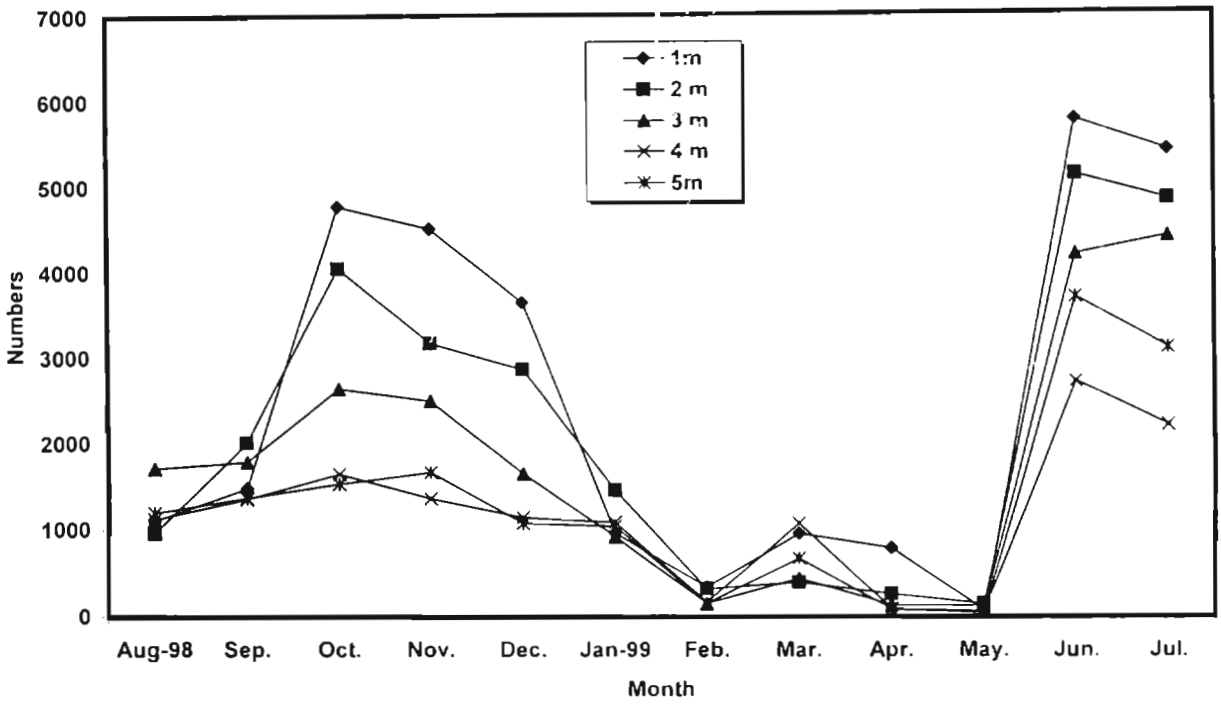


Figure 2.20. Seasonal variation of total numbers of barnacles at different depths in pearl oyster farms in non shaded panels

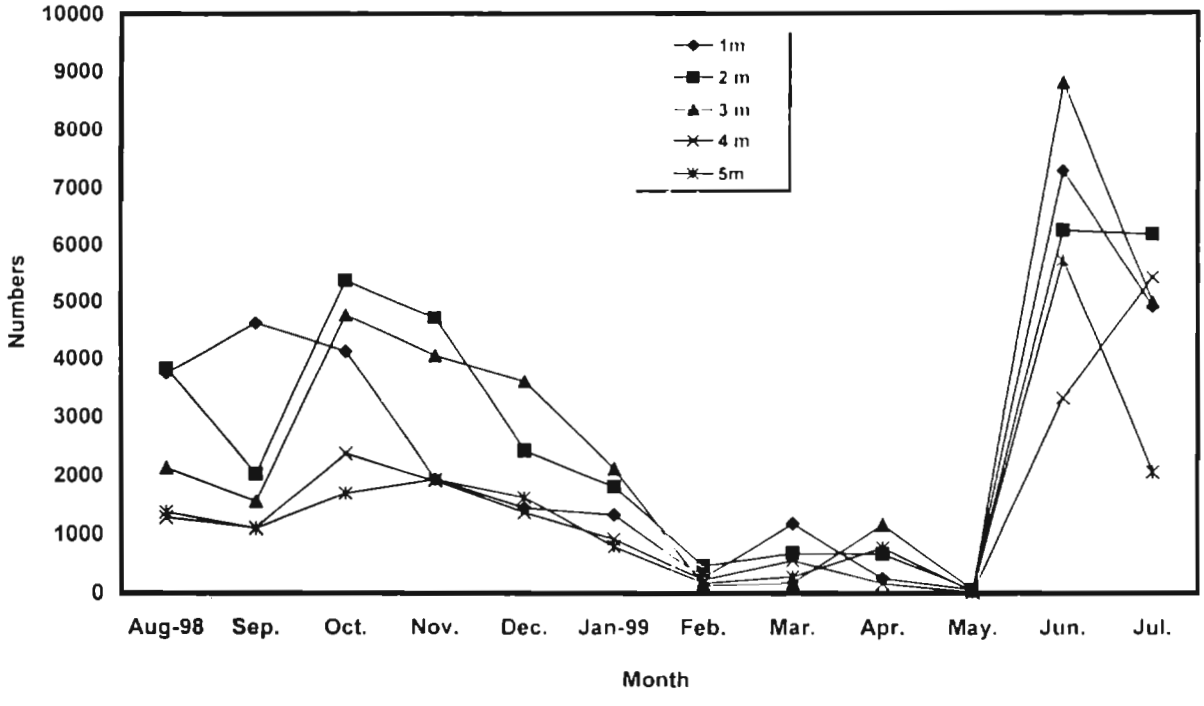


Figure 2.21. Seasonal variation of total weight of barnacles at different depths in pearl oyster farms in shaded panels

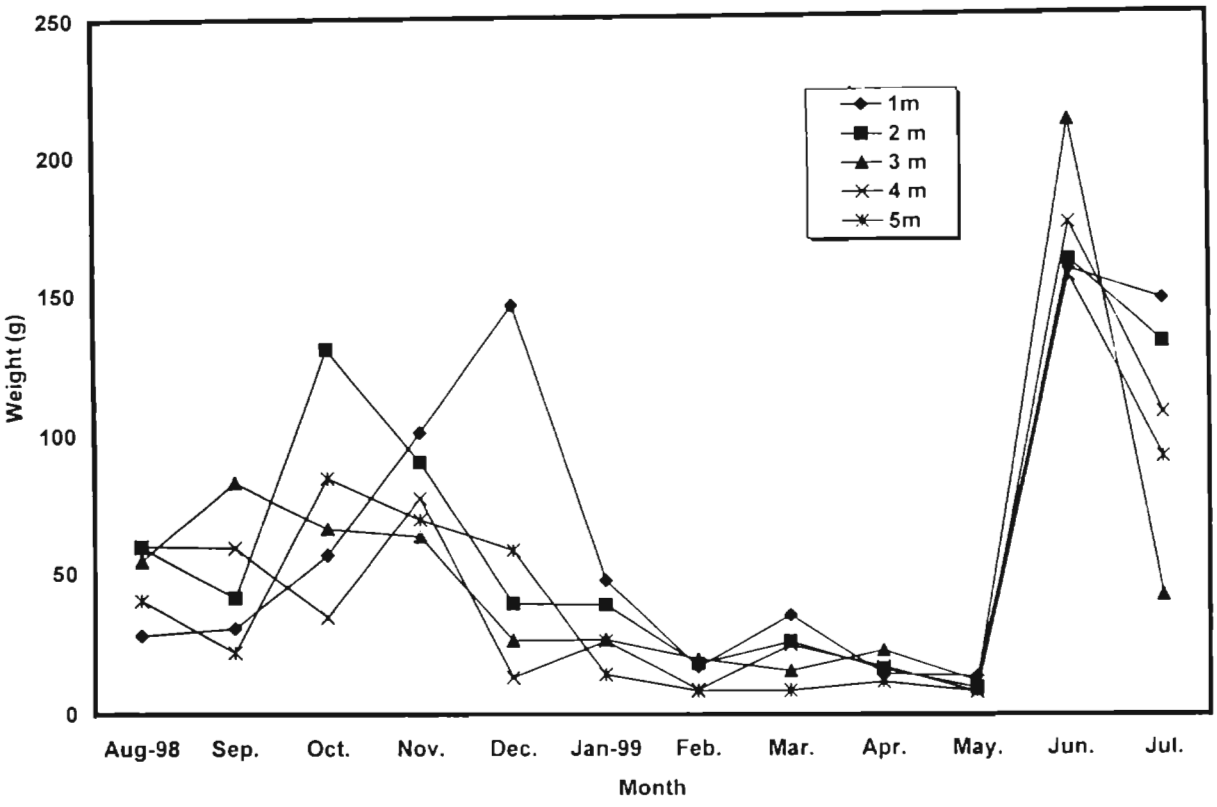


Figure 2.22. Seasonal variation of total weight of barnacles at different depths in pearl oyster farms in non shaded panels

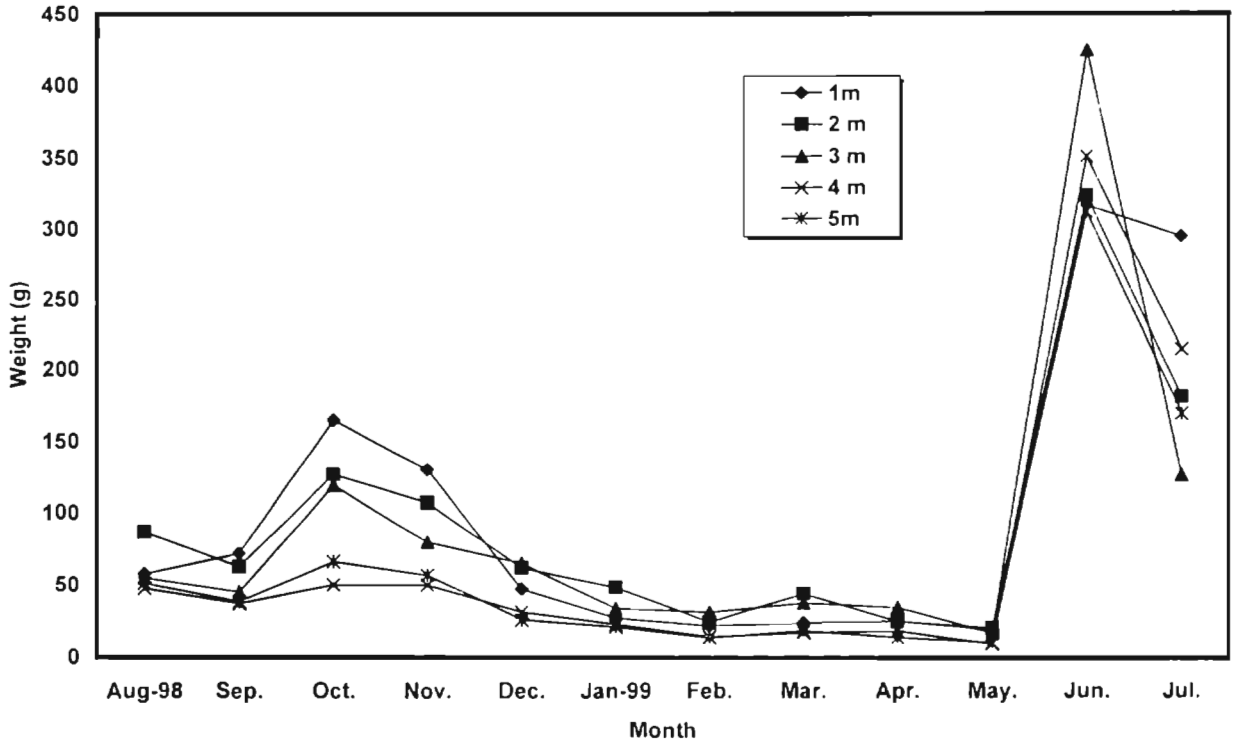


Figure 2. 23. Seasonal variation of total weight of fouling of other organisms (excluding barnacles) at different depths in pearl oyster farms in non shaded panels

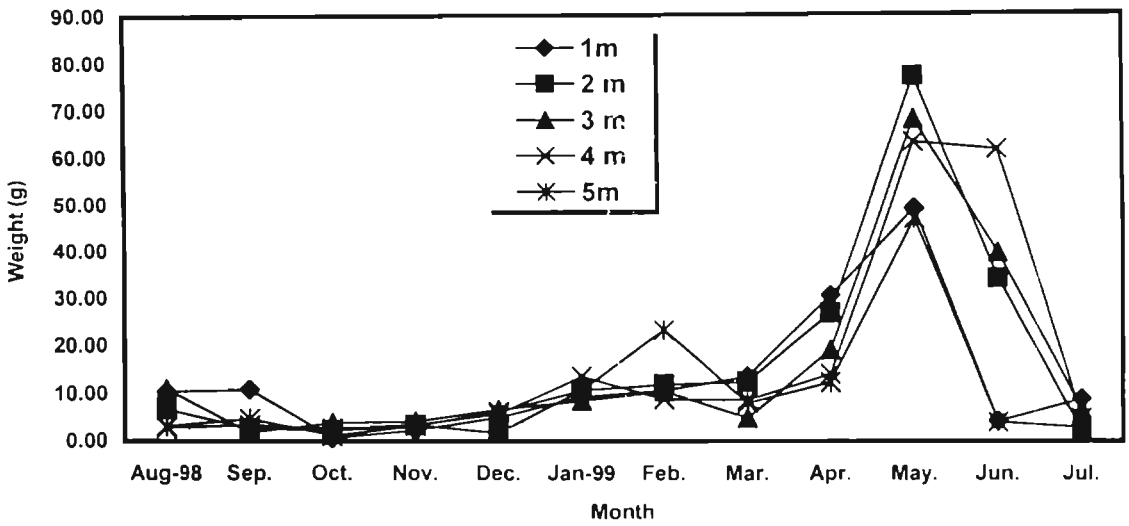


Figure 2.24. Seasonal variation of total weight of fouling of other organisms (excluding barnacles) at different depths in pearl oyster farms in shaded panels

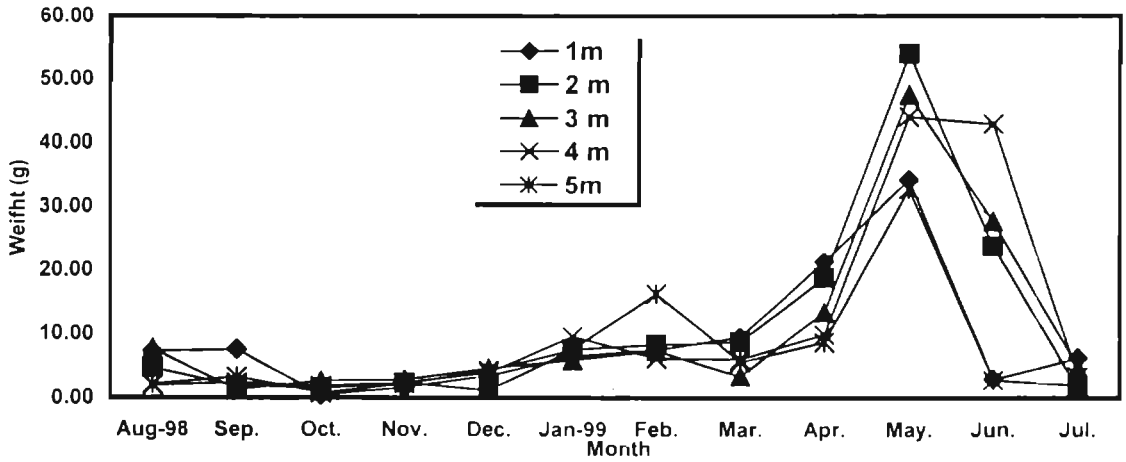


Figure 2.25. Seasonal variation of total weight of silt at different depths on the non shaded panels

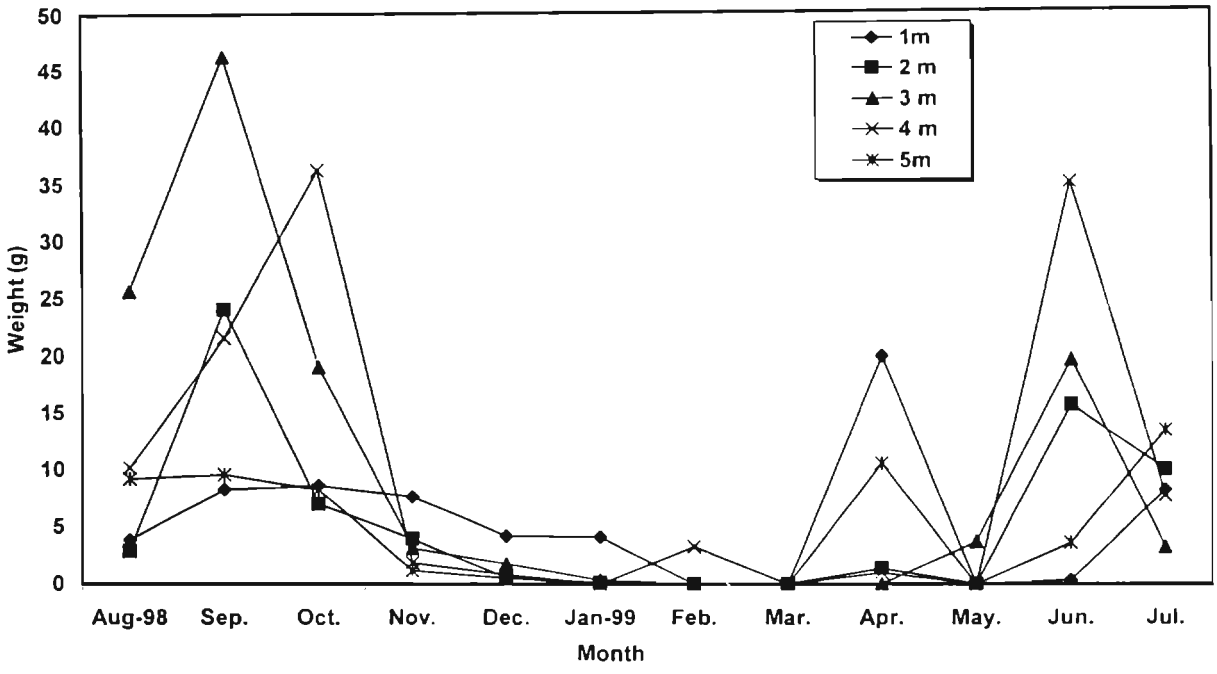


Figure 2.26. Seasonal variation of total weight of silt at different depths on the shaded panels

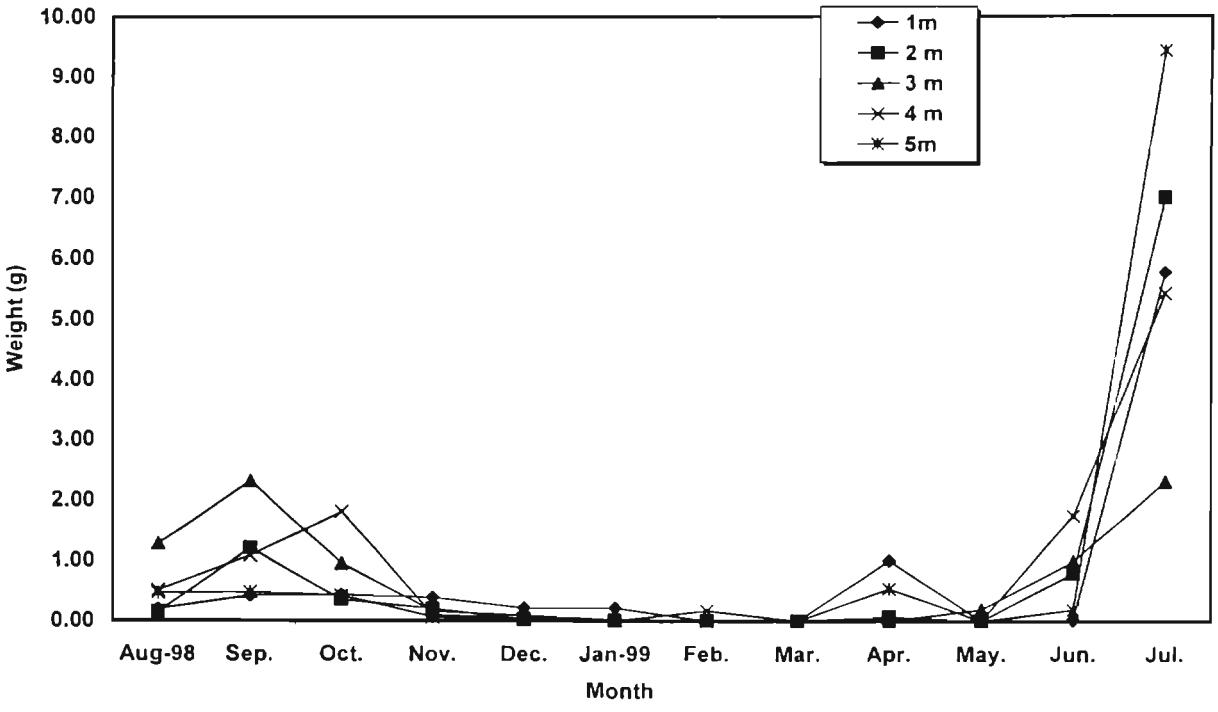


Figure 2.27. Seasonal percentage mortality of pearl oysters caused by fouling organisms at different depths in the pearl oyster farms

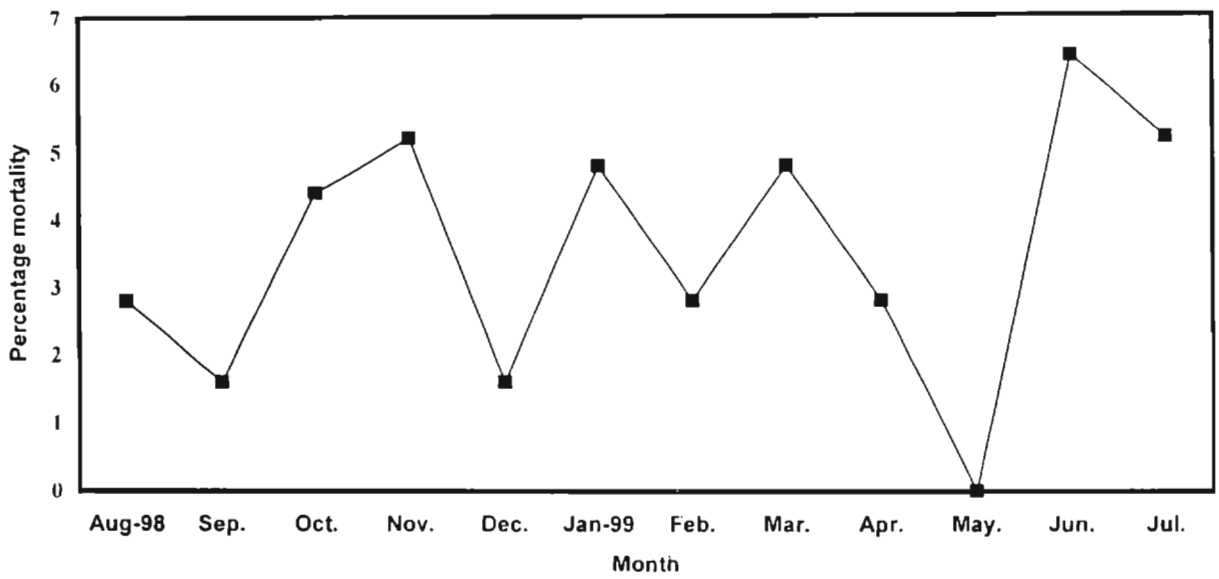


Figure 2.28. Depth wise percentage mortality of pearl oysters caused by fouling organisms at different depths in the pearl oyster farms

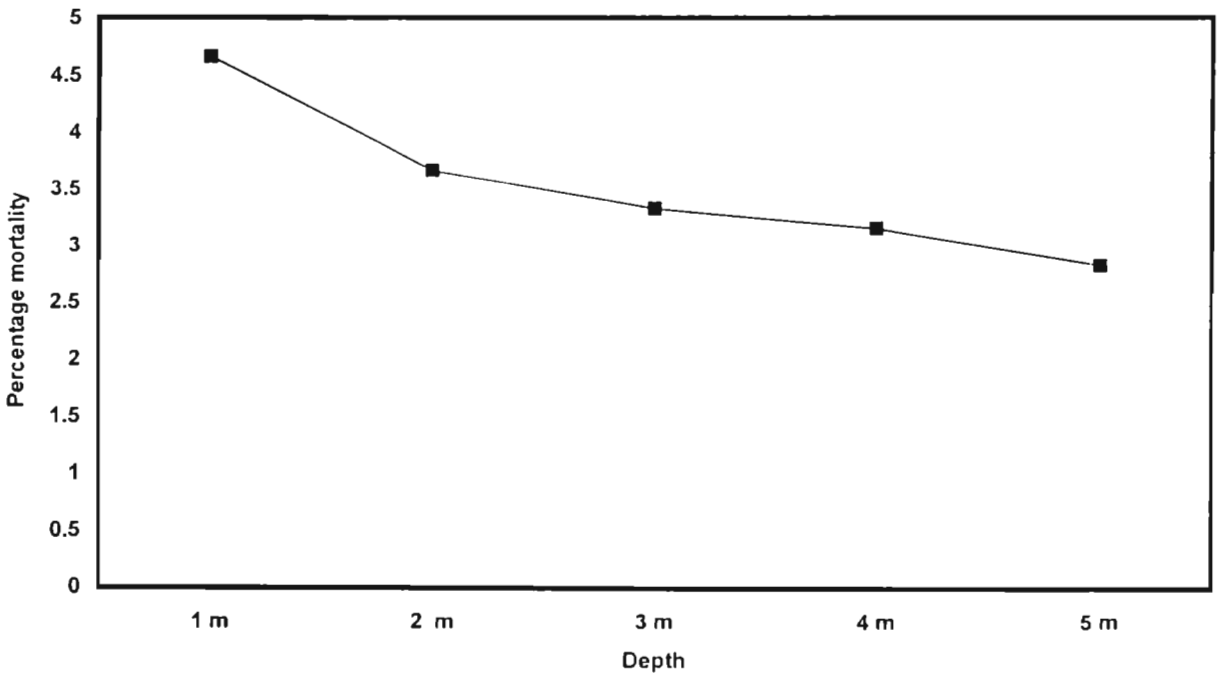


Table 2.1. Morphological characters of *P. fucata* from different parts of India (Mean ± Standard deviation)

Area	Length (DVM) (mm)	Hinge length (HL) (mm)	Depth (Thickness) (mm)	Width (APM) (mm)	Add. Muscle length (mm)	Add. muscle width (mm)	Upper shell weight (g)	Lower shell weight (g)	Upper shell thickness "A" (mm)	Upper shell thickness "B" (mm)	Lower shell thickness "B" (mm)	Lower shell thickness "B" (mm)
Gujarat	70.00 ± 0.00	65.26 ± 4.93	29.80 ± 0.34	67.72 ± 1.96	27.02 ± 0.42	14.67 ± 0.54	25.74 ± 0.82	28.71 ± 1.38	2.39 ± 0.18	1.23 ± 0.10	3.22 ± 1.19	1.18 ± 0.17
Vizhinjam	70.00 ± 0.00	55.00 ± 0.83	29.02 ± 0.60	63.11 ± 0.83	27.08 ± 0.43	14.68 ± 0.54	16.16 ± 2.22	11.48 ± 0.48	1.57 ± 0.10	0.77 ± 0.04	1.35 ± 0.04	1.06 ± 0.01
Tuticorin	70.00 ± 0.00	60.13 ± 2.54	29.41 ± 0.80	65.41 ± 2.62	27.05 ± 2.64	14.68 ± 1.64	20.95 ± 5.37	20.10 ± 0.89	1.98 ± 0.26	1.00 ± 0.31	2.28 ± 0.69	1.12 ± 0.18
Mandapam	70.00 ± 0.00	47.30 ± 1.70	25.05 ± 2.75	58.77 ± 1.99	19.05 ± 0.91	12.85 ± 1.25	8.48 ± 2.16	7.97 ± 1.04	1.69 ± 0.16	0.45 ± 0.11	1.98 ± 0.54	0.80 ± 0.19

Table 2.2. Shell and nacre colour of *P. fucata* from different parts of India

Area	Shell	Nacre colour	Periostracum colour	Hardness of the shell
Gujarat	Upper/Lower	Ivory cream/ Light cream yellow/yellow	Greenish/pale yellow	Hard
Vizhinjam	Upper/Lower	Ivory cream /Golden yellow/peacock/yellow	Reddish brown yellow on a pale yellow background	Medium hard
Tuticorin	Upper/Lower	Ivory cream /Golden yellow/peacock/yellow	Reddish brown yellow on a pale yellow background	Medium hard
Mandapam	Upper/Lower	Ivory cream/Golden yellow/ peacock/yellow	Reddish brown yellow on a pale yellow background	Less hard

Table 2.3 One Way ANOVA for hinge length of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	486.684	3.000	162.228	18.933	4.066	0.001
Within Groups	68.549	8.000	8.569			
Total	555.233	11.000				

Table 2.4 One Way ANOVA for Thickness of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	45.014	3	15.005	6.932	6.802	0.13
Within Groups	17.317	8	2.165			
Total	62.331	11				

Table 2.5 One Way ANOVA for width of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	120.556	3.000	40.185	10.456	4.066	0.004
Within Groups	30.747	8.000	3.843			
Total	151.302	11.000				

Table 2.6 One Way ANOVA for Adductor muscle length of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	154.386	3.000	51.462	25.194	4.066	0.000
Within Groups	16.341	8.000	2.043			
Total	170.728	11.000				

Table 2.7 One Way ANOVA for Adductor muscle width of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	9.174	3.000	3.058	2.534	4.066	0.130
Within Groups	9.652	8.000	1.207			
Total	18.826	11.000				

Table 2.8 One Way ANOVA for upper shell weight of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	558.029	3.000	186.010	19.019	4.066	0.001
Within Groups	78.241	8.000	9.780			
Total	636.270	11.000				

Table 2.9 One Way ANOVA for lower shell weight of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	819.29	3.00	273.10	271.74	4.07	0.01
Within Groups	8.04	8.00	1.00			
Total	827.33	11.00				

Table 2.10 One Way ANOVA for upper shell thickness "A" of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	2.41	3.00	0.80	24.27	4.07	0.00
Within Groups	0.26	8.00	0.03			
Total	2.67	11.00				

Table 2.11 One Way ANOVA for upper shell thickness "B" of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	1.073	3.000	0.358	11.708	4.066	0.003
Within Groups	0.244	8.000	0.031			
Total	1.317	11.000				

Table 2.12 One Way ANOVA for lower shell thickness "A" of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	5.796	3.000	1.932	3.543	4.066	0.068
Within Groups	4.363	8.000	0.545			
Total	10.158	11.000				

Table 2.13 One Way ANOVA for lower shell thickness "B" of P. fucata collected from different regions of India

Source of Variation	SS	df	MS	F	F crit	P-value
Between Areas	0.243	3.000	0.081	3.388	4.066	0.074
Within Groups	0.191	8.000	0.024			
Total	0.434	11.000				

Table 2.14. Fouling organisms settled on panels and Pearl oyster (*Pinctada fucata)*

Group & Species	Month of occurence
Plants *Cladophora sp.* *Codium tomentosum* *Enteromorpha compressa* *Ceramium sp.* *Gracilaria edulis* *Boergesinia forbesei*	April, September, October
Sponges *Haliclona exigua* *Callyspongia fibrosa* *Siphona chalina comminis*	Occasional
Hydroids *Abeitinaria greenet* *Companularia sp.* *Diphasia mutulata* *Lytocarpus sp.* *Obelia gracilis* *Sertularia sp.* *Thuiaria palans*	June, July/ throughout
Alcyonarians *Sarcophytum sp.* *Clavularia margaritifera*	June, July/ throughout
Actinaria Sea anemones	Occasional
Platyhelminthus *Planaria sp.*	Occasional
Bryozoans *Bugula neritina* *Bugula cuculata* *Electra bengalensis* *Thalamoporella rozeii* *Watersipora sp.* *Membranipora sp.*	February - June / throughout
Polychaetes *Unis antinnata* *Nereis sp.* *Hydroides norvevgica* *Serpula vermicularis* *Terebela ehrenbergi*	September - December
Cirripedia (Barnacles) *Balanus amphitnte communis* *Balanus amphitrite venustus* *Balanus amphitrite variegatus* *Balanus amphitrite tintinabblum*	June - July
Amphipoda *Caprella sp.* *Corphium triaenonys* *Gammarus sp.*	Throughout
Isopoda *Cirolana bovinar* *Sphaeroma walkeri*	June
Decapoda *Charybdis lucifera* *Pinnotheres sp.* *Matuta sp.* *Porcellana sp.* *Thalamita sp.*	Occasional

Continued in next page

Gastropoda *Aeolis sp.* *Cypraea sp.* *Olivia sp.* *Cymantium singulatum*	Occasional
Pelecypoda *Anomia achaens* *Avicula vexillum* *Modiolus metcalfei* *Martesia sp.* *Crassostrea madrasensis* *Pteria sp.* *Pinctada fucata* *Pinctada sugillata* *Pinna bicolor*	April - July, November
Tunicata *Ascidia sp.* *Botrylloides sp.* *Diplosoma macdonaldi* *Diandrocarpa sp.* *Depressiuscula sp* *Styela bicolor* *Symplegma vinde*	October - December / Throughout
Pisces *Blennius steidachneri*	Occasional in dead oyster shells

Table 2.15. Correlation between barnacle and other fouling organisms in the pearl oyster farm

Species settlement under different parameters	Correlation Coefficient (r^2)
Barnacle numbers (shaded & month)	0.6313
Barnacle numbers (non shaded & month)	0.6510
Barnacle weight (shaded & month)	0.6422
Barnacle weight (non shaded & month)	0.6621
Barnacle numbers (shaded & depth)	-0.9732
Barnacle numbers (non shaded & depth)	-0.9770
Barnacle weight (shaded & depth)	-0.8210
Barnacle weight (non shaded & depth)	-0.9514
Barnacle numbers (shaded & silicate)	0.8642
Barnacle numbers (non shaded & silicate)	0.8820
Barnacle weight (shaded & silicate)	0.8642
Barnacle weight (non shaded & silicate)	0.8820
Barnacle numbers (non shaded & silt)	0.2072
Barnacle numbers (shaded & silt)	0.2222
Accessory foulers (weight & month)	-0.5242
Accessory foulers (weight & depth)	-0.5555
Accessory foulers (weight & silt)	0.8315
Accessory foulers (weight & salinity)	0.5783
Accessory foulers (weight & clarity)	0.3839
Mortality of oysters & month	0.8183
Mortality of oysters & depth	-0.9396
Mortality of oysters & total fouling	0.9913
Fouling organisms & depth	-0.9913
Primary productivity & month	0.8183
Primary productivity & chlorophyll	0.8421
Primary productivity & silicate	0.8022

Table 2.16. Analysis Of Variance of number of barnacles at various months and depth

Souce of Variation	Sum of Squares	DF	Mean Square	F value
Main Effects	122882.416	15	8192.161	399.647
MONTH	120521.562	11	10956.506	534.502
DEPTH	2360.854	4	590.213	28.793
2-way Interactions	27257.125	44	619.48	30.221
MONTH DEPTH	27257.125	44	619.48	30.221
Explained	150139.541	59	2544.738	124.143
Residual	1229.911	60	20.499	
Total	151369.452	119	1272.012	

Table 2.17. Analysis Of Variance of weight of barnacles at various depths and shades

K Value	Source	DF	Sum of Squares	Mean Square	F Value
1	Replication	1	1164.501	1164.501	19.702
2	Factor A	1	11437.622	11437.622	193.51
-3	Error	1	59.106	59.106	
4	Factor B	11	279863.2	25442.109	579.96
6	AB	11	38413.378	3492.125	79.604
8	Factor C	4	5734.052	1433.513	32.677
10	AC	4	652.867	163.217	3.7206
12	BC	44	20856.178	474.004	10.805
14	ABC	44	9356.585	212.65	4.8474
-15	Error	118	5176.537	43.869	
	Total	239	372714.026		

CHAPTER 3

BIOLOGY OF PEARL OYSTER *Pinctada fucada* (GOULD)

CHAPTER 3

BIOLOGY OF PEARL OYSTER *P. fucata* (GOULD)

3.1 INTRODUCTION

Reproduction in continuously breeding tropical species of bivalve molluscs is not likely to be of the same intensity throughout the year. However, close examinations shows that several populations have periods of intense reproduction (Giese and Pearse, 1974). A breeding season usually lasts for one month or so followed by a period of another month or more of almost no reproductive activity in almost all the individuals. Many tropical species have a bi or semi annual breeding season, which is characteristic of areas influenced by monsoons (Paul, 1942; Antony 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. Although some of these factors affecting the reproduction of bivalves have been investigated experimentally, most information has been through field observations (Seed, 1976; Seed and Suchanek, 1992).

Seasonal gonad developments of molluscs restrict the spawning to a particular seasons of the year. Molluscs spawn naturally during certain seasons in a year when the environmental conditions are congenial for this activity. This physiological characteristic generally limits the process of seed production and availability to certain particular periods, which is highly disadvantageous in the commercial culture of molluscan species. In this context, the concept of induced maturation gains importance as the process can be advantageously controlled for a prolonged period of seed production. For any successful hatchery technology, the most important aspect is the complete control of the life cycle of the candidate species under captivity. The

first step in this direction is the successful induction of maturity in captivity. In the present study induced maturation refers to the accelerated gonadal development using different techniques to achieve sexual maturity, so that they can be used for seed production even when they are relatively young and out of the spawning season.

The term conditioning has been used in literature in tandem with induced maturation and is a little confusing. Several of the early works and some recent ones have used this term for induced maturation experiments. The various techniques of hatchery conditioning of broodstock of bivalves have been reviewed recently (Utting, 1993, Utting and Millican, 1997, 1998). The technique of maturation and spawning of bivalve molluscs out of season was revolutionized by Loosanoff and Davis (1950, 1952, 1963) in *Venus mercenaria*. In pearl oysters it was done in *P fucata martensii* (Kuwatani and Nishii, 1968; Kuwatani *et al.*, 1974) and very recently in *Pinctada mazatlantica* (Saucedo *et al.*, 2001).

Preliminary observations on biology and growth of Indian pearl oyster *P. fucata* have been undertaken by many workers (Chacko, 1954, 1956, 1957; Devanesan and Chidambaram, 1956; Alagaraja, 1962; Anandharaman, 1967; Narayanan and Michael, 1968; Chellam, 1978, 1987, 1988; Achary, 1982; Appukuttan, 1987; Velayudhan *et al.*, 1996).

Herdmann (1903) has studied the early development of the pearl oyster *P. vulgaris* =(*P. fucata*). Some preliminary works on the larval development of *P. fucata* was done by Alagarswami *et al.* (1980), the larvae could be reared only upto the straight – hinge stage, subsequently Alagarswami *et al.* (1983 *a*) produced successfully pearl oyster spat in hatchery.

The present work attempts to study the reproductive cycle of the pearl oyster, which will be helpful to collect mature specimens for induced maturation and spawning experiments. The work on larval development was done with a view to developing techniques for the rearing of commercially important pearl oyster *P. fucata*, and also to elucidate the principles and

problems of tropical bivalve larval rearing in general for further investigations in the future.

3.2 MATERIALS AND METHODS

3.2.1 Study Area

All the experiments of induced maturation, spawning, larval rearing and settlement, juvenile and adult growth were conducted in the shellfish hatchery (Plate 3.7) and the pearl oyster farm (Plate 2.6B) at Tuticorin Research Centre of C.M.F.R.I, Tuticorin

3.2.1.2 Maintenance of Seawater Quality

Seawater for all the experiments was the Gulf of Mannar pumped into a settling tank (Plate 3.1A) and then by gravity induced biological filter bed (Plate 3.1B) and by rapid flow through a rapid sand filter. Filtered seawater was stored in a concrete sump from where it was distributed to all parts of the hatchery through PVC pipes. For induced maturation, larval rearing, raw sea water was collected during high tide from the Tuticorin Bay, Gulf of Mannar from a seawater well.

3. 2.1.2 Water Quality Parameter Estimations

Water samples were collected daily from the rearing containers (tanks and beakers). Air and water temperatures were noted with a thermometer. The water quality parameters like salinity, pH, Dissolved Oxygen, Ammonia, Hydrogen Sulfide were estimated according to Strickland and Parsons (1972).

3.2.1.3 Induced Maturation Experiments

3.2.1.4 Selection of Animals

Pearl oysters from the same brood were selected to minimize errors related to age and genetic factors (Plate 3.2A). The size of the individual was 40 ± 2 mm in (DVM). The epifauna were removed by brushing the surface without damaging the growth process es of the oysters. Only those animals without sponge and boring sponges and polychaetes infestation were selected. Then gonad of the oysters was checked and the stages identified and fixed as per the classification of Chellam (1987). Briefly

the male and female gonads were classified into different maturity stages as follows (Plate 3.10 – 3.12)

Stage I: Inactive /spent resting (Indeterminate)

Stage II: developing /maturing

Stage III: Mature (ripe or Mature)

Stage IV: Partially spawned (Partially spent)

Stage V: Spent

3.2.1.5 Experimental Protocol

The maturation of gonad in respect to different types of feed was studied in the hatchery where pearl oysters were maintained at a temperature of 23 ± 1 ° C in an air conditioned brood stock conditioning room (Plate 3.2B). Maturation of gonad under natural conditions was also noted. The experimental design using the different types of feed is given below. 1. Mixed algae, 2. Mixed algae + Corn flour, 3. Mixed algae + raw rice flour, 4.Oysters maintained in the farm and 5. No feed (control)

For each experiment, animals of the same brood were used (Plate 3.2A). All the experiments were done in quadruplicates with 50 oysters in each set. Five animals were sampled from each set at weekly intervals and the stages of gonad were noted by sacrificing the animals and examining the gonad smears under the microscope. The results were calculated as percentage for each gonadal stage.

3.2 .1 .6 Preparation of Experimental Feeds

3.2 .1.7 Microalgal Culture

Microalgae *Isochrysis galbana*, *Chaetoceros calcitrans* were used as feed for different experiments. These algal strains were locally isolated from the bay water off Tuticorin and maintained in filtered heat sterilized sea water as pure algal stock cultures in 5 l Haffkine flasks maintained in low temperatures (24 ± 1° C) under a fluorescent lighting of 12 hour cycle (2000 lux) (Plate 3.3). Depending on the need, aseptic 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 ton white bottomed FRP tanks were also maintained for the use in conditioning and induced maturation experiments (Plate 3.2B).

3.2.1.8 Preparation of Raw Rice and Corn Flour

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. A similar procedure was followed for corn flour feed preparation also. However in this case no grinding was done.

3.2.1.9 Feeding Protocols

For each experiment one control was also kept. For each experiment 4 replicates each with 50 animals were used. The oysters were reared in 150-200 l capacity FRP tanks in 100 l of seawater. The pearl oysters were fed 2 l (cell concentration of 3.4×10^{6} cells ml^{-1}) of mixed phytoplankton; with 90 % *Chaetoceros calcitrans* was fed twice at 8 am in the morning and 6 pm in the evening.

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 other procedures followed were those that followed for the previous experiments along with mixed algae. Whereas in this case the ground food was passed through a 50 µm nylobolt cloth to the rearing tanks and proper aeration was provided for the suspension of the ingredients in the water column.

Controls without feed was also maintained to understand whether there is any maturation of animals under unfed conditions.

The animals were maintained under natural condition in other words they were in wild condition but under captivity.

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 other procedures were followed as per the previous experiments to assess the condition of the gonad of the oysters.

3.2.1.10 Brood Stock Maintenance in The Lab and Farm Conditions.

For finding out the regression of the gonad from stage III to stage IV, this experiment was conducted. Here 200 ripe (Stage III) pearl oysters (50 x 4 replicates) were maintained in the lab conditions at low temperature of 23 ± 1 ° C in air conditioned room for studying the regression of the stages of the maturity of gonad under unfed and fed conditions where a mixed micro algal feed (feeding protocols similar to the induced maturation experiments) was given. A similar set of oysters with the same stage was also maintained in the farm. The sampling and gonad examination was similar to the earlier mentioned induced maturation experiments.

Simultaneously in the farm maintenance experiments, a total of 200 oysters (50 per 40x 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 suspended from the raft in the farm for periodical observations. The sampling and gonad examination was similar to those mentioned in the induced maturation experiments.

3.2. 1.11 Larval Rearing and Settlement of Spat

3.2.1.12 Larval Rearing

In the present experiment 30 pearl oysters with mature (10 Males: 20 females) were used. The ripe oysters were thermally stimulated to spawn by increasing the temperature from 28.5 ° C to 32.5 ° C (30 minutes to a maximum of one hour duration using a (Jumo -thermometer 0-50 ° C and silicon cased gel heating mantle with temperature adjusted at 32.5 ° C) as per the protocols of Alagarswami *et al.* (1983 *b*) (Plate 3.4).

3.2.1.13 Measurement of Larvae

The beakers and tanks were checked for the presence of larvae. The streak formed in the corners of the tanks or periphery of the beakers were observed .The floating larvae were siphoned gently into 30 µm nylobolt sieve kept inside another clean plastic bucket to reduce the pressure excreted in the minute meshes of the sieves and clogging of the sieves by the larvae. This may in turn reduce the damage to the shells of the larvae. When sufficient quantities of larvae were caught in the sieve the sieve with the larvae is released gently into another 5 l beaker containing filtered seawater.

After releasing the larvae in the beakers the level of the water in the beaker is adjusted to a known volume. Using glass rod the larvae with water in the beaker was mixed thoroughly and 1 ml from the beaker transferred to a Sedgewick rafter counting chamber and one drop of 5% formalin was added to kill. The meristic characters of these larvae were recorded. The counting process was repeated for 3 times and averages were worked out to estimate the total number of larvae in each beaker. The calculated quantities of the larvae from the general stock were measured and released in each container.

The dorso ventral (DVM) and anterio- posterior (APM) measurements of 60 larvae sampled randomly were done with a calibrated ocular micrometer.

3.2.1.14 Rearing of Spat

Two hundred settled spat of 295.5 µm was segregated and reared @ 2 spat / beaker in 100 ml glass beakers with 80 ml seawater. The spat were reared for 37 days (reaching a size of 4.2 mm) till they were ready to be transplanted to the farm for further rearing.

3.2.1. 15 Larval Feeding

The micro algae used to feed the pearl oyster larvae were siphoned out from 20 l or 50 –75 l capacity perspex tanks containing the algal culture. Just before feeding, the feed was checked for any contamination,

especially the presence of ciliates. A sample of Isochrysis *galbana* was pipetted into a glass vial and fixed by using Lugol's iodine fixative. Since the cells of Isochrysis is motile and comparatively very minute in size, an estimate of the number of algal cells per ml of the culture was made by using a blood counting chamber/ haemocytometer. Depending on the experimental algal density chosen for algal rearing, the amount of culture to be added to the medium was calculated. The larval feed was maintained @ 10^3 /ml, @ 3 x 10^3 and 4 x 10^3 cells ml^{-1} in the rearing medium (Veliger to Umbo, Plantigrade to Spat and till 30 day after spat setting respectively) during the process of experiment. After spat settlement mixed feeding with *I. galbana* was continued till the spat was transferred to the farm.

3.2.1.16 Feeding of Spat

For the phase II growth, the settled spat were fed with a mixed diet with the 30,000 cells spat $^{-1}$ day $^{-1}$ (increased to 50,000 cells spat $^{-1}$ day $^{-1}$ after 15 days) *I. galbana* and an equal proportion of mixed algae consisting of Chaetoceros *calcitrans* , *Pleurosigma sp., Nizschia sp.* and *Skeletonema sp.* Were fed for a period of 37 days after settlement (day 23rd onwards after settlement). All the containers were aerated. When the spat reached 2-3 mm sizes, *Isochrysis galbana* was not offered as food. Only mixed algae in equal proportion was given @ 60,000 cells spat $^{-1}$ day $^{-1}$. This was continued till the end of the experiment.

3.2.1.17 Experiment in Tanks (Larger Containers)

Larvae were reared in two numbers of 50 l FRP tanks. Each tank was filled with 30 l of filtered seawater and totally 120,000 larvae were released at a larval density of 2 larvae ml^{-1}. The water was replaced on alternate days. During water changing the larvae were collected in 30- 40 µm, 80µm, 110 µm, 180 µm and 200 µm sieves according to the size of Veliger, umbo, eyed pediveliger and plantigrade /spat stages respectively. The larvae collected in the sieves were kept immersed in fresh seawater and washed gently by adding fresh filtered seawater into the sieves and the larvae were released in to the respective marked tanks containing required quantities of

filtered seawater. Merestic features like Dorso ventral (DVM) and Anterior Posterior (APM) measurements and the survival rates were also noticed (Plate 3.6A).

3.2.1.18 Experiment in Smaller Containers

Controls were reared in four 10 l glass beakers with 7.5 l filtered seawater. The density of the larvae was maintained at 2 larvae ml $^{-1}$ of water as in the case of previous experiment and totally 60000 larvae were reared in this experiment. The beakers were marked individually and kept half dipped in 50 l capacity FRP tanks holding 20 to 30 l seawater (The method followed was to keep the water temperature constant), and the whole tanks with 4 beakers were covered with black cloth to prevent debris falling into the tanks and also to reduce the light intensity and hence certain multiplication of microalgal feed in the rearing tanks.

All procedure like rate of food supply growth of larvae were done as followed in the earlier experiments. The larvae were reared in sand filtered seawater and no aeration was provided till settlement. The water was changed once in two days through 30, 40, 80, 110, 180 200 µm sieves during veliger, umbo, eyed, plantigrade and spat stages respectively. The growth of the larvae in each experiment was recorded once in 2- 7 days soon after the removal culture medium. Normally 3-50 larvae were sampled at random and measured.

3.2.1.19 Studies on the Growth Rate of *P. fucata*

To understand the rate of growth of *P. fucata* at different growth phases, the growth of larvae from the spawning to settlement (**Phase I**), the growth of the spat 295.5 µm to a size of 4.2 mm (**Phase II**). The growth of spat up to the adult stage of (24 months) was studied both individually and randomly (Plate 3.8A, B). For all the growth studies the larvae spawned from the same brood was used. The growth studies in Phase I and Phase II was done in the hatchery while Phase III was done in the farm. The growth studies were undertaken from August 1998 to July 1999.

3.2.1.20 Rearing of Spat in the Farm (Phase III)

3.2.1.21 Individual and Random Growth Studies

Two mm mesh velon screen of 5 m length was cut at 20 cm width and folded length wise and stitched in such a way to make five 10 x 10 cm wide individual compartments (Plate 3.9A, B). The folded screen was stitched in such a way to form compartments and to open it out towards the cut end of the velon screen pouch. Each 5 m velon screen pouch can hold 50 spat in separate pockets making it easy to take individual measurements. Four such 5 m length pouches were used to rear 200 selected spat for the study.

After taking initial measurements the spat was released in each pouch (Plate 3.8B) with respective token and in some instances tagging was done by using dymo letro tape and the adhesive anabond /feviquick to fix number on the spat. Each spat was put in each pouch with the respective number and stitched to avoid the falling of the spat from the pouch and to avoid other animals from entering the pouch. Two pouches each holding 50 spat were released in a box type cage 40 x 40 x 10 cm dimensions and lid netted with 2 mm synthetic thread. The boxes were again inserted into another bag made out of 10 mm mesh fish net. This serves as a protection to the velon screen with pearl oyster spat from predators like crabs and fishes.

In another experiment thirty spat were measured randomly from the lot of 200 spat. After taking initial measurements, the spat were released @ 100 numbers (2 replications) in 2 similar cages as in the previous experiment. The other procedures were followed as in the individual growth experiments. The experiment was carried out for 24 months.

For both the experiments, on 15th day the cages and velon screens with spat were gently cleaned of the silt and other fouling organisms which hinter the growth and survival of the spat. The process was continued till the spat reached a size of 20-30 mm in DVM. Monthly morphometric measurements were taken, where the length (DVM) dorso ventral measurement, HL (hinge length), Depth (thickness) and total weight (W) of

the animals were recorded for statistical analysis and growth rate studies. The length, hinge length and depth were taken using a digital Vernier Calipers corrected to 0.002 mm. The total weight of the oyster was taken corrected to 0.01 g with the help of an electronic balance.

3.2.1.22 Statistical Analyses

One way Analysis of Variance (ANOVA) using Microsoft Excel computer software was also done to find out the best treatment for inducing maturation of *P. fucata* in captivity. One way ANOVA was also done for the percentage survival and percentage settlement (after arcsine transformation of the percentages) and rate of during the three phases. A one way ANOVA was also used for comparing the growth in individual and randomly measured oysters in Phase III to find out whether there was any significant difference ($p < 0.05$) between the various treatments

In such cases where the treatments were significant then the F value would be significantly different. Then the best treatment was found out through pair wise (Students t – test ($p < 0.05$)) comparison of treatment using Critical Difference (CD).

Regression analysis using SPSS computer software was done to find out the relationship between shell lengths (DVM) (only for Phase I), Hinge (HL), Depth (DEP), Weight (WT) with age. The results are presented in scatter diagrams with fitted lines. The significance of the relationship was found out using correlation coefficient (r^2).

3.3 RESULTS

3.3.1 Induced Maturation in the Laboratory

Fully matured pearl oysters were obtained on day 43, 42 and 36 in oysters fed with mixed algae, (feed1) mixed algae and corn flour (feed2) mixed algae and rice flour (feed3) respectively under laboratory conditions. Whereas pearl oysters reared in the farm matured fully on the15th day. None of the non artificially fed oysters matured in the laboratory (Figure 3.1). The maturity stage II attained in different treatments is given in (Figure 3.2) Among the treatments it was observed that the treatment of mixed algae with raw rice flour gave the best results with pearl oysters maturing in laboratory conditions on the 29th day (Figure 3.2).

The water quality parameters did not record any perceivable difference. The values noted were salinity: (35 ± 1 ‰), dissolved oxygen: (4.8 ± 1 ml l^{-1}) and pH: (8.1), Ammonia: (0.00136 ± 0.01 ppm) and H_2 S: (nil.) for the rearing water.

3.3.1.1 Holding of Ripe Pearl Oysters in the Laboratory

Of the matured animals (stage III), fed with mixed algae, 43.33 % changed to stage IV within 19 days in the laboratory conditions, while the batch of (stage III) pearl oysters not fed with supplementary feed 40 ± 14.14 % changed to stage V within 26 days under laboratory conditions (Table 3.2). The water quality parameters did not record any perceivable difference. The values noted were salinity: (35 ± 1 ‰), dissolved oxygen: 4.8 ± 1 ml l^{-1}) and pH: (8.1), Ammonia (0023 ± 0022 ppm) and H_2 S: (nil).

3.3.1.2 Holding of Ripe Pearl Oysters in the Farm

Maturity (stage III) of 53.12 ± 11.97 % pearl oysters did not change when maintained in the farm within 24 days in the farm conditions (Table 3.2).

3.3.1.3 Larval Rearing

In all cases the males responded first to induced spawning by thermal stimulation (Plate 3.4).

The sizes and days given for the different larval and post larval stages above are those at which they appeared in fairly good concentrations in the rearing experimental and control condition .The results are given in (Table 3.3).

3.3.1.4 Straight – hinge Stage

The straight –hinge larva measures 67.5 µm along the antero-posterior and 52.5µm along the dorso-ventral axis). The D-shaped shell of the veliger (prodissochonch I) is transparent with conspicuous granules (Plate 3.5.7). The velum and other organs are heavily granulated.

The early straight- hinge larva measures an average size of 50.83 ± 1.89 x 77.67 ± 2.5 µm (Table 3.1A). Some of the largest larvae measure 55 x 75 µm in DVM and APM respectively. This stage is reached 20 h 40 minutes after fertilization and they grow to about 84.2 ± 3.48 x 96.53 ± 3.82 µm. with an average growth of 3.7 µm / day for 9 days Some of the largest larvae measure 90 x 100 µm in overall growth while 73.33 % were in the D-shape, 83.04 ± 3.07 x 95.5 ± 3.86 µm and 26.67 % were in the early Umbo stage with 87.38 ± 2.26 x 99.28 ± 1.77 µm at the end of the 9th day in (DVM and APM respectively) (Table 3.1B).

3.3 1.5 Umbo Stage

Transition from straight-hinge to Umbo stage is gradual and commences with the shell assuming roundish shape (Plate 3.6A). The early Umbo stage start from day 9 and typical umbo was seen from the 10th day onwards. The average size of the larvae was seen to increase up to day 20. The average size of the umbo larvae was 117.7 ± 7.84 µm x 123.89 ± 7.64 µm (Table 3.1C).

3.3.1.6 Eyed Stage

On day 15 the larva measures 158.75 ± 66.29 x 190 ± 7.07 µm (13.33 %) and develops eyespot which becomes deeply pigmented (Plate 3.6B). It persists even after metamorphosis and is visible in a spat upto 950 x 1055 µm till day 35 (16.67%) (Table 3.1D).

3.3.1.7 Pediveliger Stage

The development of a functional foot is seen on day 17 onwards when the larva attains an average size of 203.33 ± 5.77 x 213.33 ± 5.77 µm (10%). The pediveliger stage persisted five days (Plate 3.6B.3; Table 3.1E).

3.3.1.8 Plantigrade

The pediveliger, on finally settling on the substratum at the end of its wandering phase, metamorphoses into a plantigrade (Bayne, 1976) (Plate 3.6C), which begins to lead a crawling and a sessile life on day 20 onwards when it measures an average size of 215 ± 7.07 x 225 ± 7.07 µm (6.67%) (Table 3.1F).

3.3.1.9 Spat and Spat Setting

The plantigrade larva develops the characteristic adult shell and transforms to a young spat. The hinge line, anterior and posterior auricles and the byssal notch assume specific shape. With further deposition of shell materials the globular shape will be lost. The left valve is slightly more concave than the right valve. The spat attaches itself to the substratum with byssus threads and lies on one side. The typical *Pinctada fucata* spat is recognized at size 225 ± 3.39 x 236.7 ± 2.58 µm (20 %) on the 20^{th} day onwards (Plate 3.6C; Table 3.1G).

No special spat collectors were provided for the spat to settle. Dense spat setting occurred on the FRP tank bottom (Plate 3.7B), and the mean setting rate in the two tanks in experiment 1 was 1.257 spat / cm^2. The

top corners of the tank also showed good spat fall but the sides had only sparse settlement.

In both the treatment (tank) and control conditions, the settlement was high on the bottom corners of the tanks. The percentage survival and settlement was 64.4 ± 0.76 % in the tank whereas it was 24.3 ± 2.43 % in the beaker (control) in Phase I while the percentage survival was 71± 3.0 %, 86.67 ± 1.2 % in the tank and beaker respectively in Phase II, while 28 ± 0.85 % survived at the end of Phase III (Table 3.3).

One Way ANOVA showed that percentage survival and settlement was significantly better in the tank than in the control (beaker) (Table 3.5 – 3.6).

3.3.1.10 Larval Growth

From D –shell (day1) to umbo stage (day 12), the larvae grew from a mean size of 50.83 ± 1.89 µm to 141.27 ± 13.09 µm, an average growth of 7.5 µm / day. On day 15, the mean size of the total larva was 150 x 167.33 µm and that of the eyed larva 158.75 x 190 µm, with a growth of 7.19 µm DVM /day and 5.97µm /day respectively, between days 1 and 15. Pediveligers at 203.33 x 213.33 µm appeared on day 17, Plantigrade at 215 x 225 µm on day 20 and young spat at 225 x 236.7 µm on day 20. There was differential growth of larvae in each batch. On day 21 there were eyed larvae 6.67 % (mean DVM 187.5 µm), Pediveligers (3.33%, 200 x 220 µm), plantigrade (3.33 %, 210 x 240 µm) and spat (86.67 %, 302.88 x 340.9 µm) in the same type of tanks. The individual growth of each stage is given below (Table 3.4).

3.3.1.11 Studies on Growth

Phase I. The growth rate was of 12.1 ± 0.67 µm day $^{-1}$ in tank was significantly better than 10.63 ± 0.85 µm day $^{-1}$ in beaker (control) (Figure 3.4; Table 3.3). The regression analysis of larval growth showed that growth of experimental animals significantly differed from that of the control. The regression values were as follows.

DVM = 48.82 x exp (0.073 x age), APM = 57.304 x exp (0.069 x age) r^2 = 0.9412; 0.9520.

DVM = 46.65 x exp (0.079 x age), APM = 54.72 x exp (0.077 x age) r^2 = 0.9165; 0.9500 respectively.

In the **Phase II** of growth from spat (23rd day) to 60 day old spat, the growth rate was 120.83 ± 3.1 µm day $^{-1}$, in the tank (Table 3.3; Figure 3.4).

Whereas in the final phase, **Phase III** (day 60 to 2 years), The growth rate was 64.4 ± 2.1 µm day $^{-1}$ (Figure 3.4). The spat growth regression analysis for DVM, Hinge length (HL) Depth (Dep) and Weight (Wt) with age was calculated. The data obtained are given below.

DVM = 57.43 x (1-exp (-1.0576 x age) r^2 = 0.9863

HL = 53.291 x (1-exp (-1.1893 x age) r^2 = 0.9822

Dep = 42.663 x (1-exp (-0.3749 x age) r^2 = 0.9898

Wt = 17.9988 x age (-56005)

For **the random measured** spat (cage reared) the regression values were

DVM = 64.247 (1-exp (-0.8942 x age) r^2 = 0.9755

HL = 53.0482 (1-exp (-1.1893 x age) r^2 =0.9792

Dep = 39.7754 (1-exp (-0.4466 x age) r^2 =0.9820

Wt = exp (4.492 – 2.1313/age)

The corresponding scatter plots and fitted lines for each parameter is given in Figures 3.5 – 3.14

3.4 DISCUSSION

The present studies 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 too fed rice powder as a source of diet for maturing adult pearl oysters *P. fucata martensii* and observed during the same duration. 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 starch occurring in the species of algae in estuaries and sea may be important in development of meat of lamellibranch. He further stated that this might be due to the presence of the enzyme amylase, which is capable of hydrolyzing starch. Thus the induced maturation of *Pinctada fucata* obtained in this study can be attributed to the influence of starch in the diet.

Experimental results on induced maturation 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, 1965; Hrs – Brenko, 1973; Bayne *et al.*, 1982; DiSalvo *et al.*, 1983; Sprung, 1984 *a – d*; Wilson *et al.*, 1996; Numaguchi, 1997; Utting and Millican, 1997, 1998; Buchannan *et al.*, 1998; Kent *et al.*, 1998; Trotia and Cordisco, 1998; Jeffs, 1999) and also pearl oysters (Saucedo *et al.*, 2001).

In the present study maturation was obtained in specimens maintained at low temperature of 23 ± 1 °C. Conditioning and maturation of broodstock at low temperature to mature has been successful also in *P. mazatlanica* (Saucedo *et al.*, 2001) and also in many bivalve species (Nayar *et al.*, 1984, 1987, 1988; Velez and Epifano, 1978; Palaniswamy and Sathakathullah, 1992; Nair, 2001). However, the results of this study are in contrast to the results of other workers who conditioned bivalves to mature in ambient temperatures (27 – 31° C) (AQUACOP, 1979; Siddall, 1980;

Ajithakumar, 1984; Coeroli *et al.*, 1984; Sreenivasan *et al.*, 1988; Gallardo *et al.*, 1992; Chotipuntu and Pongthana, 2000).

In this study, it was observed that maturation of the gonad occurred on 15 in the farm and in 37 days in the laboratory. This is in agreement with the observations of, Haven (1965) and Gabbot and Walker (1971) of Paolobreber (1981) and Nair (2001) for other bivalve species.

According to Gabbot and Walker (1971) two factors, which differentiate the hatchery, conditions from field conditions are temperature and the quantity 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 from body reserves. Pouvreau *et al.* (2000) suggested that in a tropical environment particulate organic matter is the main determined factor for gametogenesis and temperature has only limited influences. Moreover the quantity of food they obtain is dependent on the density of the food organisms and the pumping rate (filtration rate) of the bivalve concerned. Tranter (1958 a-d, 1959) observed that generally pearl oysters take 7-8 months for gonadal development in the temperate waters of Australia.

Ripe *P. fucata* was held in the farm and laboratory conditions (in temperature of 23 ± 1° C) for 24 and 19 days respectively in this study. Alagarswami *et al.* (1987) reported successful maintenance of ripe pearl oysters of the same species in captivity even though there was no mention of the maintenance time. In a similar study Palaniswamy and Sathakathullah (1992) reported holding of matured *Crassostrea madrasensis* in controlled low temperature conditions for 6 months at temperature of 20 ° C.

Larvae of many temperate and subtropical species of bivalves have been successfully reared in the laboratory (Loosanoff and Davis, 1963; Nishikawa, 1971; Walne, 1974; Bayne, 1976). In marked contrast, there have been only a few attempts to rear pearl oysters. A literature review showed that the three species *Pinctada fucata, P. margaritifera* and *P. maxima* from the Indo-Pacific region have been studied.

Herdman (1903) studied the early life history of *Pinctada vulgaris* (= *P. fucata*) up to day 3 and bridged the stage with further larval stages obtained from the plankton. Cahn (1949) provided abstract information on the larval development of *P. martensii* and *P. maxima*. The Japanese works on breeding of pearl oyster have been sketchy and largely relate to spawning induction, artificial fertilization and early development (Kobayashi, 1948; Wada, 1953; Wada, 1961). Minaur (1969) attempted rearing of *P. maxima*. The present study, together with the work of Alagarswami *et al.* (1980a, b), provide a detailed account of the success in artificial rearing of the pearl oyster *P. fucata* in the tropics.

In the present observations the majority of larvae of *P. fucata* settled on day 23. Taking an overview of larval development, despite variations in rearing conditions and natural differences between species and zoogeography, most of the commercially important species of pearl oysters appears to have similar life history stages and are found to have a pelagic larval stage lasting about three weeks, with setting of spat between day 20 and 25. This had been reported for *P. fucata* by Alagarswami *et al.* (1983 a, b), Anuradhakrishnan (1987); P. *fucata martensii* (Ota, 1957; Shinju Yoshoku Zensho Honshu linkai Honshu, 1965); *P. margaritifera* (Alagarswami *et al.*, 1989, Doroudi *et al.*, 1999 a, b; Doroudi and Southgate, 2000), and for *P. maxima* (Baker and Rose, 1994).

Isochrysis galbana was supplied to the straight-hinge larvae at a concentration of 80—120 cells μl^{-1} the present study although higher concentration was used on certain days. This is almost equal to the food concentrations used by Minaur (1969) who fed the larvae of *Pinctada maxima* with different algal species at a density of 100 cells μl^{-1}. On the other hand the feed concentration used in this study was far more than that used by Wada (1973) of 10 cells μl^{-1} on days 1-12 and at 20 cells μl^{-1} from days 13-25. Anuradhakrishnan (1987) reported that 25 cells μl^{-1} of I. *galbana* was necessary for optimum growth, survival and settlement of the same species of larvae and she further reported that any increase from this concentration causes reduction in growth. However, this observation is contradictory to the

results of the growth rate (discussed subsequently) of the *P. fucata* larvae in this study, as this was nearly twice that obtained by Anuradhakrishnan (1987).

Individual investigators have used different larval densities in rearing experiments with pearl oysters. In the present study even though larval concentrations ranging from 2 – 30 larvae $^{-1}$ was tried, a larval concentration of 2 larvae ml $^{-1}$ was used after experimentation (personal observation). The larval concentration used in this study is equal to that used by Alagarswami *et al.* (1983 *a*) for the same species but slightly less than the concentration suggested by Anuradhakrishnan (1987) of 5 ml l $^{-1}$. The larval concentration used in this study is far more than that recommended by Nishikawa (1971) of 0.3—0.5 larvae ml $^{-1}$. However, much higher larval densities of 12 – 14 and 20 larvae ml $^{-1}$ has been used by Hayashi and Seko (1986) and Wada (1973) respectively with good results for *P. fucata* while 10 - 15 ml $^{-1}$ have been used by Minaur (1969) for rearing *P. maxima.*

The percentage of spat (24 – 64 %) obtained in this study (24 % in 500 l FRP tanks and 64 % in beakers) is slightly inferior to that obtained by Alagarswami *et al.* (1987) (31.6 % in tanks and 99.9 % in beakers), but almost similar to that reported by Anuradhakrishnan (1987) (37 – 60 % in 5 l beakers stocked at 5 larvae ml $^{-1}$) for the same species in identical larval rearing protocols. This difference could be attributed to the husbandry measures undertaken in these experiments. For other species the spat production is very low. Alagarswami *et al.* (1989) (6.3 %), Southgate and Beer (1997) (4.3 – 5.7 %), Doroudi *et al.* (1999 a) (< 10 %), Doroudi and Southgate (2000) (7 %) reported very poor spat production for *P. margaritifera* and similarly Rose and Baker (1994) (4 %) for *P. maxima* The very low spat production in these 2 species when compared with *P. fucata* is due to the fact that both of these species are very sensitive and cannot tolerate wide fluctuations in environmental parameters.

The results of the above workers showed that less than 10 % of the initial larval population successfully settled and metamorphosed into spat, which grew to 5 mm in shell length. As these low percentages suggest that the husbandry protocols used during both of these studies were less than

optimal. Recent rearing trials with related species; *P. maxima* have increased the percentage of larvae settling to 21 - 25 % (Rose personal communication). The improvement in percentage survival and settlement appears to be largely due to feeding the larvae with phytoplankton, which is morphologically more suitable for ingestion and digestion (*i.e., Isochrysis* and *C. calcitrans).* It was observed in this study that dark coloured tanks noticeably improve settlement over light coloured ones for the larvae of *P. fucata, which* is in agreement with the findings of Alagarswami *et al.* (1983 *a*, 1987).

The present study indicated that P. *fucata* larvae in each batch exhibited differential growth. This has been reported in *P. fucata* and *P. margaritifera by* (Alagarswami *et al.*, 1983 *a*, 1989), *P. maxima* (Rose and Baker, 1994), *M. edulis* (Bayne, 1965, 1983) and Loosanoff and Davis (1963) for other bivalve larvae. Thus the settlement typically lasts for 1 week. This may be associated with the following three husbandry practices used during this study. Firstly, the failure to rigorously culled out; slower-developing larvae from the population may have been a reason for the delay of settlement period over several days When faster-developing larvae are selected, the settlement period can be reduced to 3 days .The delay in metamorphosis of bivalve larvae until a suitable stimulus or substratum is available has been well documented (Pawlik, 1992; Pechenik, 1999).

Growth rate of *P. fucata* larvae appears to be in agreement with Wada's (1973) data on growth of *P. fucata*. Larval growth obtained in this study (12.1 ± 0.67 µm day $^{-1}$) is more or less in agreement with the growth rate reported by Alagarswami *et al.* (1983 *a*) and Wada (1973) of 13.2 µm day $^{-1}$ for the same species, while it was better than 2.31- 5.17 µm day $^{-1}$ reported by Anuradhakrishnan (1987). The slight variations in the growth may be species specific, due to quality and quantity of feed and environmental factors influencing growth.

Growth of P. *fucata* spat up to 13 weeks showed a curvilinear pattern. The growth curve of lamellibranch larvae is generally sigmoidal, although a linear fit to some data in the middle size ranges is not unreasonable (Bayne, 1976). Walne's (1974) figures on the growth of *O.*

edulis larvae for the first 10 days appear to give a linear relationship. Minaur's (1969) data for *P. maxima* up to about 20 days show a linear fit but the larvae did not grow further.

Growth obtained in this study (12.1 ± 0.67 µm day $^{-1}$) was better than the growth rate reported in related species of pearl oysters. For *P. margaritifera* Alagarswami *et al.* (1989) reported 10.98 µm day $^{-1}$ while Southgate (1999) observed 11 µm day $^{-1}$, for *P. maxima* Minaur (1969) observed 10 µm day $^{-1}$ and Rose and Baker (1994) reported a slightly less growth rate of 8 µm day $^{-1}$.

Growth rate in phase II is in agreement with the works of Alagarswami *et al.* (1983 *a*, 1987) and Chellam (1988). There was a 10 fold increase in the growth rate (120.83 ± 3.1 µm day $^{-1}$) in phase II of *P. fucata* spat in this study. This spurt in the post settlement growth rate of spat is in agreement with the growth rate of most other bivalve molluscs (Loosanoff and Davis, 1963).

In this study the individual oyster growth rate was 102.79-µm day $^{-1}$ and 37.15 µm day $^{-1}$ for the first and second year respectively. Whereas in the random growth rate in the first and second year the growth rate respectively was 120.69 µm day $^{-1}$ and 37.29 µm day $^{-1}$. This is more or less in agreement with the work of Devanesan and Chidambaram (1956), Narayanan and Michael (1968), Chellam (1978, 1988) and Jeyabhaskaran *et al.* (1983) who reported (130.5, 122.36, 124, 130.5, 102.77 µm day $^{-1}$ respectively in the first year and 48, 48.97, 19.8 47.2 µm day $^{-1}$ respectively in the second year. However the growth rate was inferior to that obtained by Appukuttan (1987) of 50 µm day $^{-1}$, Nalluchinnappan *et al.* (1982) (72.5 µm day $^{-1}$) and Muthuraman and Dev (1988) (72.5 µm day $^{-1}$) in the same species and Numaguchi (1994) for *P. fucata martensii*.

Gokhale *el al.* (1954) and Narayanan and Michael (1968) have observed that pearl oysters from Gulf of Kutch grow fairly fast during the first three or four years and thereafter show little growth. Observations of Devanesen and Chidambaram (1956) show that the oysters grow to a height

of about 36 mm in six months, 35-45 mm at the end of one year, 50-55 mm at the end of second year, 55-60 mm at the end of third year, 60-65 mm at the end of fourth year and 65-70 mm at the end of fifth year.

Observations by Appukuttan (1987) at Vizhinjam also show that young oysters show faster growth and later growth rate is very slow. He reported that compared with other areas the pearl oysters register a fast growth in first year itself at Vizhinjam. Another feature observed in the present study was the effect of monsoon season on growth. Growth rate of oysters during November was more than other months. This phenomenon was also observed by a few workers (Gokhale *et al.*, 1954; Pandya, 1975; Chellam, 1978; Jeyabhaskran et *al.*, 1983; Traithong *et al.*, 1997; Urban, 2000). According to these workers growth rate is inversely proportional to salinity and the fast growth rate attributed by these workers is due to a salinity drop during these months, which was also observed in tnis study. Also it was seen that the chlorophyll content in the sea water in Tuticorin farm area during this period was also high when compared to other months hence it is also inferred that in addition to a salinity drop a substantial increase of chlorophyll in the water also acted synergistically for the fast growth observed by oysters in this season. This could be due to the flushing of the nutrients into the sea by rainwaters thus reducing salinity and also adding to the productivity of the sea. On the contrary Traithong *et al.* (1997) found no obvious relationship between growth, and chlorophyll content in the bay while rearing *P.maxima* in cages in the Thai waters.

Chellam (1978) was of the opinion that more than these parameters sediment suspension, velocity of the currents and availability of food, fouling and boring also affect the growth of pearl oysters in totality.

The present work on the larval rearing of pearl oyster in India has given the basic knowledge for the mass scale production of pearl oyster spat. Further investigations are required to determine optimum larval density, critical cell concentration of feed, use of local tropical species of microalgae and diatoms as food, and factors influencing spat setting in order to improve and standardize the procedures for commercial production.

PLATE 3.1

A. View of pipe lines for seawater intake system for pearl oyster hatchery operations at Karapad bay Tuticorin

B. Filter bed, seawater storage and pumbing system

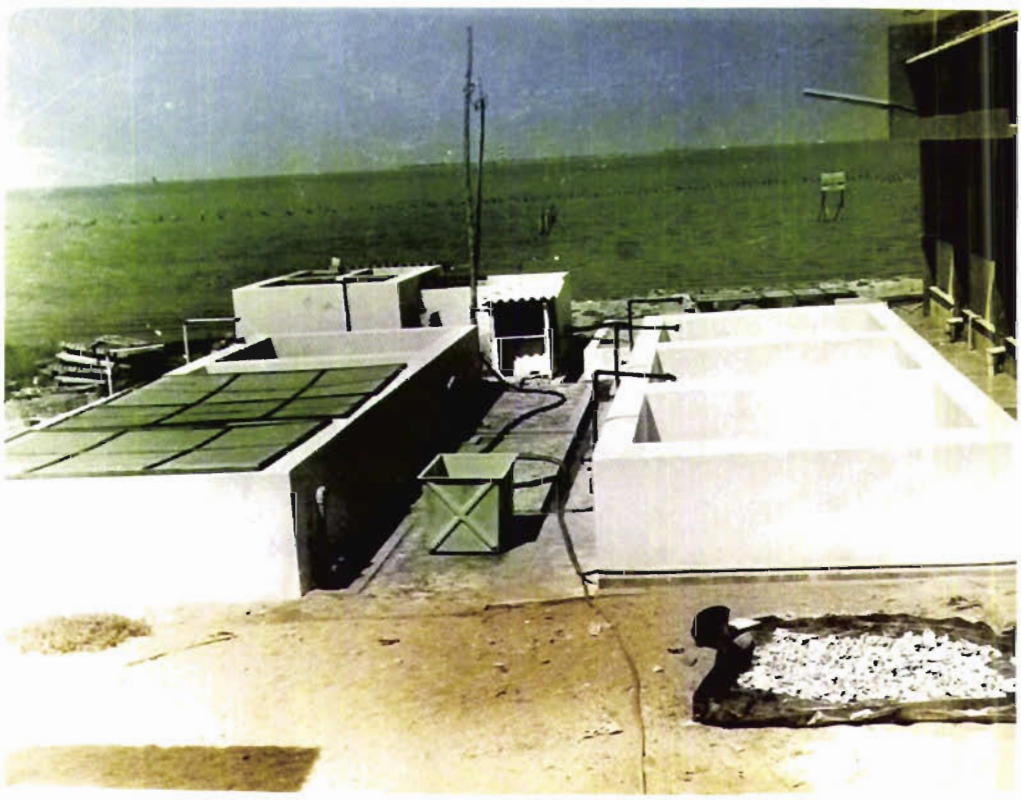


PLATE 3.2

A. *Pinctada fucata :* selected for induced maturation

B. Conditioning room : tanks holding pearl oysters for maturation

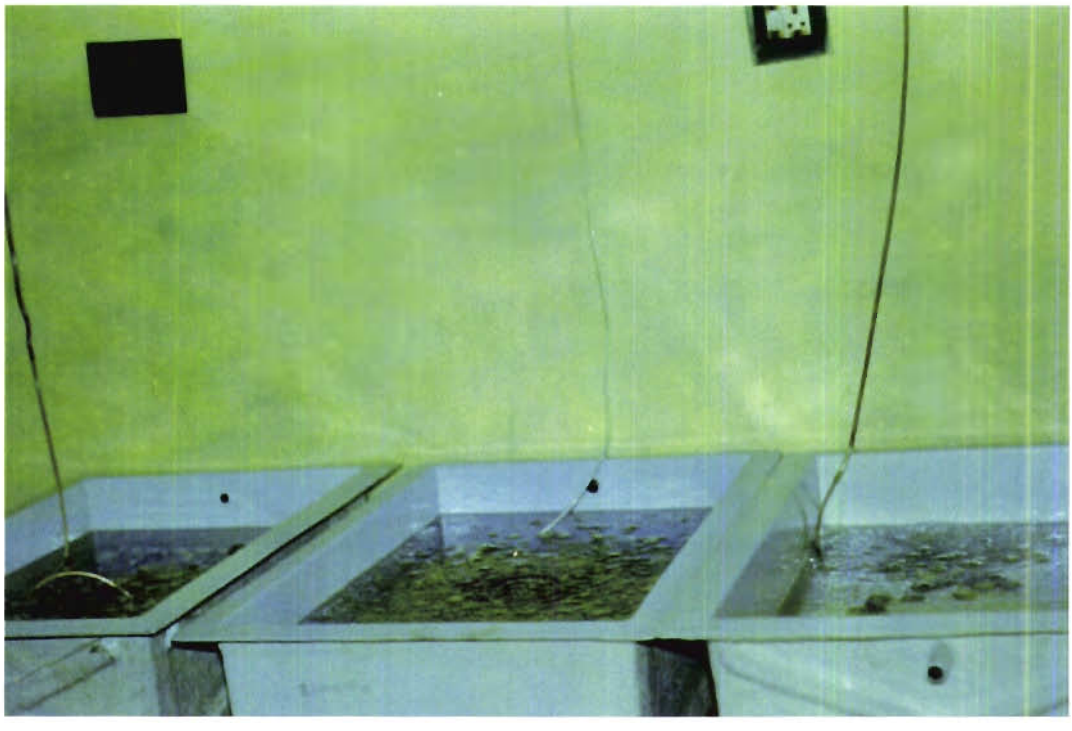
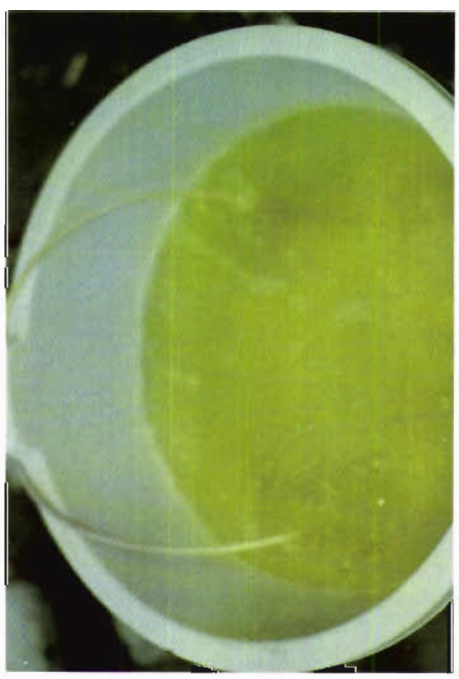


PLATE 3.3

A. View of microalgal culture room setup

B. Mass microalgal culture, C. Mass culture of Chaetoceros



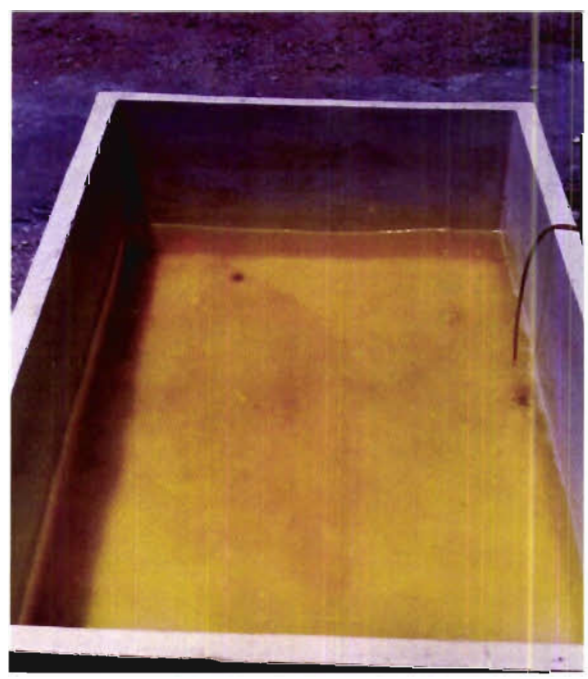
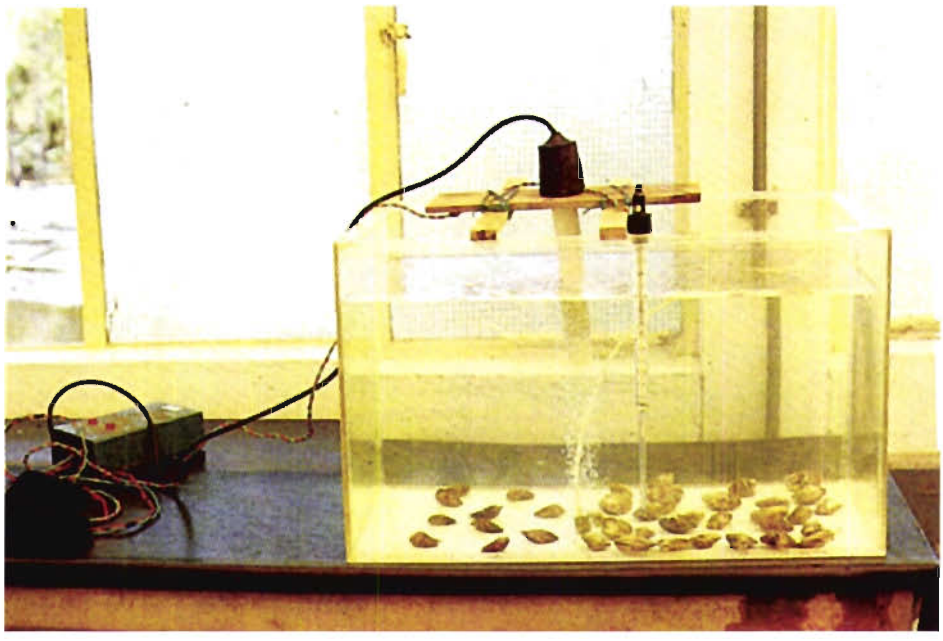
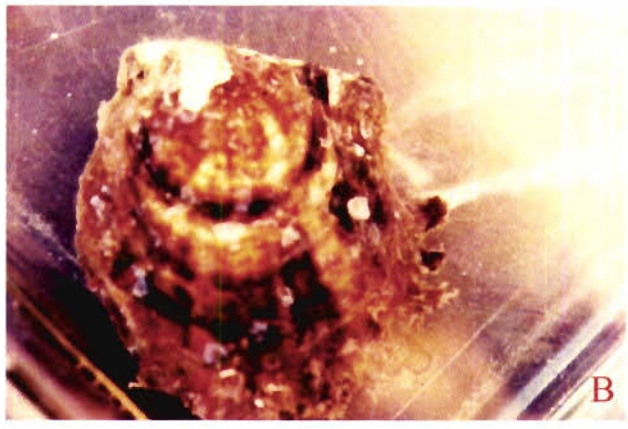


PLATE 3.4

A. Thermal induced spawning tank (with heating element Jumothermometer & matured pearl oysters



B. *Pinctada fucata* : Spawning female



C. *Pinctada fucata* : Spawning male

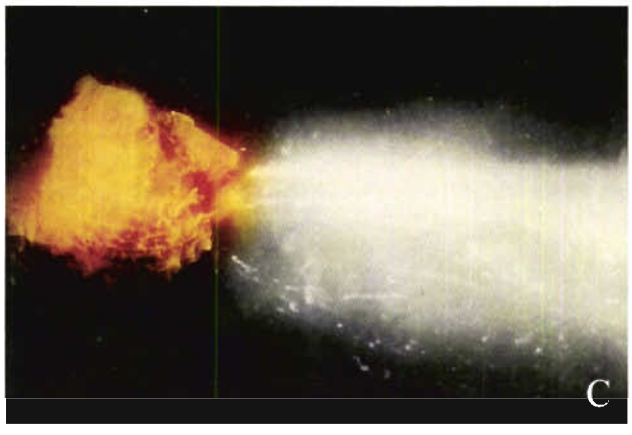
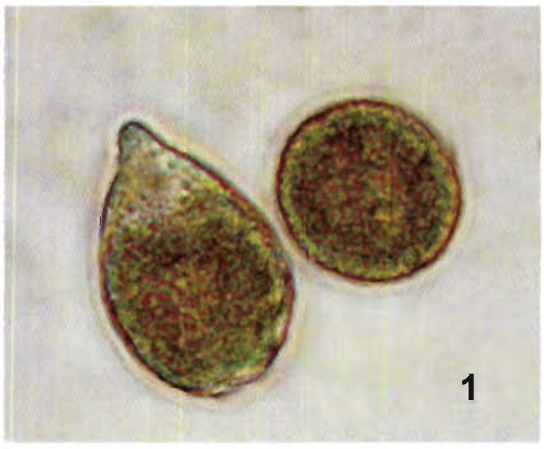


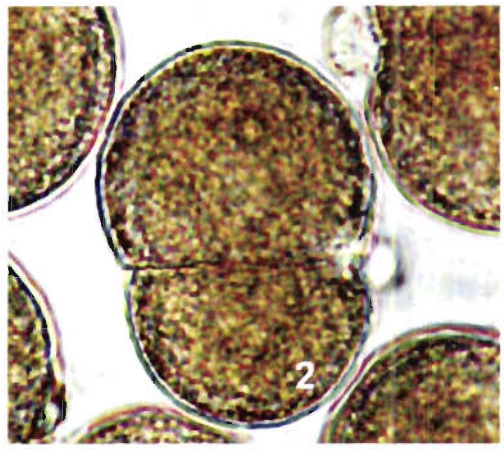
PLATE 3.5

A. *Pinctada fucata* : Early developmental stages

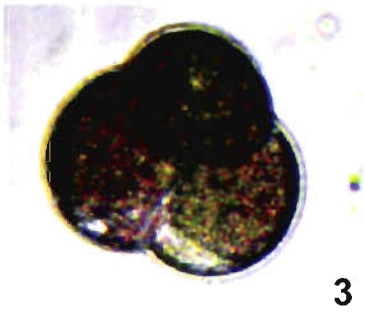
1. Unfertilized & fertilized Egg



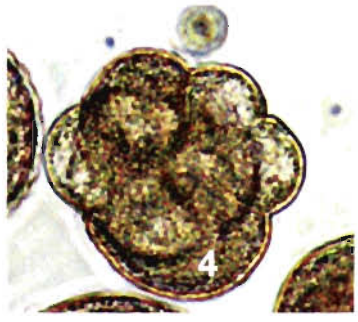
2. Cell stage



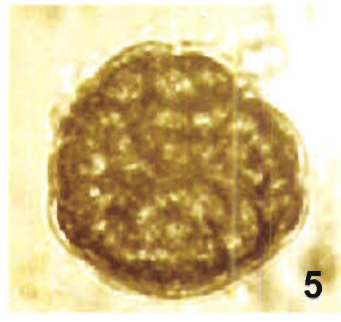
3. Trefoil stage



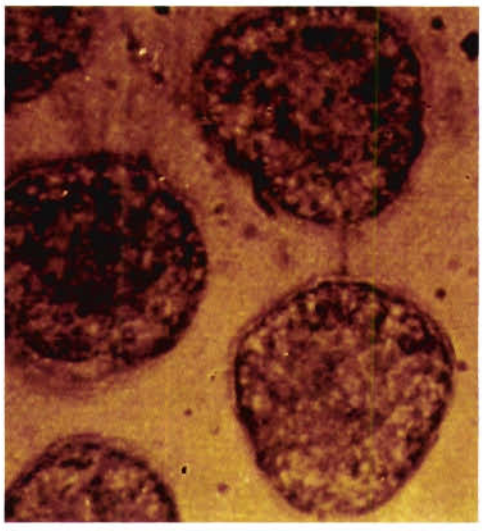
4. 8 cell stage



5. Morula stage



6. Trochophore larva



7. Veliger larva

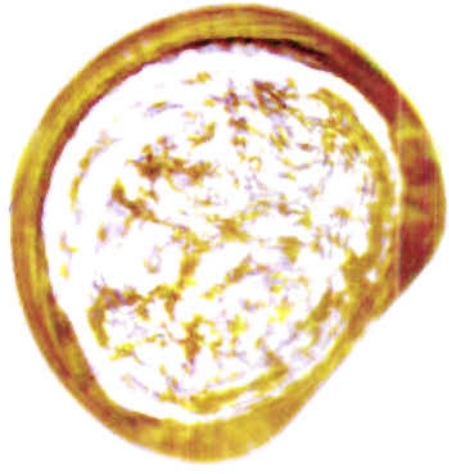
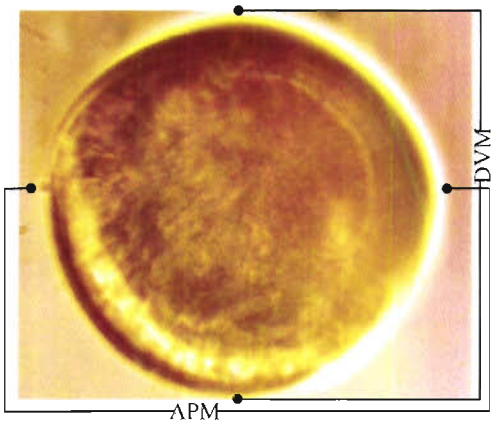


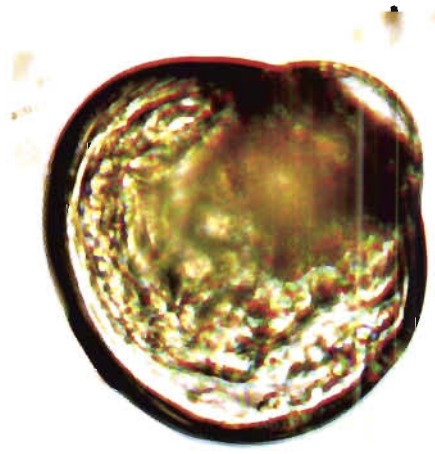
PLATE 3.6

A. *Pinctada fucata* : Developmental stages

Early umbo larva

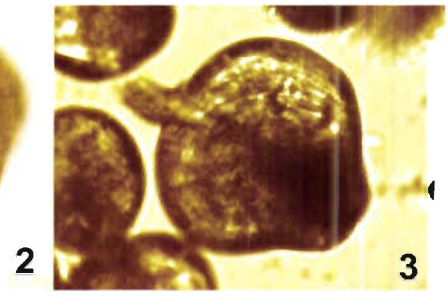


Late umbo larva

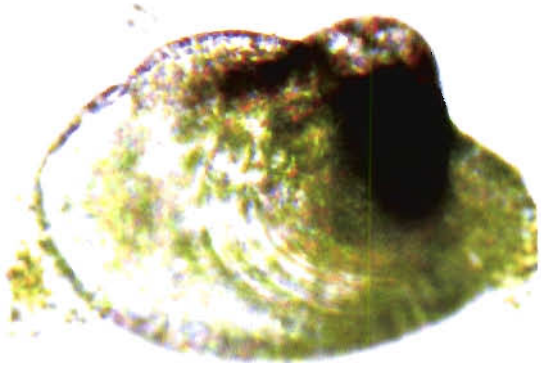


B.1&2 Eyed stage

3.Pediveliger stage



C. Plantigrade



Spat

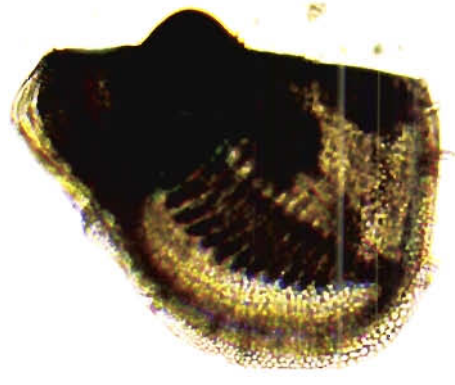
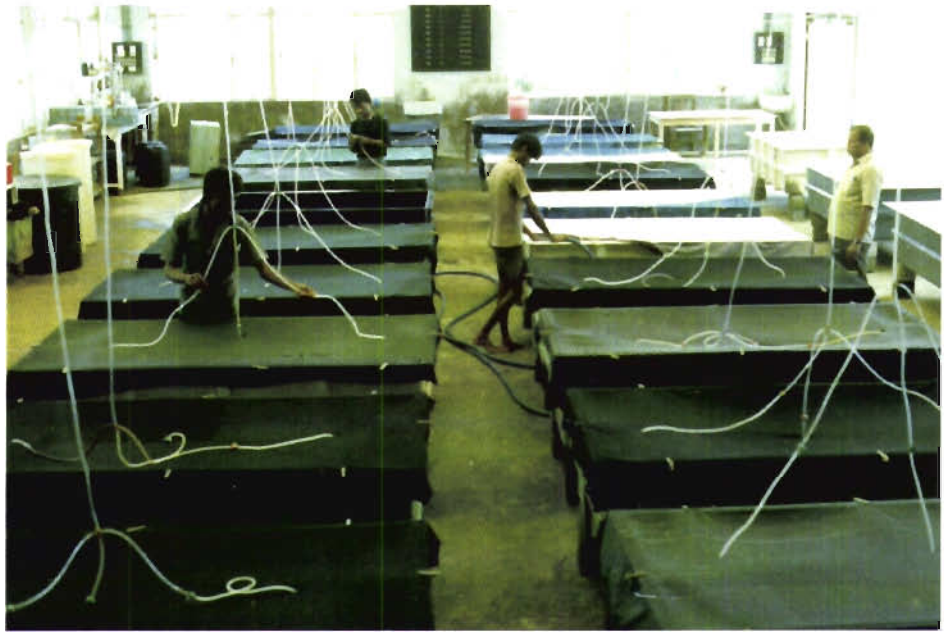


PLATE 3.7

A. *Pinctada fucata* : View of hatchery for larval rearing



B. Spat settled on the sides,
corners and at the bottomof the FRP tank

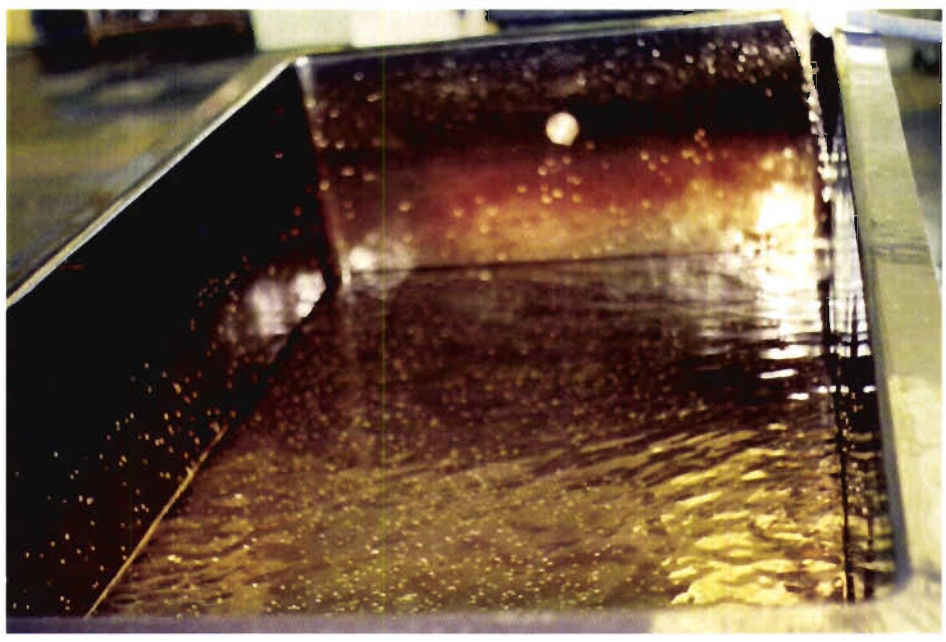
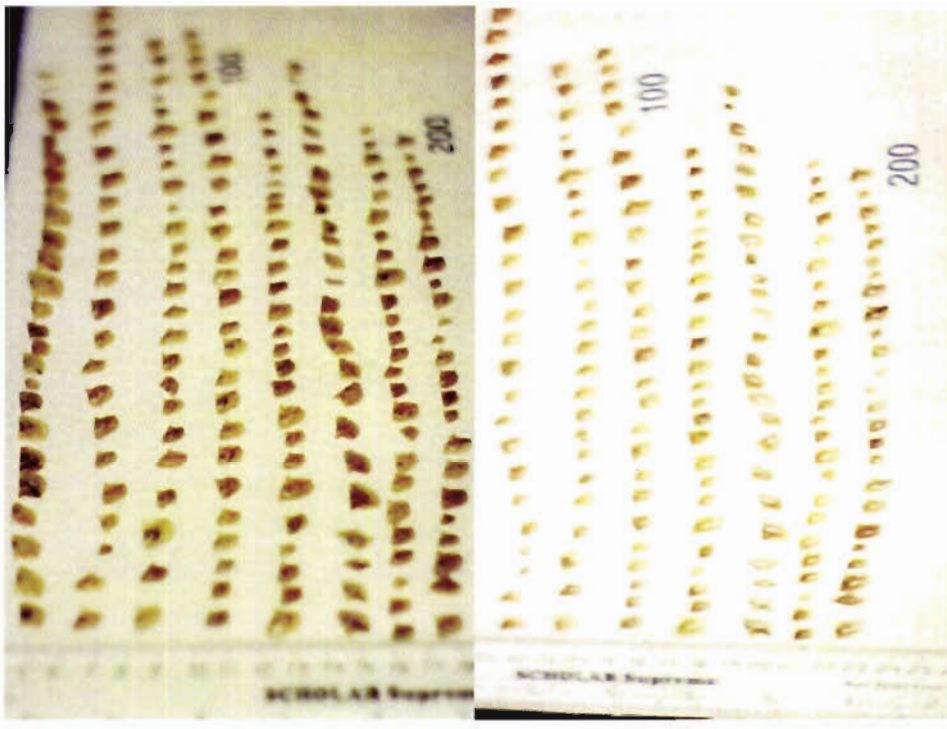
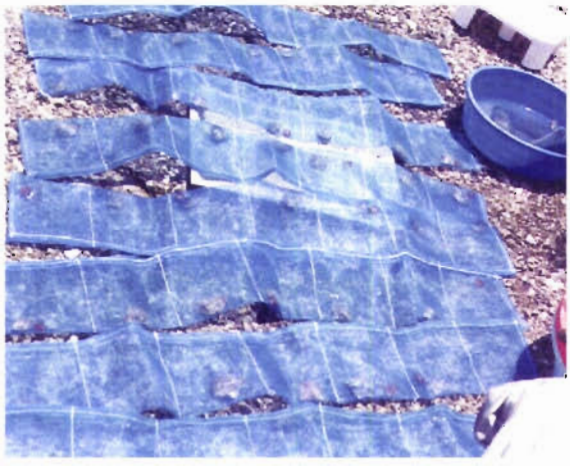


PLATE 3.8

A. *Pinctada fucata* : Spat selected for individual and random growth studies



B. Pouch for individual and iron frame cage covered with velon screen for random growth studies



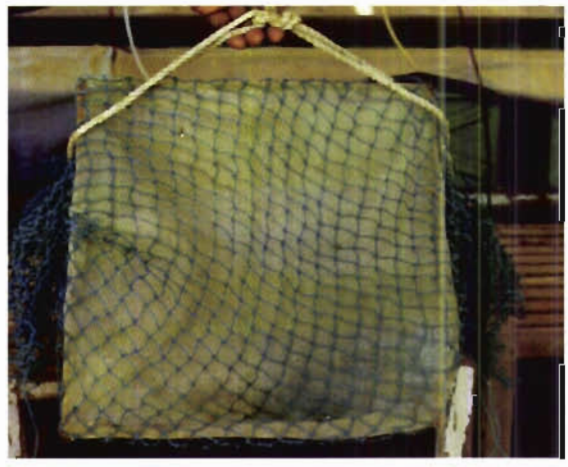


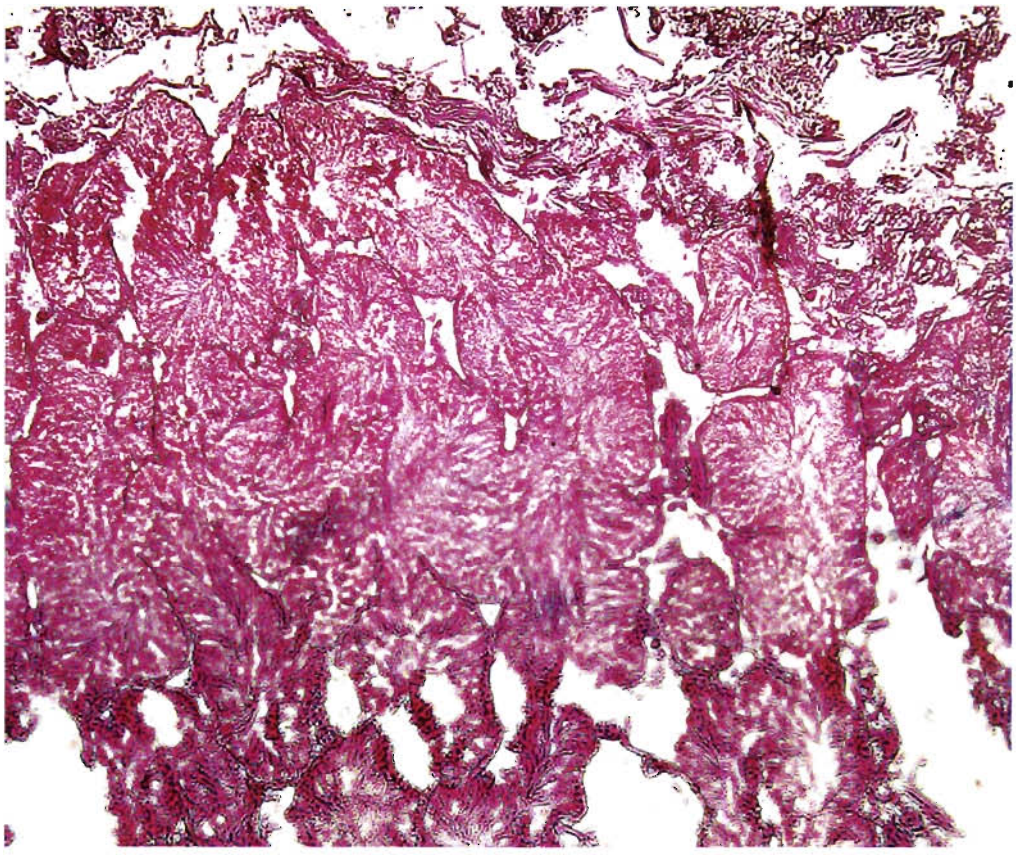
PLATE 3.9

A. *Pinctada fucata* : Tagged for individual and growth studies

B. Pearl oysters selected for random growth studies

PLATE 3.10

A. *Pinctada fucata* : Ripe male gonad (x 10)



B. Ripe female gonad (x 10)

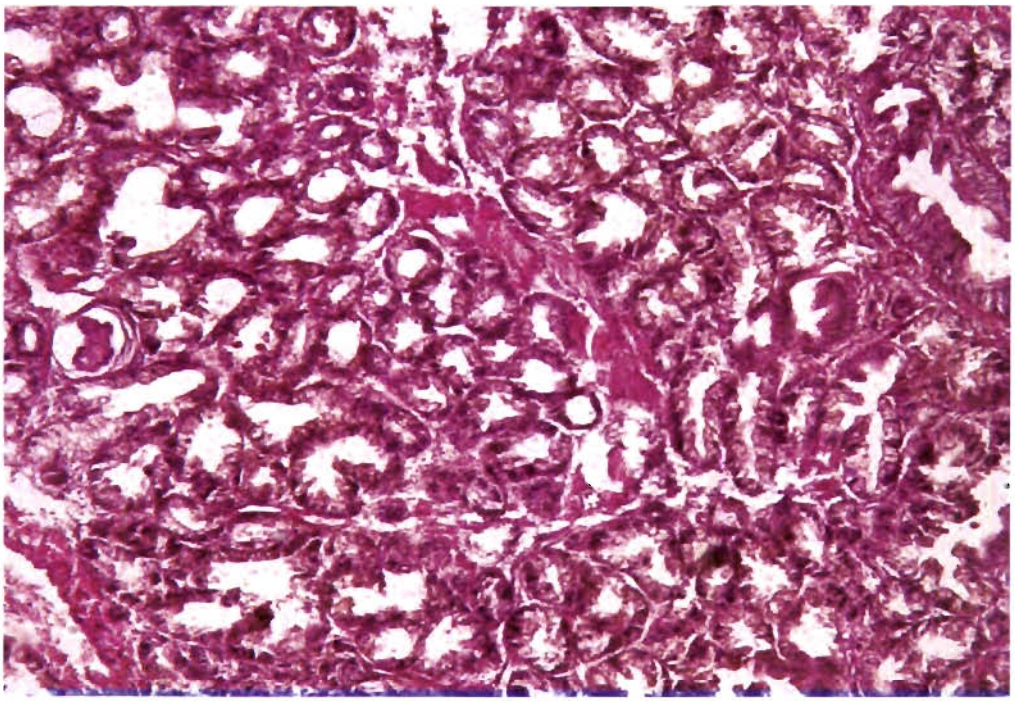
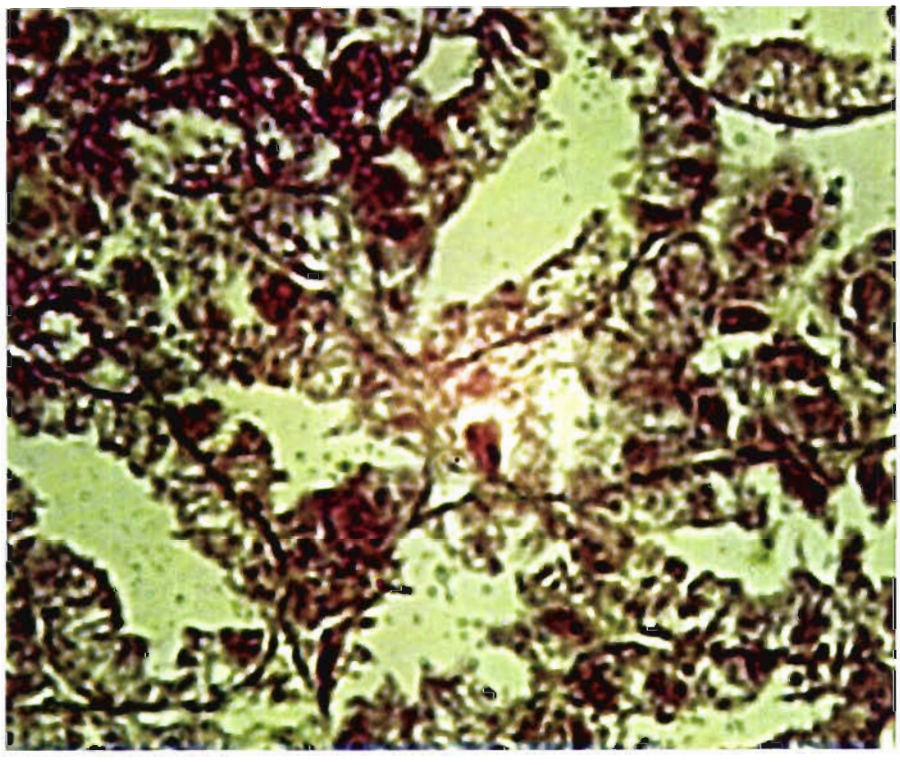


PLATE 3.11

A. *Pinctada fucata* : Histological section of spend male gonad (x 40)



B. Histological section of spend female gonad (x 40)

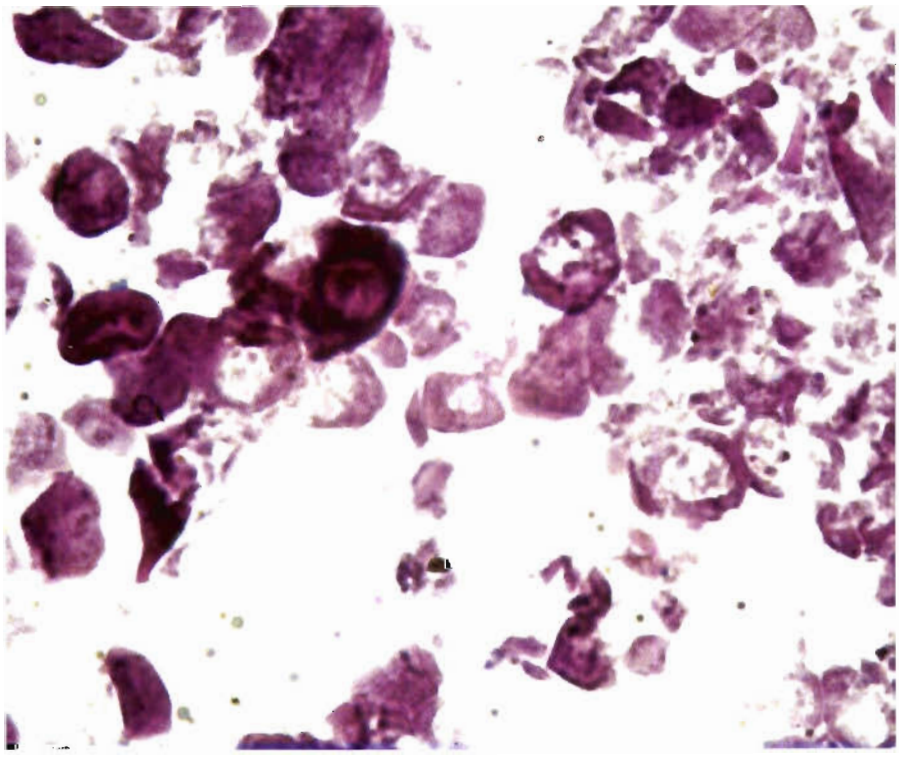
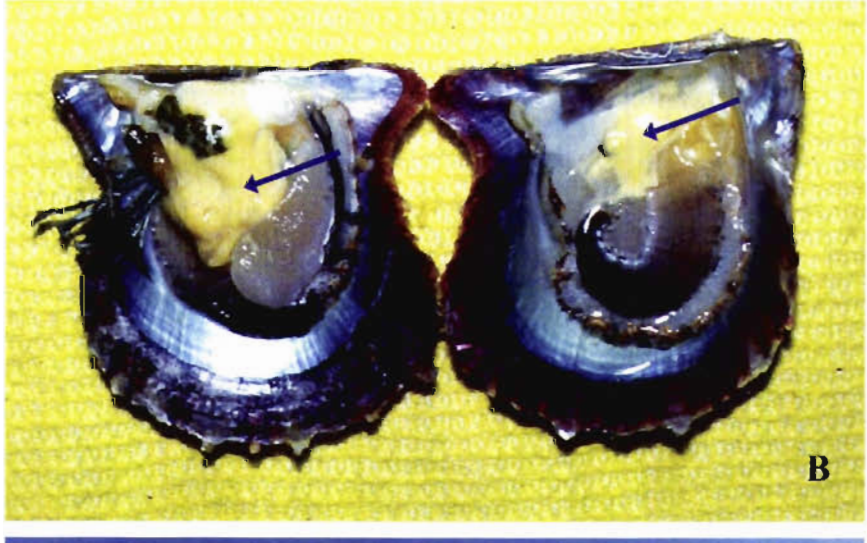


PLATE 3.12

***Pinctada fucata* : Internal view arrow A. Spend male gonad, B. Maturing female gonad & C. Ripe female gonad**



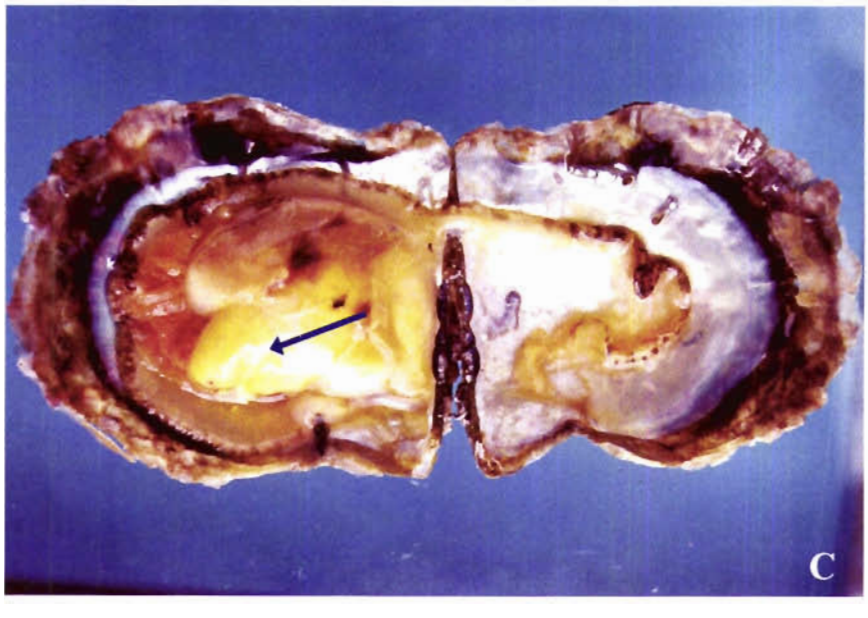


Figure 3.1. Effect of different feeds on the maturation of pearl oyster *Pinctada fucata* (Gould) - pecentage of stage III animals

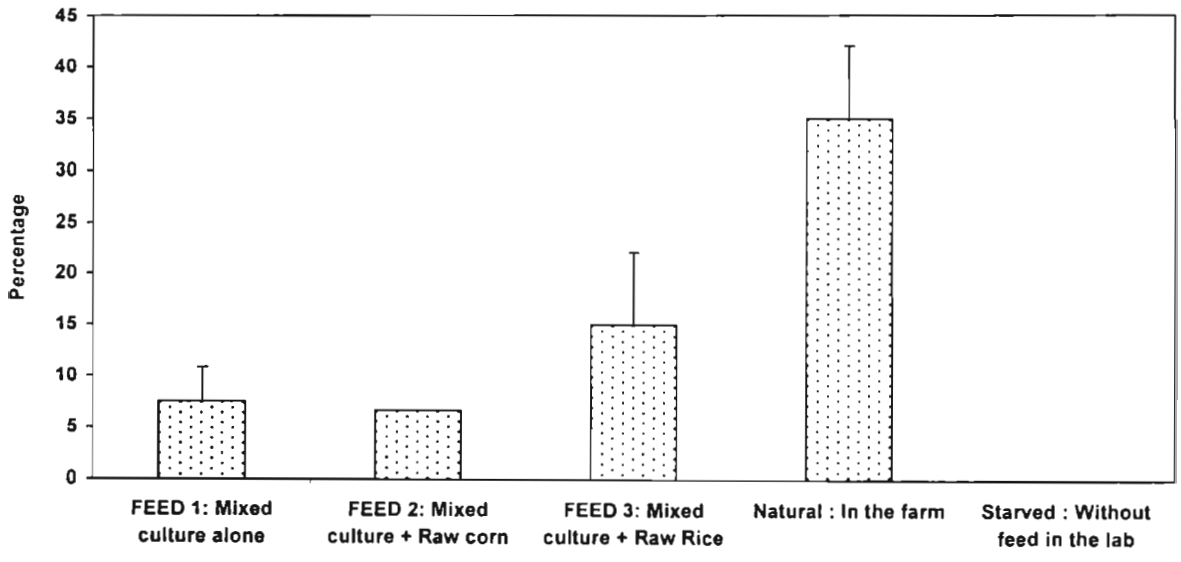


Figure 3.2: Effect of different feeds on the maturation of pearl oyster *Pinctada fucata* (Gould) - percentage of stage II animals

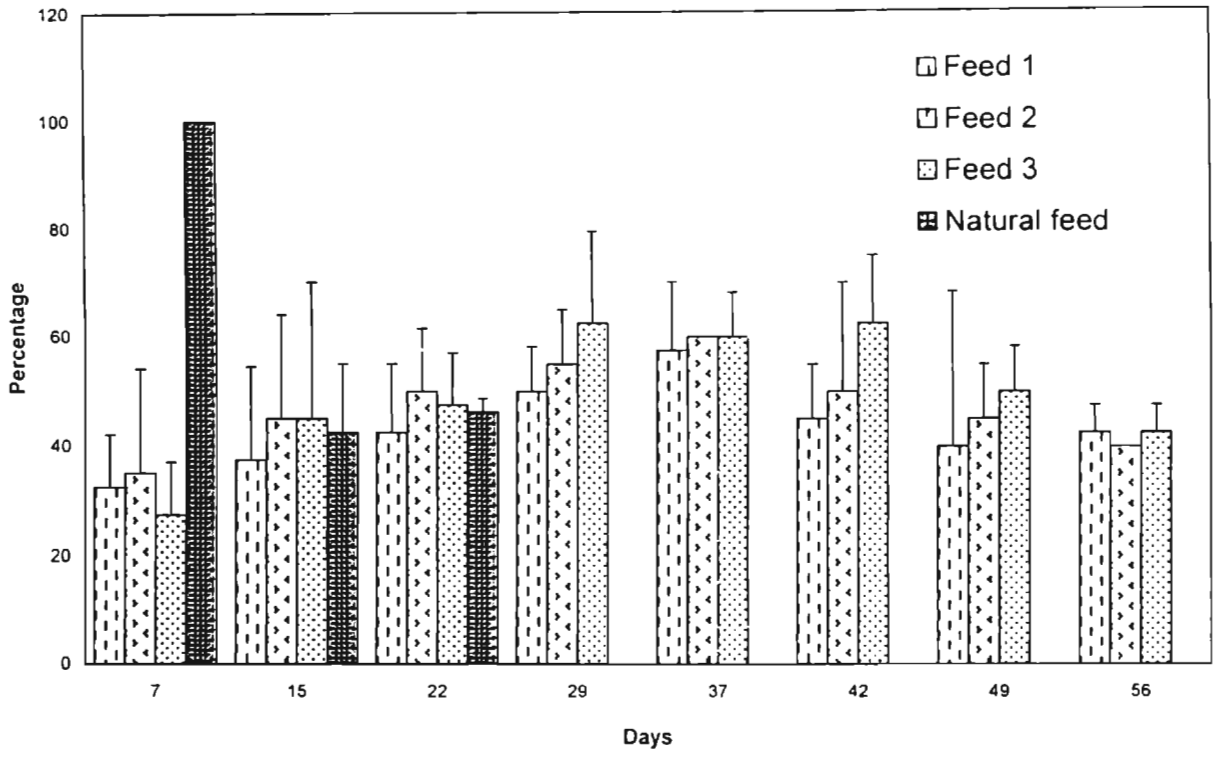


Figure 3.3. Effect of different feeds on the maturation of Pearl oyster *Pinctada fucata* (Gould) - percentage of stage III animals

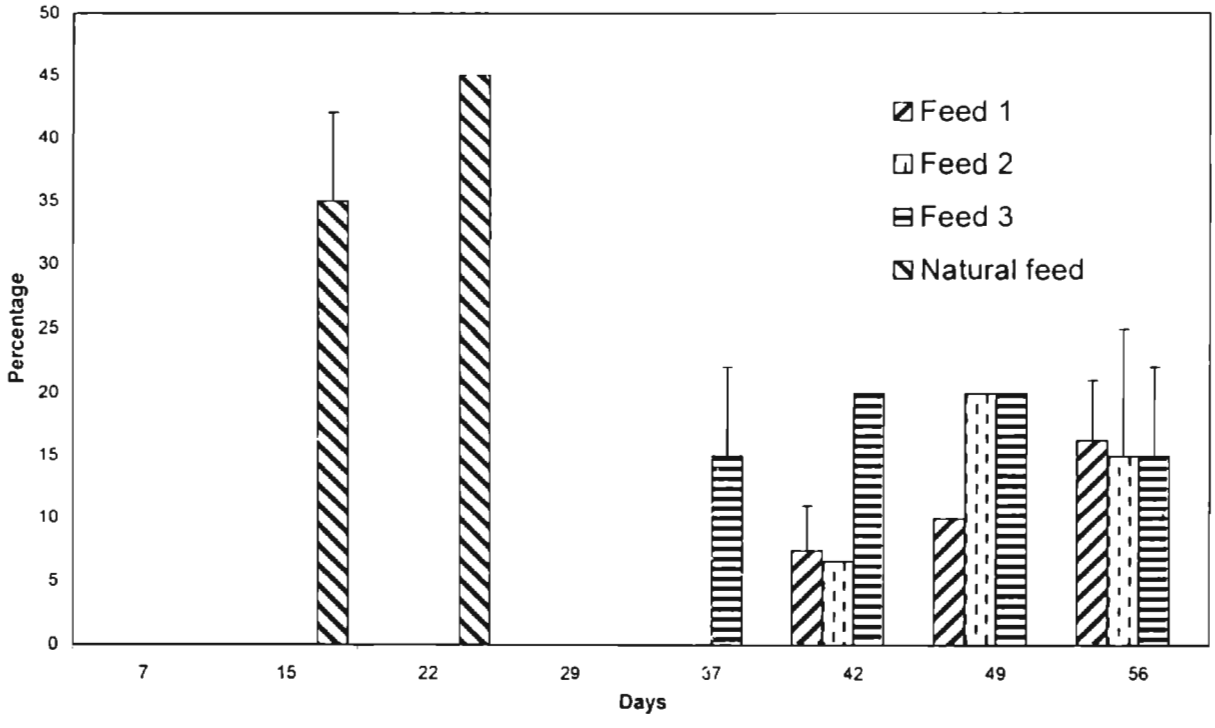


Figure 3.4. Growth rate of Pinctada fucata (Gould) larvae

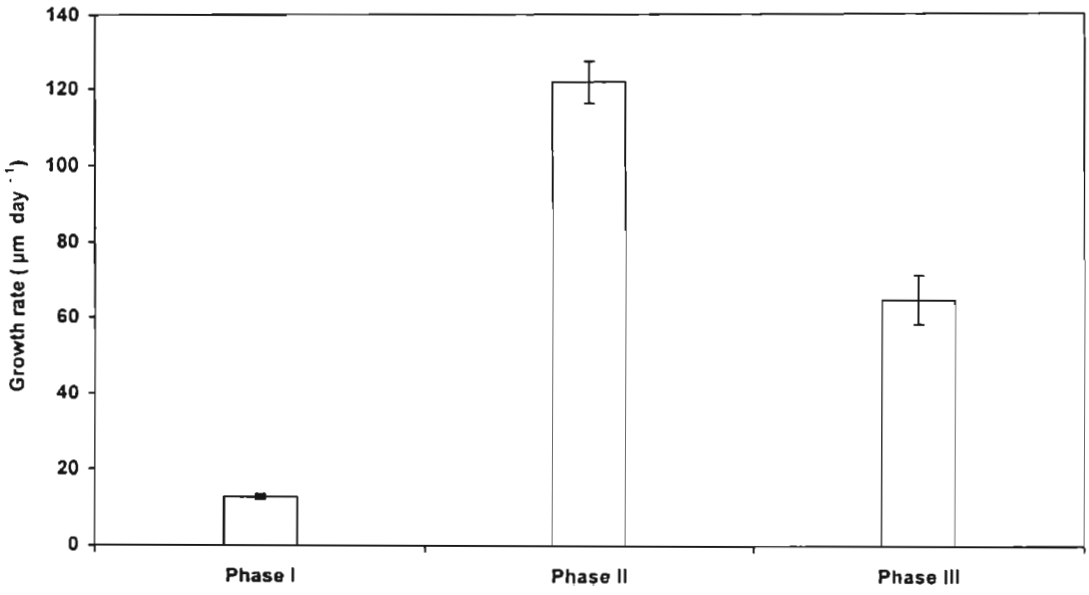


Figure 3.5. Dorso Ventral Measurement (DVM) of the larvae of *P. fucata* (Gould)

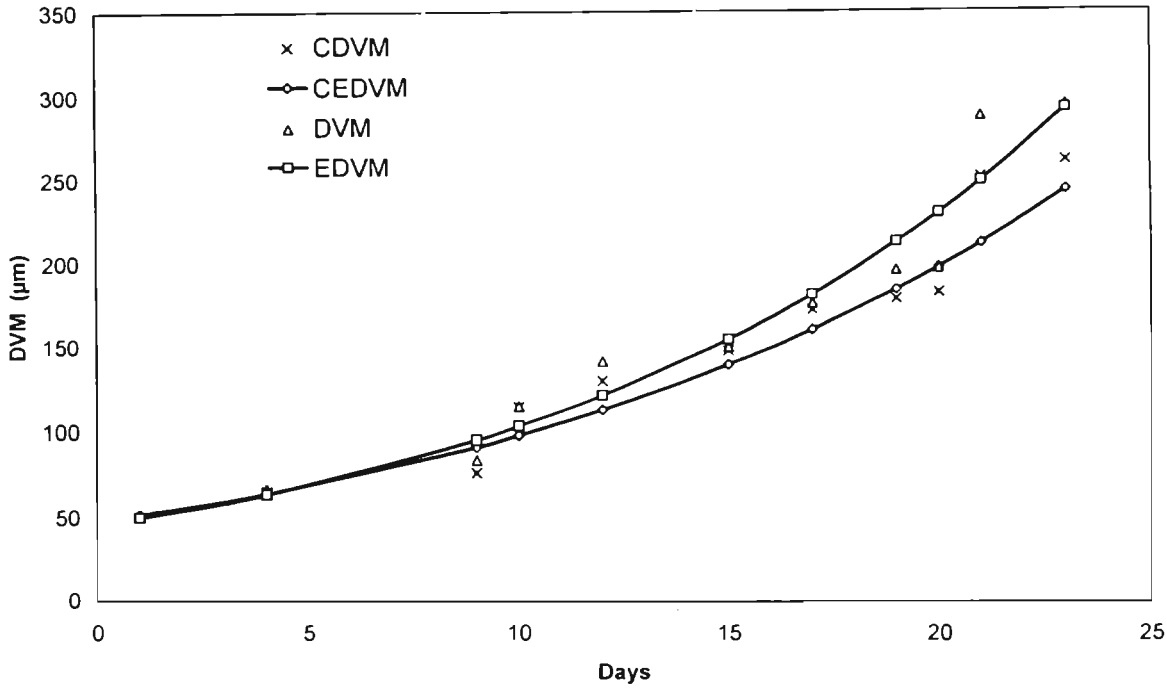


Figure 3.6. Anterior Posterior Measurement (APM) of the larvae of *P. fucata* (Gould)

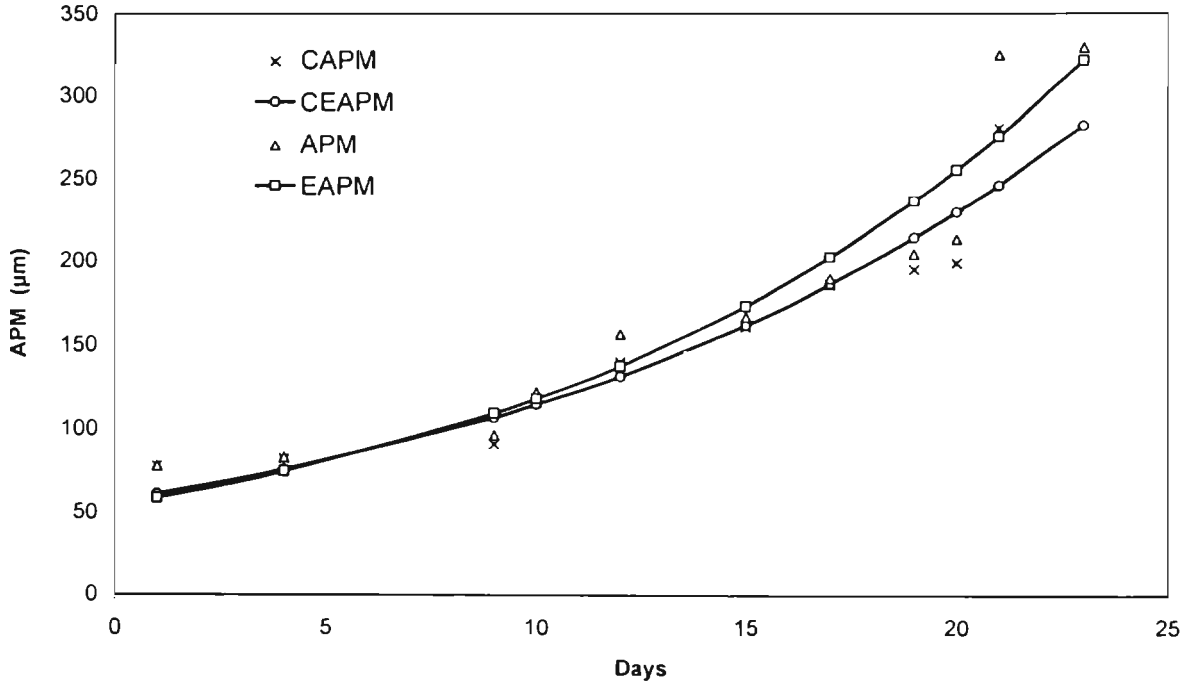


Figure 3.7. Growth in Dorso Ventral Measurement (DVM) of *Pinctada fucata* (Gould)

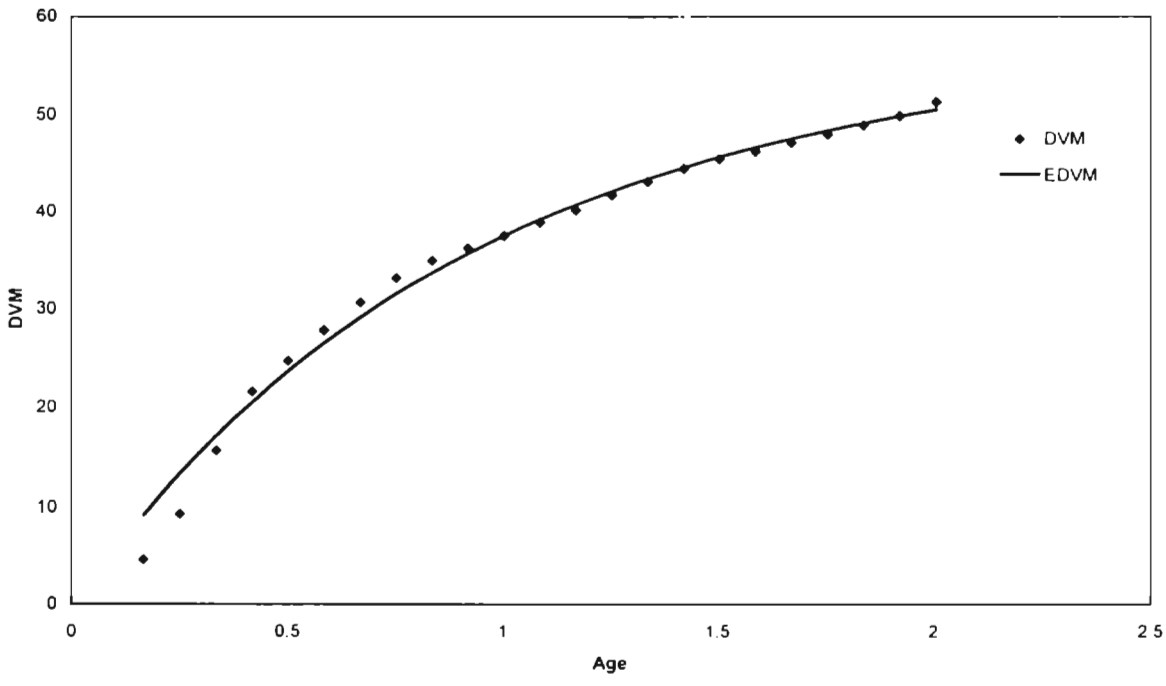


Figure 3.8. Growth in Hinge Length (HL) of *Pinctada fucata* (Gould)

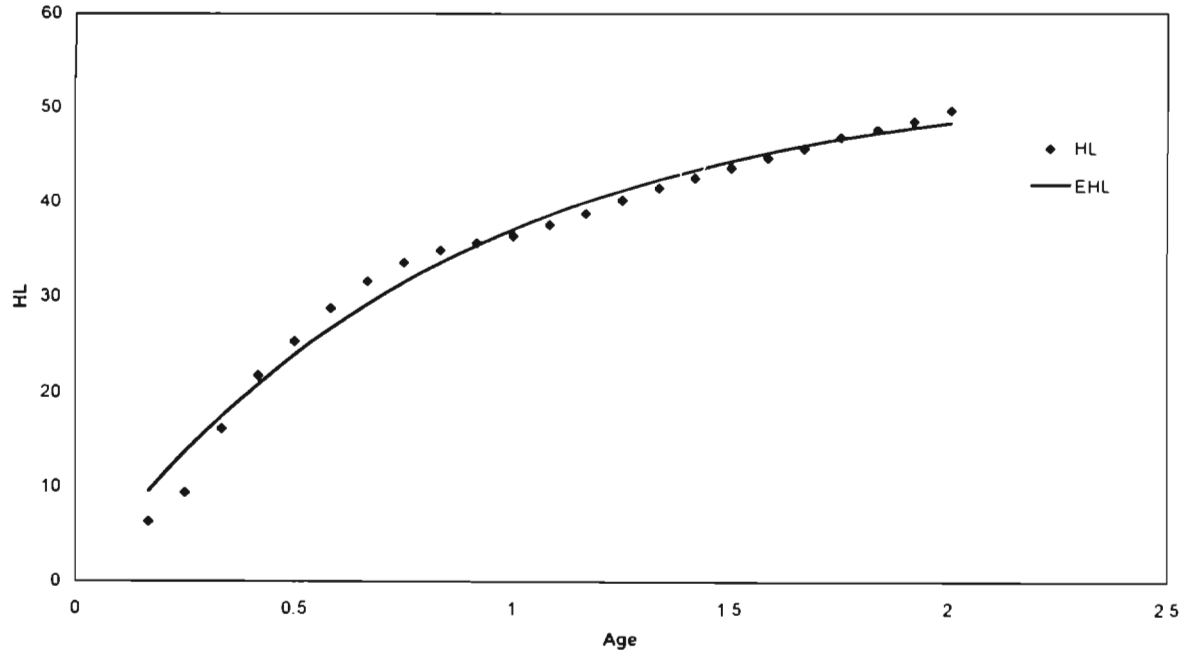


Figure 3.9. Growth of *Pinctada fucata* (Gould) in terms of depth

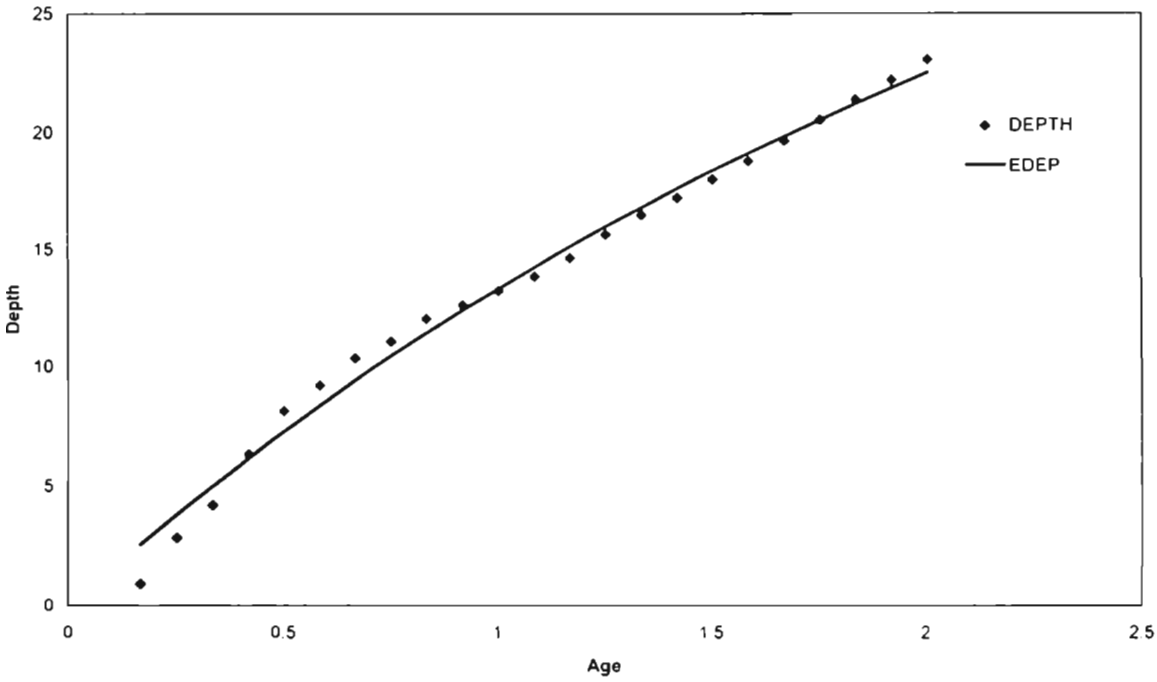


Figure 3.10. Growth in Weight of *Pinctada fucata* (Gould)

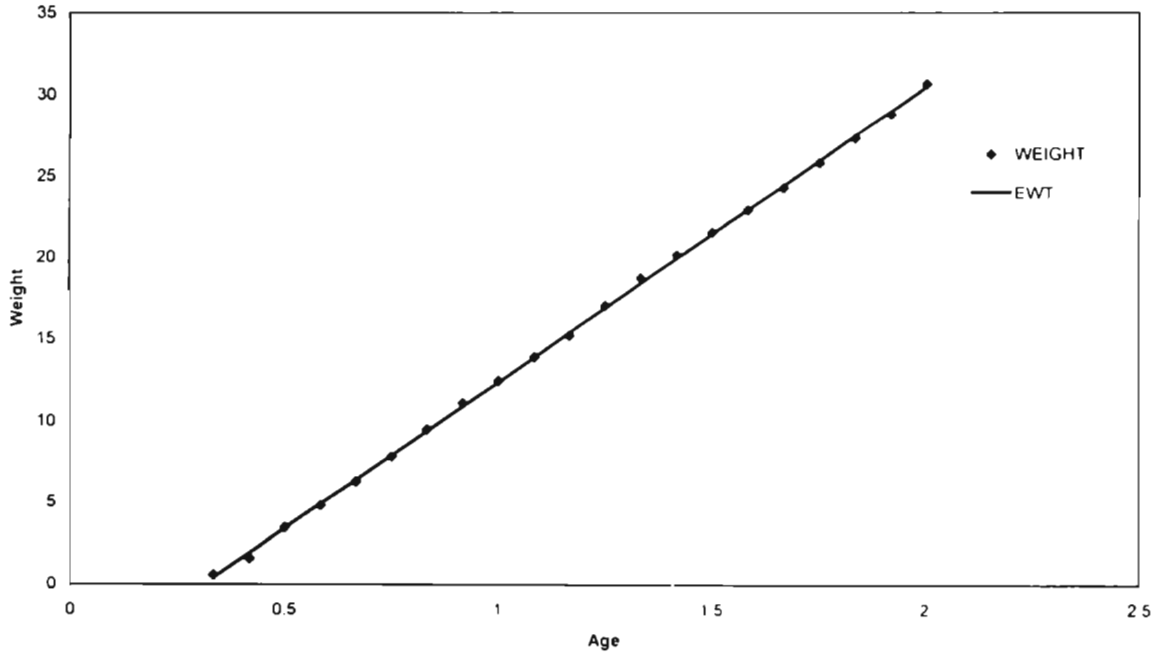


Figure 3.11. Growth in Dorsoventral Measurement (DVM) of *P. fucata* grown in cages

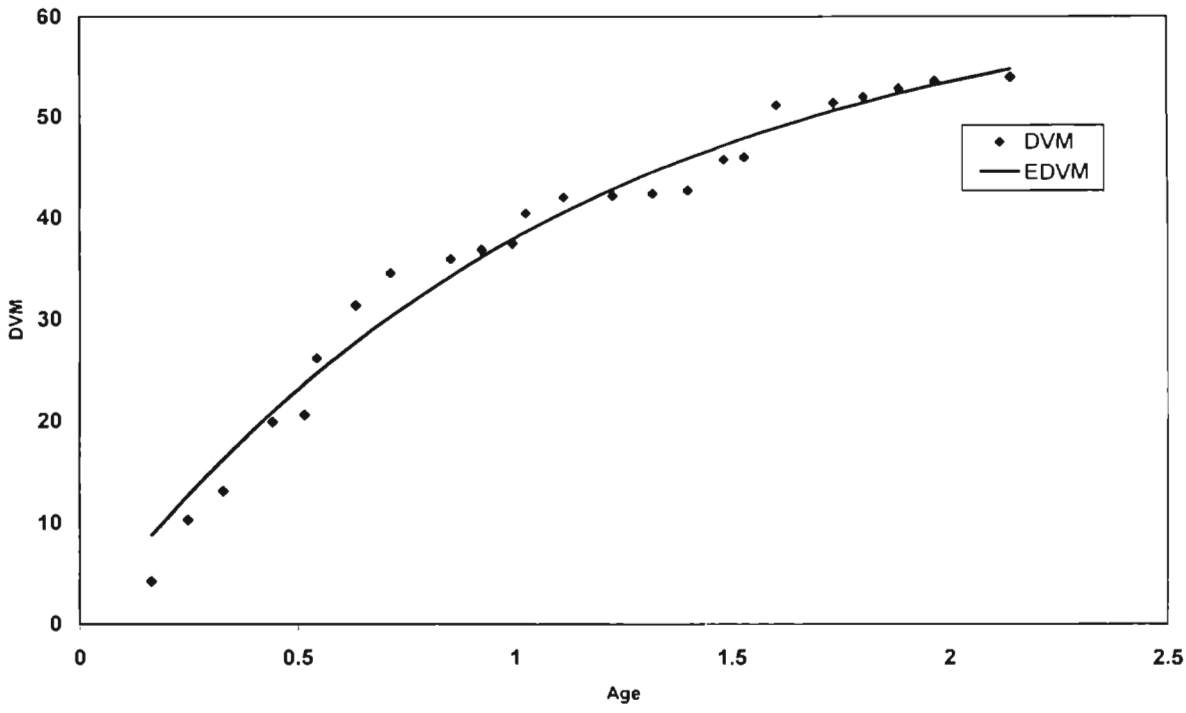


Figure 3.12. Growth in Hinge Length (HL) of *P. fucata* grown in cages

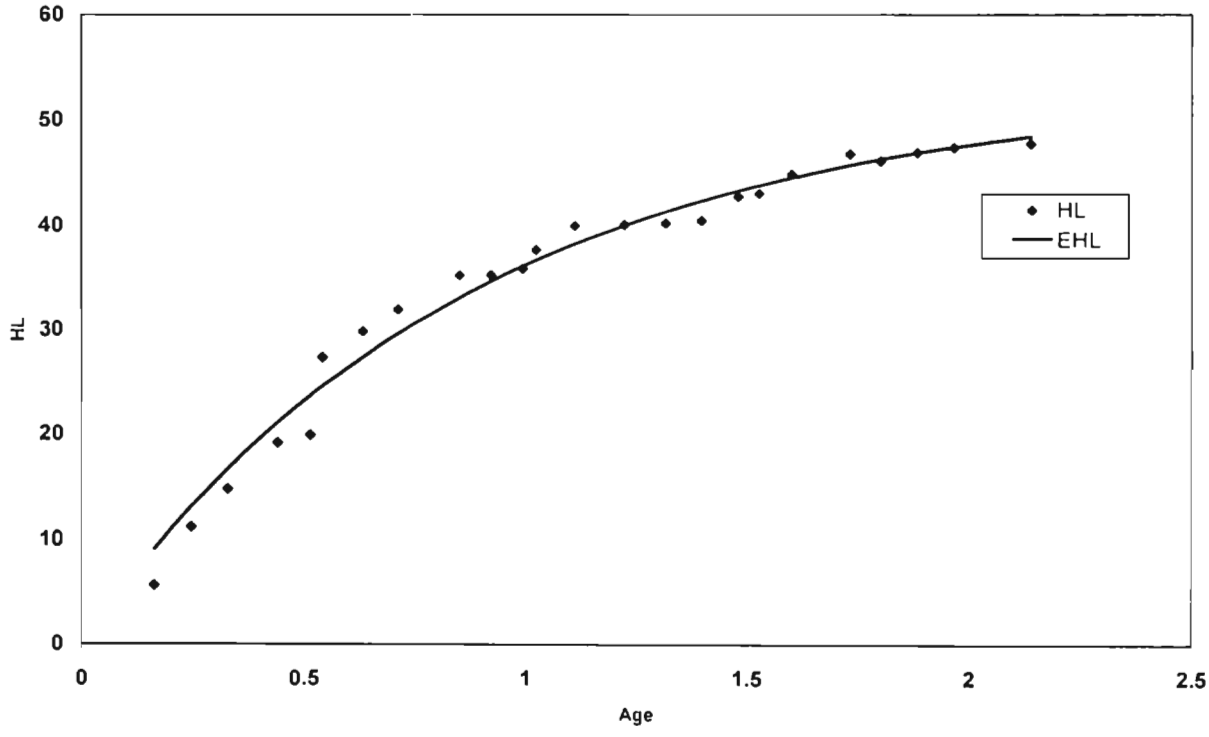


Figure 3.13. Growth of *P. fucata* grown in terms of depth in cages

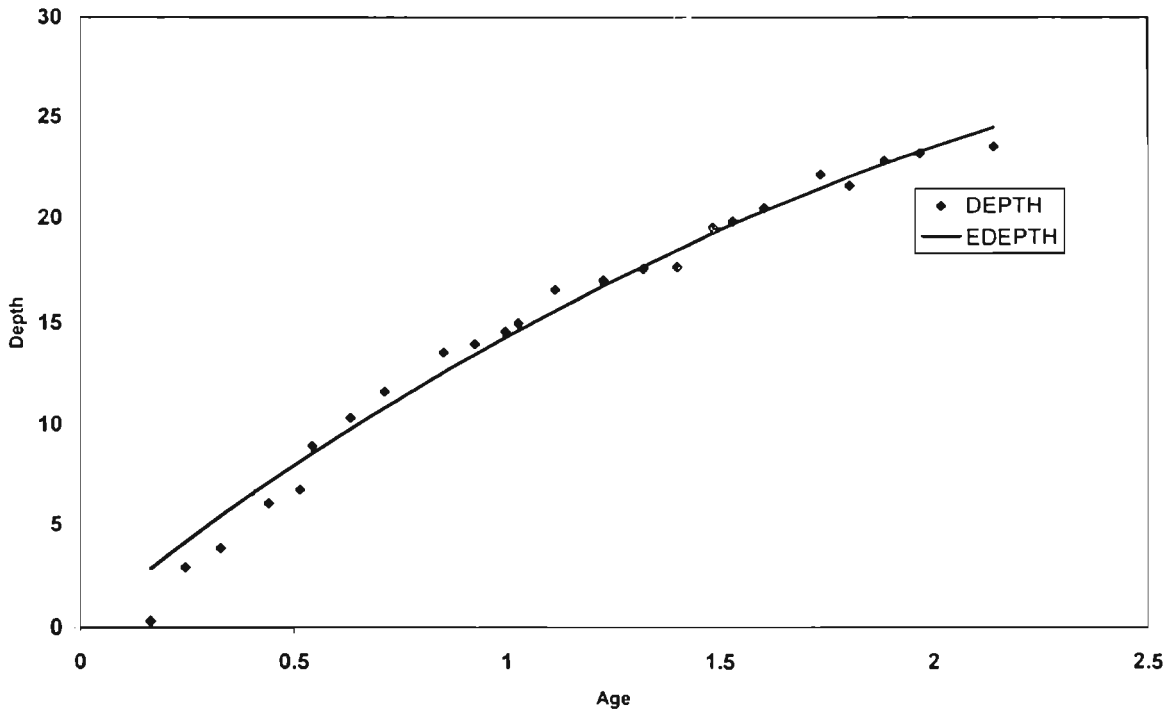


Figure 3.14. Growth in Weight of *P. fucata* grown in cages

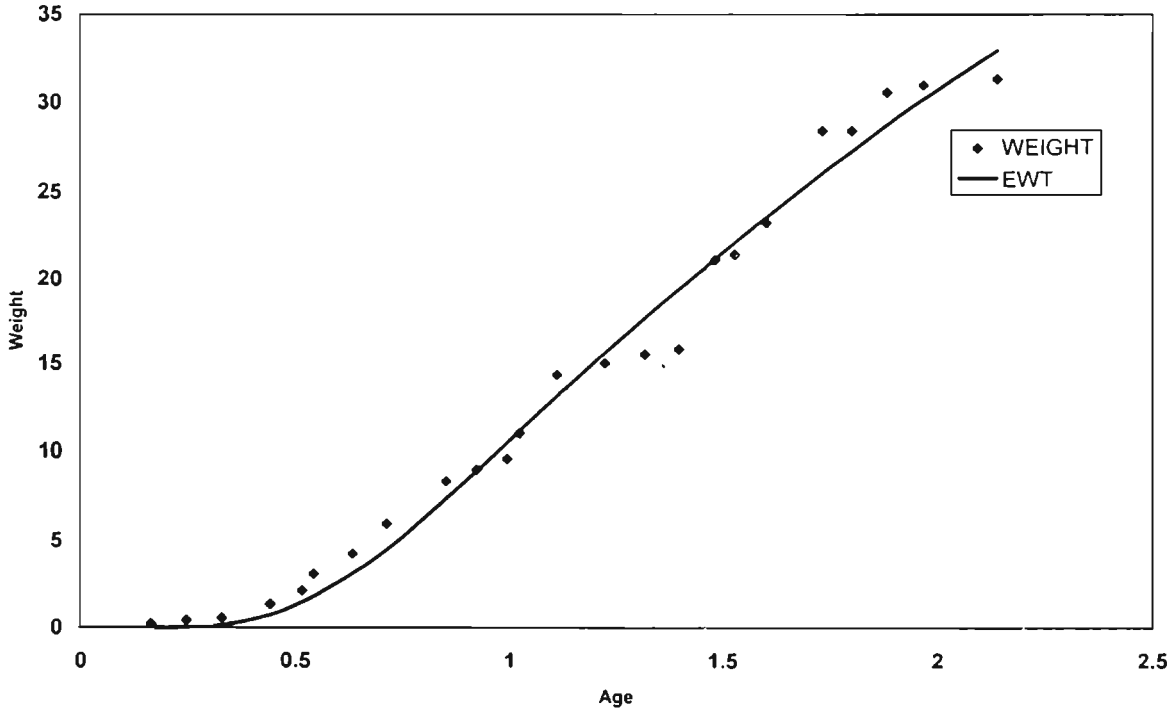


Table 3.1. Percentage survival and size of larvae of *Pinctada fucata* (Gould)

A: 'D' Shaped larvae

Age (days)	Size (Mean μm ± SD)	Size Range (μm)	Percentage survival
1	50.83 ± 1.90	50 - 55	100
4	67.03 ± 1.96	64 - 70	100
9	83.05 ± 3.08	80 - 90	73.33

B: Early Umbo stage

Age (days)	Size (Mean μm ± SD)	Size Range (μm)	Percentage survival
9	87.38 ± 2.26	84 - 90	26.67
10	93.33 ± 2.89	90 -95	10

C: Late Umbo stage

Age (days)	Size (Mean μm ± SD)	Size Range (μm)	Percentage survival
10	117.70 ± 7.85	100 -130	90
12	141.27 ± 13.09	120 - 160	100
15	148.65 ± 9.75	125 - 160	86.67
19	175.83 ± 3.76	170 - 180	20

D: Eyed stage

Age (days)	Size (Mean μm ± SD)	Size Range (μm)	Percentage survival
15	158.75 ± 6.29	150 - 165	13
17	187.50 ± 5.84	180 - 195	40
19	191.07 ± 7.64	180 - 205	46.67
20	187.00 ± 5.28	180 - 195	53.33
21	187.50 ± 3.54	185 - 190	6.67

E: Pediveliger stage

Age (days)	Size (Mean μm ± SD)	Size Range (μm)	Percentage survival
17	203.33 ± 5.77	200 - 210	10
19	216 ± 3.94	210 - 220	33.33
20	195 ± 5.00	190 - 200	16.67
21	200 ± 0.00	200 - 200	3.33

F: Plantigrade stage

Age (days)	Size (Mean μm ± SD)	Size Range (μm)	Percentage survival
20	215 ± 7.07	210 - 220	6.67
21	210 ± 0.00	210 - 210	3.33

G: Spat

Age (days)	Size (Mean μm ± SD)	Size Range (μm)	Percentage survival
20	225 ± 3.39	220 - 230	20
21	302.88 ± 38.99	220 – 350	86.67
23	295.5 ± 47.15	220 - 335	100

Table 3. 2. Transition of gonad of pearl oyster *Pinctada fucata* under different feeding conditions

Sl No	Experiment	% Change from stage III to stage IV	Days
1	With feed	43.33 ± 11.55	18
2	Without feed	40.00 ± 14.14	26
3	Farm conditions	53.12 ± 11.97	24

Table 3. 3. Percentage survival of *Pinctada fucata* during different growth phases

Growth phase	Survival percentage (Mean ± SD)	
	Tank	Beaker (Control)
Phase I (0- 23 days)	64.463 ± 0.768	24.3175 ± 2.437
Phase II (23 – 60 days)	71.37 ± 3.041	
Phase III (Day 60 – 2 year)	28 ± 0.85	

Table 3.4. Growth of *Pinctada fucata* larvae

Age	Size (Mean μm ± SD)	Size range
1	50.83 ± 1.90	50 – 55
4	67.03 ± 1.96	64 – 70
9	84.2 ± 3.45	80 – 90
10	115.27 ± 10.54	90 – 130
12	141.27 ± 13.09	120 - 130
15	150 ± 9.91	125 – 165
17	176.68 ± 17.68	145 – 210
19	196.33 ± 16.34	170 – 220
20	197.43 ± 17.03	170 – 230
21	288.67 ± 51.71	185 – 350
23	295.5 ± 47.15	220 – 355

Table 3.5. Analysis of variance of growth rate of *Pinctada fucata* larvae in tanks and beaker

Source of variation	SS	df	MS	F	F Crit	P value
Between treatments	5.581	1	5.51	25.29	7.71	0.0073*
Within groups	0.87	4	0.22			

* indicates significant difference at ($p < 0.05$)

Table 3.6. Analysis of variance of survival and settlement of *Pinctada fucata* larvae in tanks and beaker

Source of variation	SS	df	MS	F	F Crit	P value
Between treatments	1568.17	1	1568.17	87.93	7.71	0.0072*
Within groups	71.33	4	17.83			

* indicates significant difference at ($p < 0.05$)

CHAPTER 4

STUDIES ON THE MORPHOLOGY, ANATOMY, HISTOLOGY OF MANTLE AND PEARL-SAC OF PEARLOYSTER *Pinctada fucata* (GOULD)

CHAPTER 4

STUDIES ON THE MORPHOLOGY, ANATOMY, HISTOLOGY OF MANTLE AND PEARL – SAC OF PEARL OYSTER *Pinctada Fucata* (GOULD)

4.1 INTRODUCTION

Jameson (1901) stated that the identity and distribution of pearl oyster species of the genus *Pinctada* were difficult to separate form one another by hard and fast line owing to the absence of well marked diagnostic characters and the extraordinary geographical and ecotypical variations. However to achieve satisfactory results in the production of cultured pearls, it is absolutely essential to have a precise knowledge of the morphology, and the anatomy of the animal. Examination of the morphology and structure of the foot, gonad, viscera and the gonad show that they play a very important role in the production of a pearl and that the gonad has got another vital role in the nourishment of the pearl sac.

Herdman (1904) has given a detailed account of the anatomy of Indian pearl oyster *Pinctada vulgaris* (= *P. fucata*). Hynd (1955) and Kuwatani (1965 a, b) also contributed to our understanding of the pearl oysters of Australian and Japanese waters, in addition to throwing light on the functional role of digestive and reproductive organs.

Many workers explained the theory of pearl formation as the defence reaction the system when a foreign particle gets into the shell causing irritation. In due course the epithelial cells of the mantle form a sac and secrete pearly coating over this particle, which forms the pearl. The same principle was adopted in the production of cultured pearl by implanting a piece of the mantle along with a shell bead nucleus made out of molluscan shell. The histology of mantle and pearl-sac formation in Japanese pearl oyster *Pinctada martensii* (= *fucata*) has been studied in detail by Ojima (1952), Aoki (1956), Nakahara and Machii (1956), and Machii (1968) that of the Australian pearl oyster *Pinctada maxima* by Dix (1973); Zahab *et al.* (1992) and Comps *et al.* (2000) that of *Pinctada margaritifera*, Garcia-Gasca *et al.* (1994) that of

Pinctada *mazatlanica*. Awaji and Suzuki (1995) and Wada (1996) studied the histology and process of pearl formation in *Pinctada fucata martensii*. Ojima (1952) studied the histochemistry of the calcium in the mantle to understand the processes of shell and pearl formation in the pearl oyster *P. martensii*. Regular production of free spherical cultured pearls in the Indian pearl oyster, *Pinctada fucata* began in 1973 (Alagarswami 1974); however, subsequent work has shown that the quality of the cultured pearls produced differed considerably in individual oysters (Alagarswami 1991).

The pearl-sac, which is responsible for the formation of cultured pearl, is derived from the mantle tissue. Hence, some basic studies were carried out on the structure of mantle, its histology, growth of the grafted mantle and formation of pearl- sac in the Indian pearl oyster, *Pinctada fucata* (Gould) to analyse the aspects that would help to increase the percentage of survival and the quality of pearl.

4.2 MATERIALS AND METHODS

4.2.1 Morphology and Anatomy

For the study of the anatomy and morphology of the pearl oyster, fresh healthy *P. fucata* (DVM 57 ± 1 mm) collected from the farm of CMFRI at Tuticorin was used. Thirty animals were narcotized using menthol and preserved in neutral formalin. The animals were dissected out aseptically and each organ was carefully examined and thoroughly studied. In some cases live animals were also examined. To under stand course of the arterial system, Alizarin Red stain was injected through the auricles to locate the arteries and veins before dissection the nervous system, the animals were treated in dilute picric acid solution for ½ hour before dissecting and tracing the nerve innervations.

4.3 HISTOLOGY

4.3.1The Mantle

Fresh healthy *P. fucata* (DVM 57 ± 1 mm) collected from the farm of CMFRI at Tuticorin were used for the study. The mantle, tissues from ventral fold, isthmus of mantle, pallial mantle and central mantle near the gill attachment were cut out, fixed in neutral formalin, treated, sectioned 6-7 µm thick, stained, processed and mounted following the histological procedures explained by Weesner (1960).

4.3.1.2 Pearl - sac formation

4.3.1.2.1 Preparation of wax nuclei

The wax was put in 250 ml glass beaker and kept in a bath at 58° C for melting. The cooled wax was balled into 3, 4 and 5 mm diameter spherical nuclei and floated in cooled filtered seawater before implantation. On cooling the radius of the wax ball showed minor variations. 3 mm, 4 mm and 5mm nuclei were 3.12 ± 0.11, 4.09 ± 0.1 and 5.09 ± 0.06 mm respectively. The variations were as follows 3 mm – 3.12 ± 0.11 mm to 4.09 ± 0.10 and 5mm - 5.09 ± 0.06.

Pearl-sac formation was studied in 480 pearl oysters (Plate 4.10B) graft tissue of dimension 2 ± 0.05 mm x 2 ± 0.05 mm (Plate 4.11) and wax nuclei of 3, 4 and 5 mm diameter (Plate 4.12A). The implantation of pearl oysters was done by using the surgical instruments designed and fabricated in India (Plate 4.10). The implanted oysters were maintained in the laboratory in 6 numbers of 200 l FRP tanks each holding @ 100 l sea water at water temperature ranging from 29± 1° C, pH 8.2 salinity 35 ppt .The animals were fed on a diet of mixed algae and cultured diatoms at a feeding intensity of 2 l oyster day for each oyster till the termination of the experiment. Two oysters were cut open from each lot every day and the gonad with wax nucleus *in situ* was preserved in neutral formalin. Six hours after fixation, the mantle, gill and foot of the animals were carefully cut and removed. After ascertaining the "A" position of the gonad (Plate 4.16B) where in the nucleus with the pearl sac is situated, it was cut carefully using a surgical blade and preserved in neutral formalin for 18 hrs. The nucleus and pearl sac were then sectioned 6-7 µm thick stained and mounted as per the procedures explained by Weesner (1960).

To study the progress of the graft tissue to form pearl sac over the wax nucleus the same procedure was followed. The change in the structure and size of the graft tissue was observed and recorded. A set of 30 pearl oysters were implanted with 4 mm (4 ± 0.02) shell bead nuclei and 2 ± 0.05 mm x 2 ± 0.05 mm (as in the case of wax nucleus) was kept in the farm for 4 months for studying the formation of nacreous and abnormal pearls. For the abnormal pearl formation mantle graft tissue was taken out of the marginal zone of the mantle

To study the histology of the nacreous pearl-sac, abnormal pearl sac, the gonad portion of the oyster with the quality and abnormal pearl inside were carefully fixed in neutral formalin and Bouin's fixative. After 6 hrs, the pearls were carefully removed without damaging the pearl-sac and connective tissues of the gonad. The gonads were refixed in fresh fixative for another 18 hrs. The sections were cut as above and processed as per protocols mentioned before.

In all the cases, the slides were examined and photomicrographs were taken with the help of Erma scope and computerized prints were taken with the help of Zeiss binocular microscope with digital camera attachment under different magnifications from 5 x to 100 x (oil immersion objective). For morphological studies the detailed drawings of the dissected organs were drawn and for anatomical studies prepared slides were examined, photographed and printed as mentioned above.

4.4 RESULTS

4.4.1 Morphology and Anatomy of Pearl Oysters

The details of shell characters have already been explained in detail in Chapter II

4.4.1.1 Foot

The foot is a highly mobile, tongue shaped organ capable of great elongation and contraction (Plate 4.1B). It arises from the anterior region of the visceral mass midway between the mouth and the intestinal loop and the anterior branchie flanking it on either side. The major part of the foot is composed of network of fibres running in various directions ensuring a wide range of contractibility. The foot is provided with blood spaces and is innervated by nerve fibres making the organ a highly sensitive and active one.

4.4.1.2 Byssus Gland

The byssus gland (Plate 4.2B) is lodged at the proximate end of the foot ventrally. The byssal gland lodges the common root of a bundle of stout laterally compressed bronze green fibres, the byssal threads. Each fibre of the byssus anchors the pearl oyster to rocks and other objects by means of a discoid attachment at the distal extremity. The anterior edge of the byssal gland passes into the pedal groove extending medially along the whole of the remaining length of the ventral surface of the foot. The byssus threads are secreted by this gland and attachment is effected by the highly motile foot.

4.4.1.3 Muscular System

The pearl oyster is monomyarian, possessing only the posterior adductor the largest and the most important muscle in the body.

4.4.1.3.1 Adductor muscle

The adductor muscle (Plate 4.2A) stretches transversely across the body form the valve to valve. It is a massive wedge shaped bundle. The narrow end points upwards and lies immediately behind the ventricle of the heart. The terminal part of the rectum runs in the middle line along the

posterior surface. Two distinct regions of the muscles are obvious; one a narrow tendonous strip made up of white glistening fibres forming the posterior border and the other, a broad and massive semi-translucent fibres occupying the remainder of the mass. The power exerted by the contraction of this muscle is considerable, the rapid action of which resemble ratchet mechanism.

4.4.1.3.2 The Retractor

The retractors of the foot are a pair of symmetrically disposed muscles lying in the horizontal plane of the body (Plate 4.2B). The V- shaped muscles originate from the byssal gland. The ends of this muscle are attached to the right and left valves without making a separate scar on the nacre.

4.4.1.3.3 Levators

The levators of the foot are four, two anterior and two posterior (Plate 4.1B). Each of the anterior pair has its insertion at the apex of the umbonal recess of its respective valve pressing vertically downwards on either side of the mouth spreading laterally, fan – like as they go. The left anterior levator is strong and by contraction of the strong cord of fibres, the foot is drawn over the left side of the valve, which is convex and more spacious. The posterior levators are two short insignificant bundles, which originate high upon the anterior levator, exactly on level with the mouth passing through the visceral mass to be attached to the valves behind the anterior levator scar. The contraction of the anterior levator causes the foot to be retracted and dorsally raised. The intrinsic muscles of the foot are diffused forming a muscular enveloping sheath in the foot, with ill –defined muscle bundles passing from side to side, providing a framework wherein the tubules of the digestive glands ramify. The branchial muscles cause the shortening of the gills and withdrawal of their posterior extremities. They run within each ctenidial axis (Plate 4.2B) from end to end, close to the dorsal edge.

4.4.1.3.4 Pallial muscles

The pallial muscles (Plate 4.1B) are all retractors, and together constitute the orbicular muscle of the mantle. They are a series of fan-shaped

muscles radiating towards the mantle edge from a number of insertion centres (15-18) (Plate 4.IB) of various sizes arranged semi-circularly. Together these form the well marked pallial line scars on the shell. With the exception of heart and indistinct striation on the larger portion of the adductor, the muscle fibres are non-striped.

4.4.1.4 Digestive System

The oesophagus (Plate 4.2A), stomach (Plate 4.2A) and the greater portion of the intestine lie within the viscero- pedal mass. Two horizontal lips conceal the aperture of the mouth. The labial palps (Plate 4.2A) are smooth on the surface, turned away from the mouth and grooved on the opposed faces enclosing the mouth aperture. The mouth (Plate 4.2A) is a large, slit like depression placed transversely between the anterior levator muscles of the foot. The cavity contracts inwards to the narrow width of the short conducting tube, the so called oesophagus, which is straight, dorso-ventrally compressed and ciliated (Plate 4.2A). The hinder end opens into the anterior end of the stomach, which is an organ of surprising elaboration. Folds and depressions diversify the walls and floor of -the stomach and break them *into* definite areas. The tissues consist largely of greenish brown masses often termed as liver (Plate 4.2A) termed as digestive diverticula (Plate 4.2IA). Dense clusters of secreting alveoli open into ductules and these larger ducts lead into the cavity of stomach. The most conspicuous portion of the stomach is a slightly projecting vertical fold arising from the posterior wall marking out the cardiac stomach into a right and left chamber. This fold disappears towards the roof where it is smooth and unbroken, except for a well marked pit. The wide bipartite opening into the intestine and intestinal caecum marks the hinder end of the pyloric chamber. A gelatinous rod flattened and oblique occupies the sub- central position anterior to where the postero-ventral fold disappears midway along the floor. To the right of this area of the dendritie plate is a ridge with a furrow running up to the antero-lateral bile duct. A deep rugose-sub-oesophageal pit is well marked, anterior to the dendritic plate and high upon the right lateral wall. The posterolateral furrow leads from the posterolateral duct towards the intestinal aperture. On the left side, a short anterolateral fold lies between the pre-intestinal

depressions of suboesophageal pit.

There are eleven terminal ducts opening into the intestine (a) anterolateral duct, (b) posterolateral duct opening to the posterior third stomach, (c) the postero- ventral duct, (d) three subventral ducts, (e) two pre-intestinal ducts opening within preintestinal depression and (f) three small suboesophageal ducts below the oesophageal aperture.

The head of crystalline style (Plate 4.2A) projects out of the sac where it is formed and across the cavity of the stomach where it bears against an irregular area of cuticle bearing a projecting tooth, known as the gastric shield. This area is not ciliated.

The intestine can be divided into three sections of approximately equal length such as the descending, the ascending portion and the rectum (Plate 4.2 A). The first portion passes ventrally through the posterior part of the visceral mass. Then it passes behind the base of the byssal gland and between the two pedal retractor muscles wherefrom it changes its direction curving forwards and downwards to the visceral mass passing on as ascending branch. A longitudinal fold projects inwards from the anterior and one from the posterior wall of the descending intestine. The apices of the two folds are so close together at the lower third so as to form two distinct tubes. The larger cavity is completely filled with a clear gelatinous solid cylinder, the crystalline style (Plate 4.2A). The narrow tube on right side is the true intestine, the wider left being the sheath of the crystalline style, which is imperfectly separated from the anterior portion of intestine, with which it communicates by a longitudinal cleft. The upper end of the style certainly projects into the stomach. The valvular folding of the intestinal ridge gives entrance to the ascending intestine which curves back- wards along the base of the visceral mass to the left of the descending intestine). The ascending portion crosses to the right at the posterior extremity of the ventral surface of the visceral mass where the two intestinal divisions intersect. The intestinal loop thus formed is the visceral loop. From the point of intersection the ascending intestine turns sharply upwards, running parallel with and closely adjacent to the upper part of the descending portion .The portion of the

intestine forming the second limb of the visceral loop is continued into it as a somewhat undulating ridge disappearing midway. At the point where this diversion of the intestine assumes a dual course, an increase takes place in diameter, side by side with the appearance of a long longitudinal fold-typhlosole, projecting from the anterior wall, curving over to the posterior side of the tube, and then running vertically upward without further change of course. Longitudinal furrows channel the surface. As it approaches the level of the floor of the stomach, the typhlosole thins down rapidly to a low ridge, and the intestine itself then curves posteriorly in the direction of the heart. This change in direction and thinning of typhlosole marks the beginning of rectum.

The rectum runs parallel posterior through the upper part of the pericardium. Beyond this it curves ventrally and passes round the posterior aspect of adductor muscle in the median line ending in an erectile ear like process, the anus, situated opposite the exhalent orifice of the mantle. The anal process is comparatively larger and slightly curved. Anal pappila is trifold (Plate 4.2A). It stands out at right angle to the last section of the rectum, and the tip is directed posteriorly. The anal aperture is situated at the base on the ventral aspect.

4.4.1.5 Respiratory System

The gills (Plate 4.2B) consist of four crescent shaped plates, two half gills on each side which hang down from the roof of the mantle cavity like book leaves. They represent a series of ciliated sieves the whole constituting a feeding surface of utmost efficiency. Two rows of long delicate branchial filaments (Plate 4.2B) are inserted at right angles along the whole length of the axis or vascular base which extends from the ventral border of the palps anteriorly curving round ventrally and posterior to a point opposite the anus with its convexity first forwards and then downwards. Where they terminate the mantle lobes of the two sides are briefly united by way of the inner mantle folds thus dividing the mantle cavity into a large inhalent chamber containing the gills and a much smaller exhalent chamber. Water enters by the one and leaves by the other. The outwardly directed parallel filaments of each series are folded upon themselves, so that they are V shaped, the folding being in

such a way that external filaments turn outwards and internal inwards. Consequently each branchial plate furrow of the double filaments consists of two lamellae, the direct and the reflected, which enclose narrow interlamellar space. The common base of each ctenidium is a vascular attached ridge reaching from the anterior end of gills. Hollow outgrowths, inter-lamellar junctions, containing branches from the afferent vessels (AV) (Plate 4.2) convey blood from the axial trunk to the base of reflected lamellae. The blood enters certain of the individual filaments, flows outwards to the free margin, passing over to the direct filaments returning inwards to the branchial or ctenidial axis (Plate 4.2B) where it joins the different vessel by openings along each side. Neighboring filaments are joined by continuous organic union mainly at the lower and the upper ends of the reflected filaments, where there are longitudinally running blood vessels. Elsewhere the filaments are joined chiefly by the interlocking stiff cilia of the large ciliated discs, which occur at intervals, throughout their length. The normal function of the ordinary cilia on the branchiae is to create a current of water, which enters the pallial chamber and passes over and through the branchial lamellae so as to purify the blood flowing in the filaments and to convey the food particles to the mouth. Younge (1960) has elaborately dealt with the mechanism of the food movements to the palps from the water current generated by the ciliary movement of the ctenidia.

4.4.1.6 Excretory System

The excretory system consists of the paired nephridia and numerous small pericardial glands (Plate 4.2B) projecting from the walls of the auricles. The nephridia consist of two large symmetrical pouch-like sacs occupying either side of the hinder half of the viscero-pedal mass. Each opens into the pericardium by a wide duct and to the exterior by a minute pore. They intercommunicate by a wide channel beneath the auricles. In outlines each is roughly triangular, the apex passing into the channel under the auricle, while the elongated base looks towards and forwards coinciding with the base of the anterior third of the gill of that side, and thus conforming to the inclination of the gill.

The outer wall of the nephridium (Plate 4.2B) is thin and membranous; it is fused with the body wall, as is also the most anterior portion of the inner wall, namely, that strip extending from the base of the gill to the viscero-pedal mass. From this line it runs back, overlying and in contact with the hinder part of the gonad, gradually narrowing as it approaches the auricle. The renal aperture is a minute oval slit like opening with sphincter muscle. It opens immediately below the genital aperture within an inconspicuous lipped slit placed at the junction of the inner plate of the inner gill with the visceral mass at a point about midway between the ventral border of the latter and the base of the foot.

Each nephridium consists of a glandular and non glandular portion. By separating the right and left ctenidia and reflecting each, the glandular region is seen as narrow, elongated coloured strip, yellow or pale brown or even dark dull red, bordering the anterior part of the inner base of each gill. It consists of spongy tissue, occupying the anterior angle formed by the meeting of the inner and outer walls of the organ, and the secretion passes from the cavernous chambers of the glandular region directing into the spacious cavity of the main or non-glandular portion. The passage connecting the right and left nephridia lies beneath the auricles. It is a wide tunnel with thin membranous walls, bounded behind by the lower part of the pericardium. While in front its wall lies against the visceral mass below fusing with the body wall and forming part of the root of the adductor embayment of the suprabranchial chamber. The renopericardinal tubules are a pair of wide lateral prolongations of the precardiac part of the pericardium, thin walled and membranous and directed forwards. Each gradually narrows towards the anterior end where it opens into the non-glandular part of nephridium.

The aperture is a curved slit, with the concavity facing towards the ventral aspect. It has got one lip, the tube opening at a very acute angle. It is situated upon the inner wall of the nephridia. A small area around is tinted with brown pigment. The presence of accessory pericardial glands on the walls of auricles is said to have excretory function. These glands are dark brown in colour. The lower or auricular end of the pericardium is also glandular. Its epithelium is thrown into folds formed of granular vacuolated

cells of the same character as those of the nephridium.

4.4.1.7 Circulatory System

This consists of a heart with a series of arteries, which lies above the adductor, being contained in a pericardium (Plate 4.3A) and consisting of a single ventricle, (Plate 4.3A) a pair of contractile thin walled auricles (Plate 4.3A), one on each side. Blood circulation is by contraction into the anterior and posterior aorta (Plate 4.3 A). The latter is short and serves the adductor, rectum and the anus; the rest of the body is supplied by the anterior aorta, which gives off a series of minor arteries (Plate 4.3A). These open into the sinuses or blood spaces in which blood slowly circulates. The aorta finally communicates with a pair of large blood vessels that run around the margin of each mantle lobe. Deoxygenated blood is collected in veins (Plate 4.3A), which carry it either into the gills or excretory organs. Blood flows round the organs and is purified by removal of waste products of metabolism. From the nephridium (Plate 4.3A) a pair of accessory hearts pumps it into the marginal vessel of the mantle. Finally blood, from the mantle together with that from the gills returns to the heart through efferent branchial vein (Plate 4.3A) by way of auricles, the blood is colourless.

4.4.1.8 Reproductive System

The sexes are separate except in occasional cases. The gonads are paired but asymmetrical they form a thick envelop covering the stomach, liver and the first two sections of the intestine, connecting a greater part of the outside of the proximal portion of the viscero- pedal mass (Plate 4.2). The gonads do not hide the byssal gland. When the viscero-pedal mass is viewed from the right side of the byssal gland it is seen as a broad band reaching from the base of the foot back-wards to the right retractor muscle. This band appears to divide the left gonad into a larger part dorsally and a ventral smaller portion. No portion of the reproductive glands extends into the foot proper or into the mantle.

The male and female gonads are practically indistinguishable. Both are creamy yellow in colour. The male gonad in some cases is rather

paler than the female. The gonads, testes or ovaries as the case may be, consist of branched tubuli with myriads of succate caeca, the alveoli. The spermatozoa and ova develop in these. The accumulated ripe gametes fill these alveoli and tubuli and later pass into three trunks, which converge into one just within the external genital aperture (Plate 4.2B). The genital aperture is a horizontal slit located at the junction of the inner plate of the inner gill opening dorsal with renal aperture.

4.4.1.8 Nervous System

The laterally symmetrical nervous system has three pairs of ganglia (Plate 4.3B) the cerebral ganglia at the sides of the oesophagus, (2) the pedals joined to form a single ganglion at the base of the foot and (3) a pair of large visceral or parieto - splanchnic ganglia lying upon the anterior surface of the adductor. The stout paired cerebrovisceral connectives (Plate 4.3B) link the cerebral ganglia with the parieto-splanchnic ganglia, while a pair of cerebro-pedal connectives (Plate 4.3B) joins the cerebral ganglia with the pedal nerve mass. The cerebral ganglia are supra-oesophageal in position, and a nerve cord or commissure passing over the oesophagus connects the two cerebral ganglia (Plate 4.3B). A single stout transverse visceral commissure forms the two parieto-splanchnic ganglia (visceral ganglia) (Plate 4.3B). The cerebro-visceral connectives taking their rise at the posterior end of the cerebral ganglion, each passes backwards and downwards bound within the visceral mass till it merges opposite the upper angle of the base of the foot. It passes ventrally over-laid by the renal sinus entering the tissue at the base of the gills. It turns slightly forwards still passing ventrally and ends in its respective parieto-splanchnic ganglion. The cerebral ganglion of each side gives off anteriorly a stout nerve, the anterior common pallial. This bifurcates forwards. The outer branch (external pallial nerve) runs along the pallial edge, uniting and anastomosing with the corresponding external pallial branch of the postetior common pallial trunk. The cerebral ganglia innervate the lateral palps and the otocysts.

The cerebro-pedal connectives arise from the posterior and outer sides of the cerebral ganglia and run downwards within the visceral

mass just behind the levator muscles of the foot to the pedal ganglion. They lie close together in their course. Four principal nerves arise from the pedal ganglion (Plate 4.3B), which innervates the foot and the byssal gland. Each of the visceral or parieto-splanchnic ganglia receives from above the stout cerebro- visceral connective, the two ganglia being themselves united by a single transverse visceral commissure (Plate 4.3B). Each ganglion also gives off two stout distributory nerves an anterior lateral and a posterior lateral. Each branchial nerve (Plate 4.3B) leaves the ganglion at the anterior lateral corner, turns down into the base of the gills and then backwards to the posterior tips following the afferent vessels. The posterior pallial nerves (Plate 4.3B) emerge from the posterior end of the visceral ganglion; from the base of each, a stout nerve passes straight back till it reaches the pigmented pallial sense organs of its respective side, a little anterior to the anus.

The common pallial trunk passes backwards and out- wards bifurcating; the external branch, the larger, is the external pallial nerve. The inner branch follows a median course but divides. The outer of the resultant nerves becomes the pallial nerve; the inner, internal pallial nerve. By the ramification of these three nerves in the muscular marginal region of the mantle and by their anastomosing, a complex network of nerves termed ' pallial plexus ' is formed.

4.4.2 HISTOLOGY OF THE MANTLE

4.4.2.1 Mantle Histology

The mantle of pearl oyster consists of two identical lobes, right and left, united dorsally along the hinge line to form the mantle isthmus. The mantle lobe is divided into (i) marginal zone, (ii) pallial zone and (iii) central zone (Velayudhan and Gandhi 1987) (Plate 4.4).

4.4.2.1.1 Marginal Zone

The free margin of the mantle lobe was thick, pigmented and fringed with tentacles. The marginal mantle was composed of the inner (IF), middle (MF) and outer (OF) folds (Plate 4.4). The outer and middle folds were separated by the periostracal groove (PG). Morphologically the folds were

similar but functionally different. The periostracal groove has got stratified epithelial cells (SEP) and periostracal secretions (PS).

Inner fold: the inner fold (Plate 4.4) was larger than the other two folds of the marginal mantle. It was covered with a single layer of ciliated epithelium (CE) (25 µm high) with basal nuclei. The inner portion of the mantle showed prominent pigmentation (PE). A strong band of longitudinal and transverse pigmented muscles (MS) was present below the epithelial layer. Acidophillic secretory cells (AS) measuring 3-5 µm were less while the wandering cells (WC) were more in the sub-epithelial cells.

Middle fold: The inner margin of middle fold (Plate 4.9A) was constituted like the inner margin of inner fold, but in the latter the epithelium was ciliated (20 µm) and columnar (CC) (Plate 4.9) in nature with pigmentation. Wandering acidophilic mucous cells (BS) were comparatively more at the tip of the middle fold (Plate 4.6B). Granulated acidophillic cells (AS) were also present. The ciliated columnar epithelium (CE) (25 µm) of the inner margin of the fold further elongated near the periostracal groove (PG) (Plate 4.5B) and reached the size of 35 µm, while at other places, they were cuboidal, brush-bordered (7-15 µm) and non-ciliated.

Outer fold: The outer surface of the fold was covered with specialized cells (NE). Elongated (30-35 µm) stratified columnar epithelial cells (SEP) occurred close to periostracal groove (PG) on the inner surface of the fold. Non-ciliated and non-pigmented low columnar epithelium (10-15 µrn high) (NE) (Plate 4.5A) containing basophilic cytoplasm was present on major part of the outer fold, becoming elongated (10-20 µrn) towards the tip. Basophilic cells (BS) (Plate 4.5B) occurred more on the sub-epithelial layer near the periostracal groove. Mucous cells (MU) (Plate 4.5B) were more on the inner margin of the fold, and the acidophillic cells (AS) (Plate 4.5A) towards the tip.

Mantle isthmus: Mantle isthmus (Plate 4.9B) or dorsal mantle consisted of non-ciliated columnar epithelium (NCC) (30-45 µm) with muscle fibres scattered below. Orı the dorsal side a few secretory cells (SC) were

present, and can be observed when stained in Ehrlich's haemotoxylin eosin. Sub-epithelial secretory cells were totally absent.

4.4.2.1.2 Pallial Zone

The outer epithelial cells were low columnar, nonciliated (LC) (Plate 4.8A) and small (4 - 8 µm) than the ciliated inner epithelial cells (CE) (10- 30 µm) (Plate 4.8A). In between was the muscular connective tissue (MC) (Plate 4.8A). In the outer epithelial cells of the pallial mantle, sub-epithelial layers with secretory cells (SC) and large vacuolized / porous secretory cells (VC) were present. The sub-epithelial secretory cells (SC) (Plate 4.8B) were present in the inner epithelium of the pallial mantle. The acidophilic mucous cells and granulated acidophilic secretory cells (AS) were encountered both in the outer and inner epithelial cells of the pallial mantle (Plate 4.8A & B).

4.4.2.1.3 Central Zone

The outer (shell) side of the central mantle was lined with low columnar epithelium (CE) (10- 15 µm) (Plate 4.7A). The epithelial layer contained acidophilic secretory cells (AS) and basophilic mucous cells (MU) (Plate 4.7 A). The inner margin of the central mantle is non pigmented having low columnar epithelium (CC) cells (Plate 4.7 B). Subepithelial secretory cells (SC) (Plate 4.7 B) are also seen in the inner margin of the central mantle region. Histologically, the secretory cells (SC) of inner epithelium of the central mantle (Plate 4.7B) looked similar to those of the inner epithelial cells (Plate 4.7B) of the pallial mantle.

4.4.3 PEARL-SAC FORMATION AND ITS HISTOLOGY

Formation of pearl-sac was observed in the wax nucleus implanted in the gonad of the oysters within 3-7 days after implantation in case of 3 mm nuclei, 4-10 days in the case of 4 mm nuclei and 6-12 days in the case of 5 mm nuclei (Table 4.1).

The histological studies of the implanted graft on day 2 (Plate 4.13 A) showed that the inner epithelial cells (IEP) was not fully disintegrated

while the outer epithelial cells (OEP) facing the wax nucleus was observed to be slightly proliferating.

The graft tissue on day 4 showed proliferation stage (Plate 4.13B) (roundish, acidophilic and larger sickle and spindle shaped) basophilic secretory cells (AS, BS) in the subepithelium. These secretory cells were seen to be coming out of the broken walls of the vacuolated porous cells (VC) of the outer epithelium towards the wax nucleus (WN).

A thin film of nacreous coating was found deposited on the nucleus within 18 days on a 4 mm wax nucleus (Plate 4.14). Histological studies of the nacreous pearl-sac epithelium (PE) in the male gonad (GN) of the pearl oyster showed acidophilic cells (AS) in low magnification. Under higher magnification (Plate 4.14B) the hexagonal crystalline secretion (CR) on the wax nucleus, and acidophilic secretory cells (AS) in the pearl sac epithelium (EP) covering the wax nucleus were observed.

In further higher magnification, this nacreous pearl sac (Plate 4.15 A) with pearl coating on the wax nucleus (WN) was observed that the concentration of granular acidophilic secretory cells (AS) in the sub epithelium of the nacreous pearl sac. The proliferated cells (haemocytes) (HC) have replaced part of the nacreous peal sac epithelium and pearl coating (PWN) showing that the pearl sac has become a part of the gonad of the host oyster.

Histological studies of the nacreous pearl sac (Plate 4.15B) formed on the shell bead nucleus which had produced good and lustrous pearl showed, the presence of more cuboidal, flattened non-ciliated epithelial cells (CP) along with large secretory cells (4-6 µm) (Plate 4.15B). The cells were similar to the cells of the muscular tissues of the gonad (GN). The haemocytes of the gonad tissue extended into the pearl-sac epithelium. The nucleus was in the centre and occupied much of the cell space. The secretory cells were scattered within and beneath the pearl-sac epithelium. The acidophilic secretory cells were more with large granules (AS) and the basophilic mucous cells were few and these two types of secretory cells were located within and beneath the pearl-sac epithelium.

In case of periostracal pearl-sacs (Plate 4.16A), which were produced with the wax nuclei in the laboratory, tall, ciliated columnar epithelial cells (CCP) (30-35 µm) were well distributed. Congregations of cells resembling haemocytes (HCC) were also present in some areas of the epithelium (Plate 4.16A). Basophilic mucous cells (BS) with granular inclusions were common. Acidophilic cells (AS) with large secretory granules were present in some parts of the periostracal pearl-sac.

The pearl oyster implanted with 4 mm shell bead nucleus and 2 mm x 2 mm graft has produced good lustrous pearls (Plate 4.16B) after 4 months whereas the abnormal pearl sac produced "D" quality pearls.

4.5 DISCUSSION

The anatomy of Pinctada fucata is comparable to that of *P. vulgaris* by Purchon (discussed in Kuwatani, 1965 a) who compared the anatomy of *P. vulgaris* and *P. martensii* and of Herdman (1904). Herdman (1904) described the anal pappila of *Margaritifera vulgaris* as five fold. Shiino (1952) had drawn the structure of the anal pappilae of *P. martensii* (Dunker). In *P. fucata* (Gould) the anal pappillae is trifold. In *M. vulgaris* there are three nerves enervating from the pedal ganglion wherein *P. fucata* (Gould) has 4 nerves. Herdman (1904), Shiino (1952) noticed the renogenital aperture in M. *vulgaris* and *P. martensii*. In *P. fucata* (Gould) the aperture is very difficult to trace. Kuwatani (1965 *a*) differentiated the stomach of *P. martensii* from *P. vulgaris*. According to him, in *P. vulgaris* the left intestinal groove arise from the pouch leadingto the origin of the groove. In *P. martensii*, the groove however is at the point of coming to the tongue from the major typhlosole in the second embayment. Chellam (1983) stated that the stomach content of *P. fucata* contained straight hinge stage bivalve larvae from 27.5 to 115 µm in dorso ventral axis and 37.5 to 125 µm in antero – posterior axis. The larvae with umbo ranged in size from 162.5 to 232.5 µm in dorso ventral axis and 200 – 275 µm in antero – posterior axis. In *P. martensii* the charcoal particles taken into the oesophagus were 30 µm and 17.5 µm respectively. It is inferred from this study that the stomach width as well as the oesophagus is larger in *P. fucata*.

The regional as well as functional differentiation of the mantle is marked in *Pinctada fucata*. The marginal mantle consisted of 3 folds, inner, middle and outer folds. The inner fold was muscular, the middle sensory and the outer shell fold secretory in function (Dix, 1973). The specialized, elongated columnar epithelial cells, occurring close to the periostracal groove, may be the ones, which secrete the periostracum in *Pinctada fucata*. A similar type of stratified columnar cell has been recorded in *P. maxima* (Dix, 1973) and in *P. margaritifera* (Zahab *et al.*, 1992). The non-ciliated non-pigmented low columnar epithelial cell, scattered on the other parts of the outer fold suggested a different function.

The inner fold was larger than the middle and outer folds. It had strong longitudinal and transverse pigmented muscles. Wandering cells, which might be sensory in function, were distributed in the sub-epithelial cells of the inner and middle folds. Apart from the wandering cells, the presence of a large number of acidophilic cells in the middle fold suggested probably a sensory function for this fold.

The outer epithelial cells of the pallial and the central mantle were small and non- nucleated with large vacuolized / porus secretory cells. Basophilic mucous cells and granulated acidophilic secretory cells were found scattered in the inner and outer epithelial layers of both pallial and central mantle. These and their proximity to the shell suggested their secretory function, particularly of the inner nacreous layer. The epithelial cells of the marginal mantle along with the inner surface area were ciliated with secretory cells suggesting a different function for these cells. This was same in *P. maxima* (Dix, 1972) However, Zahab *et al.* (1992) found that the cells of the outer fold of the pallial epithelium was mucous secretory in nature in *P. margaritifera*.

The epithelial cells of the nacreous pearl sac differed in size and shape from that of the periostracal pearl-sac. Some similarity was seen between the periostracal pearl-sac and that of the outer epithelial cells of the pallial and central mantle regions. The presence of haemocytes in the epithelium and sub-epithelium, the large acidophilic secretory cells with granules, and the few number of the basophilic mucous cells were the other similarities. According to a recent study by Compos et al. (2000) who studied the ultra structure of the pearl sac epithelium of *P. margaritifera*, the abnormal pearl sac was due to mineralisation disturbances related with the production of laminated organic structures in nacreous layers of the shell and the pearl. They further put forward a hypothesis that the epithelium which supplies the crystal deposit may also consists of areas of cells involved in periostracum production. This cell could be originally present in the mantle tissue used as graft or differentiated during the formation of pearl sac epithelium owing to some specific factors. They were of the opinion that the abnormal secretion

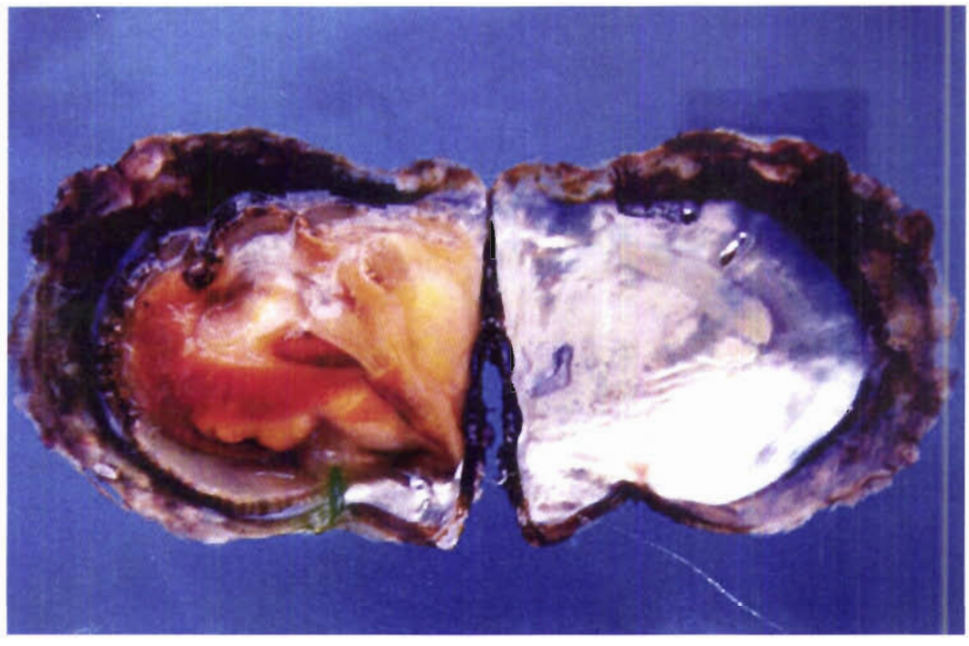
was a response to either a wound healing mechanism or by the introduction of a foreign body during the grafting.

From the histological studies of the implanted graft tissue on day 2 in the gonad of the oysters the inner epithelial cells were not fully disintegrated while the outer epithelial cells facing the wax nucleus almost is in proliferating stage for the formation of pearl sac over the wax nucleus. The most significant observation was the histology of the 4^{th} day graft on the wax nucleus in P. *fucata* gonad, which showed formation of pearl sac from day 4 onwards. This is in agreement with the works of Awaji and Suzuki (1995) and Wada (1996) for *Pinctada fucata martensii* and Garcia-Gasca *et al.* (1994) for *Pinctada mazatlanica.* The sickle and spindle shaped larger basophilic muscle cells and roundish acidophilic cells formed from the graft was similar to those type of cells produced by *in vitro* tissue culture of outer mantle epithelium of *P. fucata* by Machii (1974)

The tall ciliated columnar epithelial cells with basal nuclei and small granules, the irregularly arranged cells with projections and the presence of basophilic mucous cells with granular inclusions are the characteristic features of the periostracal pearl-sac. To a certain extent, these characters are common in epithelial cells found in the periostracal groove, the function of which is to secrete the periostracum of the shell. The presence of both types of secretory cells in the mantle regions and in the pearl-sacs indicates their dual function, in the secretion of conchiolin. Ojima (1952) is of the opinion that the middle fold of mantle is the main portion for secretion of conchiolin. The presence of secretory cells in other parts of the mantle indicates that the shell formation is not restricted to the middle fold. The mucus takes a significant part in the secretion and deposition of the conchiolin and calcium (Ojima 1952).

PLATE 4.1

A. *Pinctada fucata* (Gould) : Figure Illustrating the morphology and anatomy of the oyster



B. Figure Illustrating the right shell remove showing morphology and anatomy of the oyster

1. Central part of right mantle lobe ; 2. Right retractor of foot ; 3. Smooth part of adductor ; 4. Striped part of adductor muscle ; 5. Right levator of foot ; 6. Right anterior levator of foot ; 7. Right pallial muscles ; 8. Pallil muscle ; 9. Converged end of pallial muscle ; 10. Papillae of middle lamellae of pallial margin ; 11. Outer end of pallial margin ; 12. Pallial fold ; 13. Exhalent orifice ; 14. Left mantle lobe ; 15. Byssus ; 16. Pedal groove ; 17. Foot ; 18. Oral aperture ; 19. Mid dorsal line ; 20. Hinge line ; 21. Hinge ; 22. Ligament ; 23. Anterior ear ; 24. Posterior ear ; 25. Growth process ; 26. Dorsal margin ; 27. Ventral margin ;.28. Left anterior levator of foot.

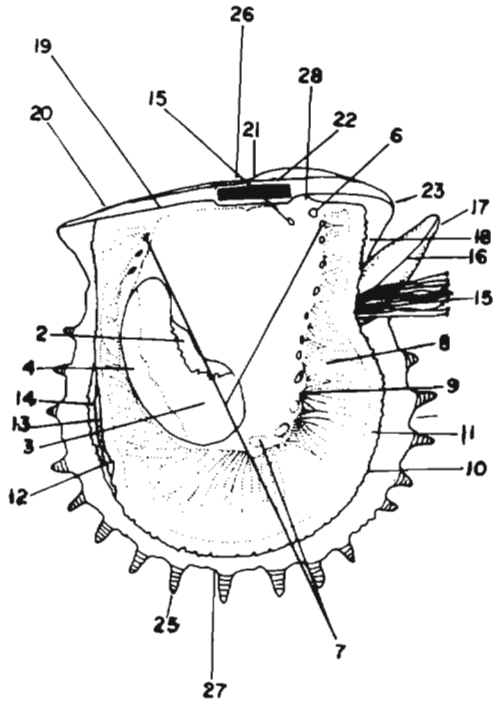
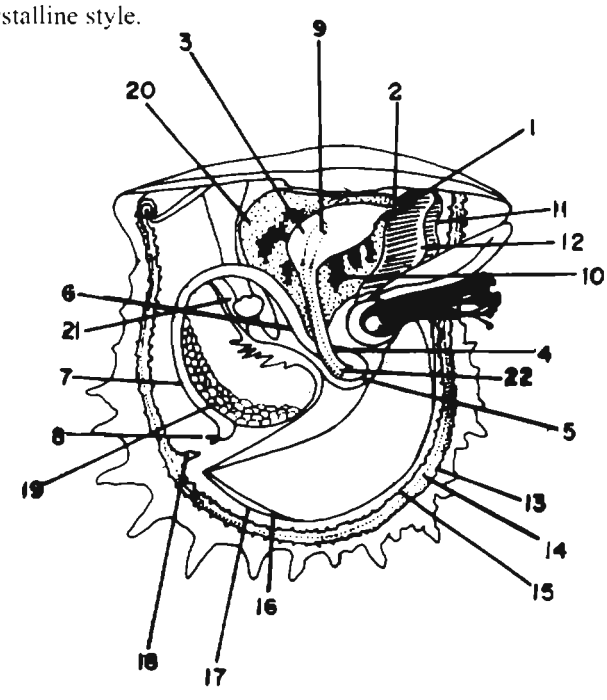


PLATE 4.2

A. *Pinctada fucata* (Gould) : *Digestive system*

1. Mouth ; 2. Oesophagus : 3. Stomach ; 4. Descending portion of intestine ; 5. Intestinal loop : 6. Ascending portion of intestine ; 7. Rectum ; 8. Anal process ; 9. Anterior part of crystalline style ; 10. Liver diverticulum ; 11. Left outer labial palp ; 12. Left inner labial palp ; 13. Outer fold of left mantle ; 14. Middle fold of left mantle ; 15. Inner fold of left mantle ; 16. Left inner ctenidium ; 17. Left outer ctenidium ; 18. Pallial fold ; 19. Striped part of adductor muscle ; 20. Gonad ; 21. Pericardial chamber ; 22. Posterior part of crystalline style.



B. Respiratory, excretory and reproductive systems

1. Renogenital aperture ; 2. Pericardial gland ; 3. Nephredium ; 4. Efferent branchial vein ; 5. Afferent branchial vein ; 6. Ctenidial axis ; 7. Muscular ctenidial axis ; 8. Anal process ; 9. Pericardium ; 10. Byssal gland ; 11. Root ; 12. Byssus ; 13. Pedal groove ; 14. Foot ; 15. Left anterior levator ; 16. Right posterior levator ; 17. Right outer labial palp ; 18. Retractor ; 19. Gill ; 20. Gonad and 21. Gill filament.

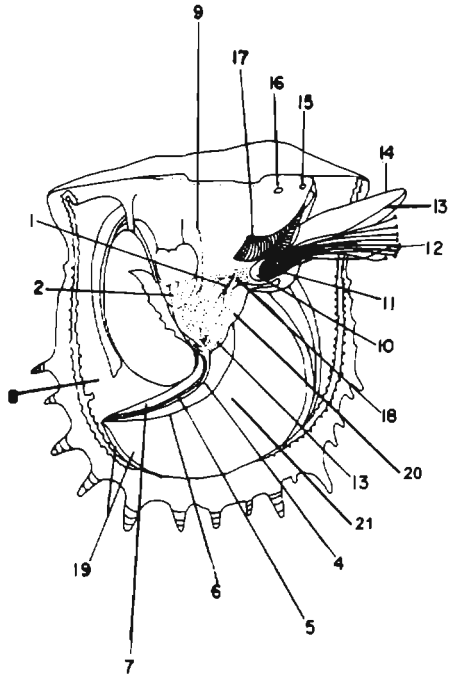
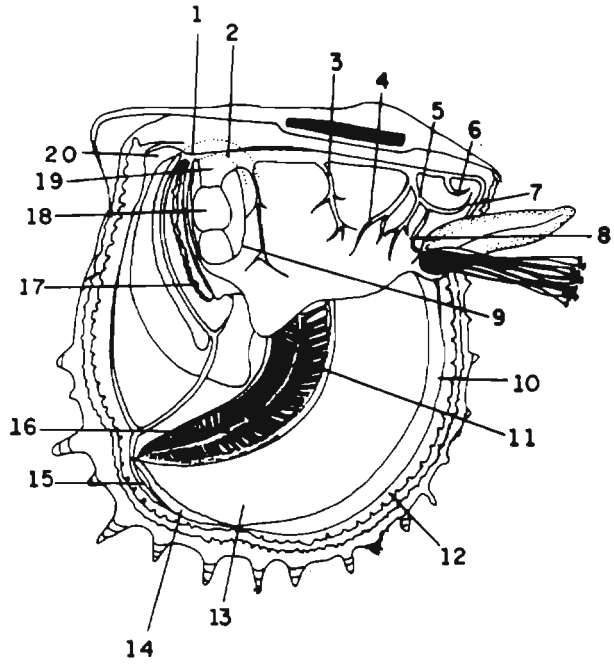


PLATE 4.3

A. *Pinctada fucata* (Gould) : Circulatory system

1. Posterior aorta ; 2. Anterior aorta ; 3. Posterior hepatic artery ; 4. Anterior hepatic artery ; 5. Hepato pedal artery ; 6. Upper labial artery ; 7. Lower labial artery ; 8. Pedal artery ; 9. Pericardium ; 10. Anterior pallial artery ; 11. Efferent branchial vein ; 12. Inner side of right mantle; 13. Right inner gill ; 14. Righ: outer gill ; 15. Anterior pallial artery ; a6. Afferent branchial vein ; 17. Right mantle lobe ; 18. Auricle ; 19. Ventricle ; 20. Posterior pallial artery ; 21. Visceral artery.



B. Nervous system

1. Cerebral ganglion ; 2. Cerebral commissure ; 3. Cerebropedal connective ; 4. Pedal ganglion ; 5. Visceral ganglion ; 6. Visceral commissure ; 7. Cerebrovisceral connective ; 8. Branchial nerve ; 9. Left mantle ; 10. Left inner gill ; 11. Posterior pallial nerve ; 12. Right mantle ; 13. Right inner gill ; 14. Adductor muscle.

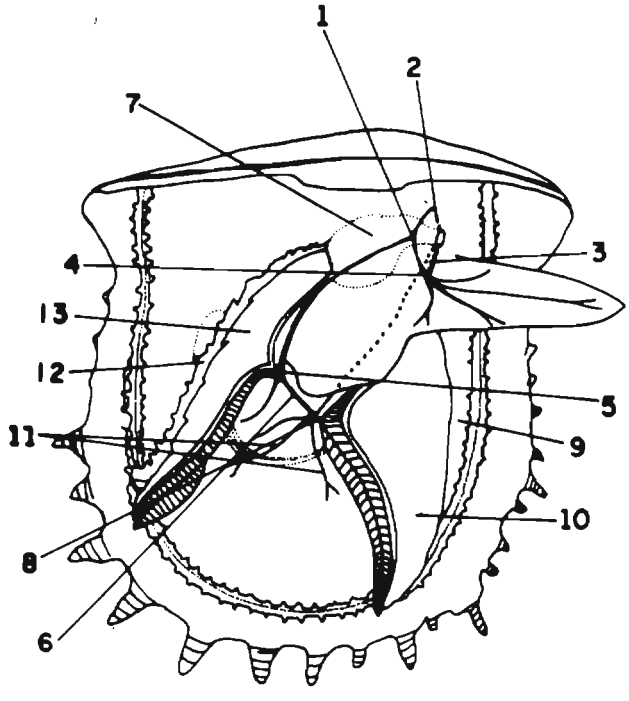


PLATE 4.4

A. *Pinctada fucata* (Gould) : Digrammatic view of sites and different regions of mantle selected for histological studies

Central mantle (CM) ; Pallial Zone (PZ); Marginal Zone (MZ); Central Zone (CZ); Adductor muscle (AM); Pallial mantle (PM); Marginal mantle (MM); Inner fold (IF); Middle fold (MF); Outer fold (OF); Periostracal secretion (PS) and Mantle isthmus (IS).

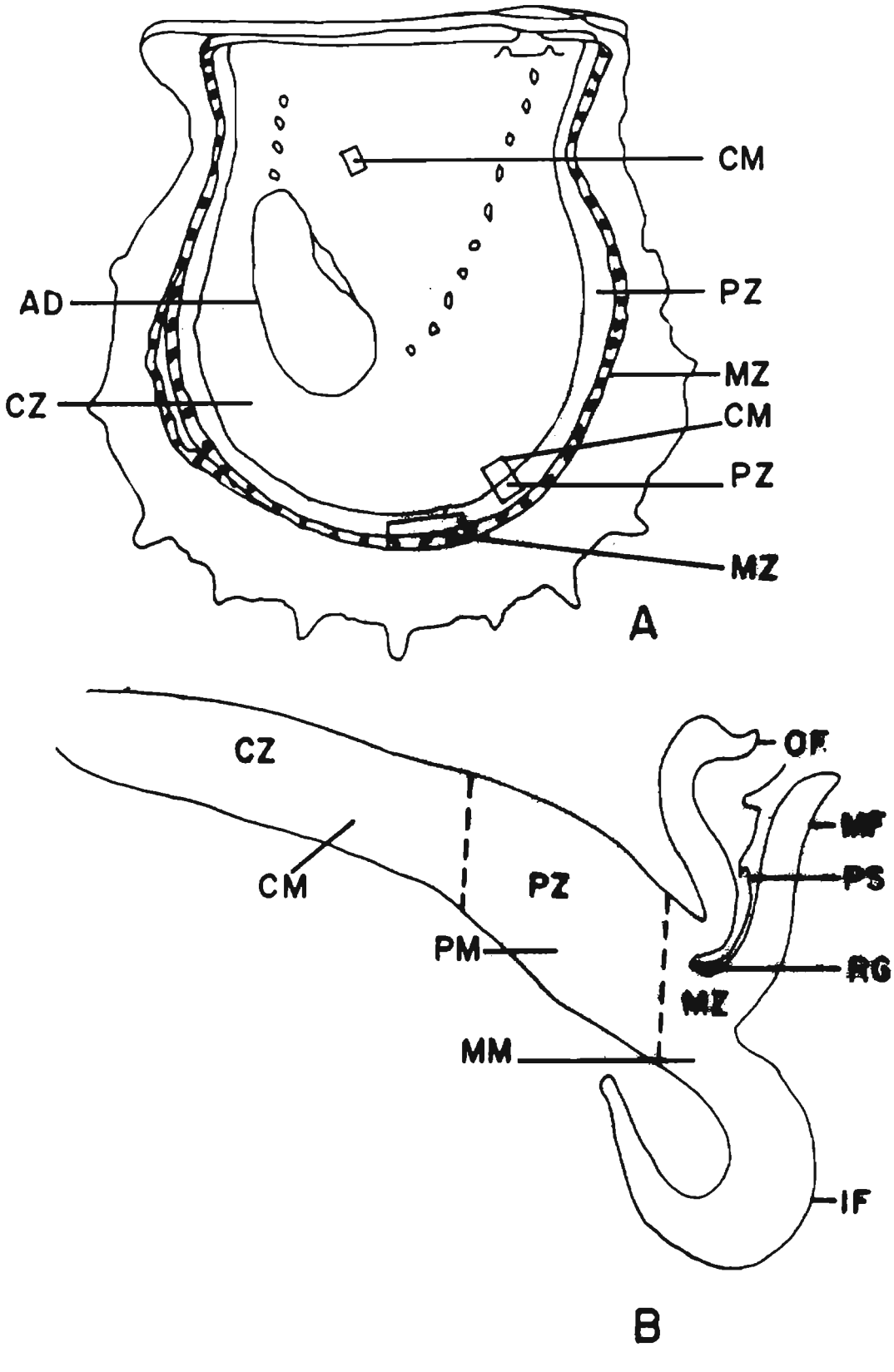
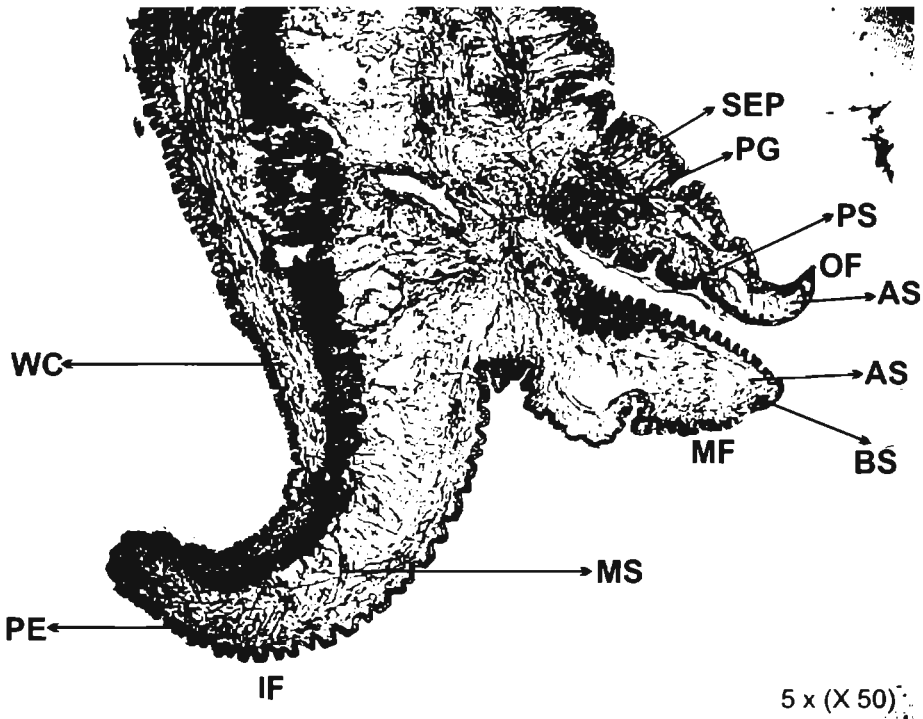


PLATE 4.5

A. *Pinctada fucata* (Gould) : Histological section of the three folds of the mantle, inner (IF) middle (MF) and outer (OF)

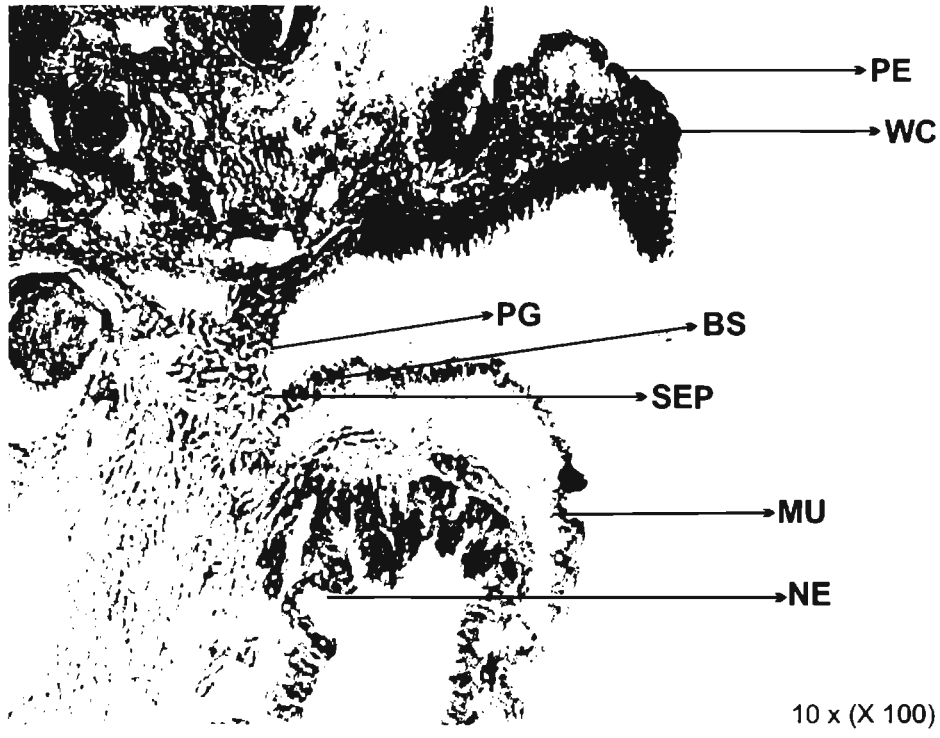
Wandering cells (WC), strong band of muscles (MS), and pigmented epithelium (PE), of the inner fold of the marginal mantle, periostracal groove (PG), stratified epithelial cells (SEP), and periostracal secretion (PS).



5 x (X 50)

B. Histological section of the middle and outer folds of the marginal mantle

Pigmented epithelium (PE), Wandering cells (WC), of middle fold and non pigmented epithelium (NE), basophilic cells (BS), and (MU) mucous cells.

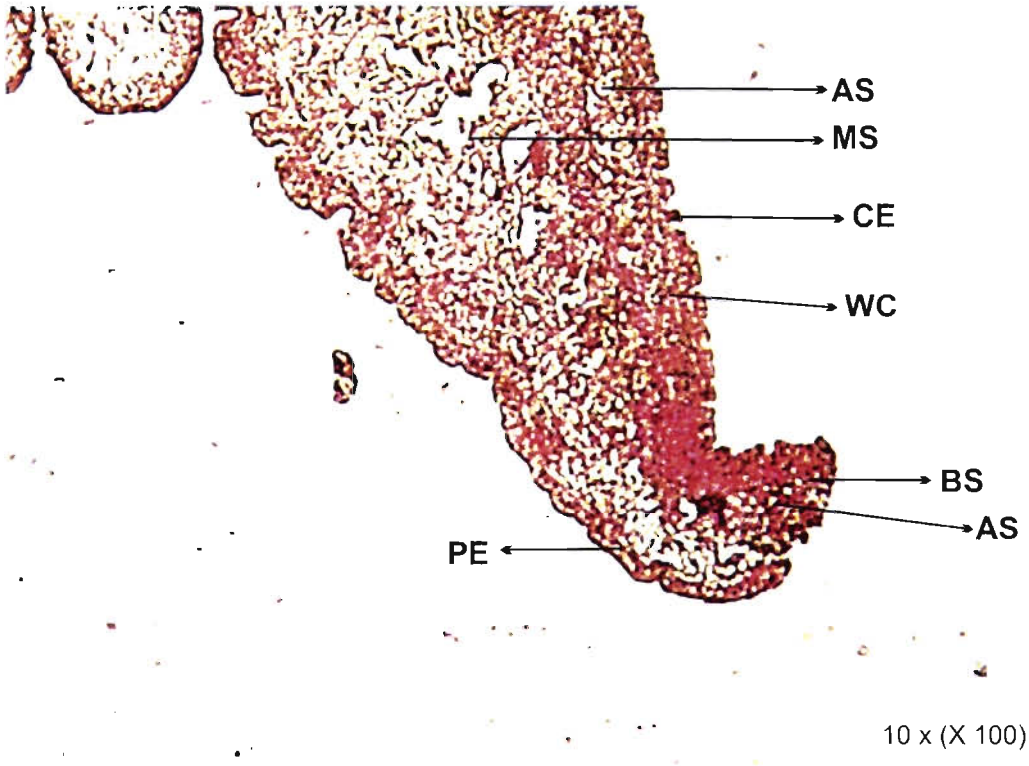


10 x (X 100)

PLATE 4.6

A. *Pinctada fucata* (Gould) : Histological section of the inner marginal mantle fold

Wandering cells (WC), strong band of muscles (MS), and pigmented epithelium of the inner fold of the marginal mantle (PE), Acidophilic cells (AS), strong band of basophilic cells (BS), and (CE) ciliated epithelium.



B. Histological section of middle mantle fold inner marginal mantle fold

Basophilic cells (BS) found in the tip of the middle mantle fold

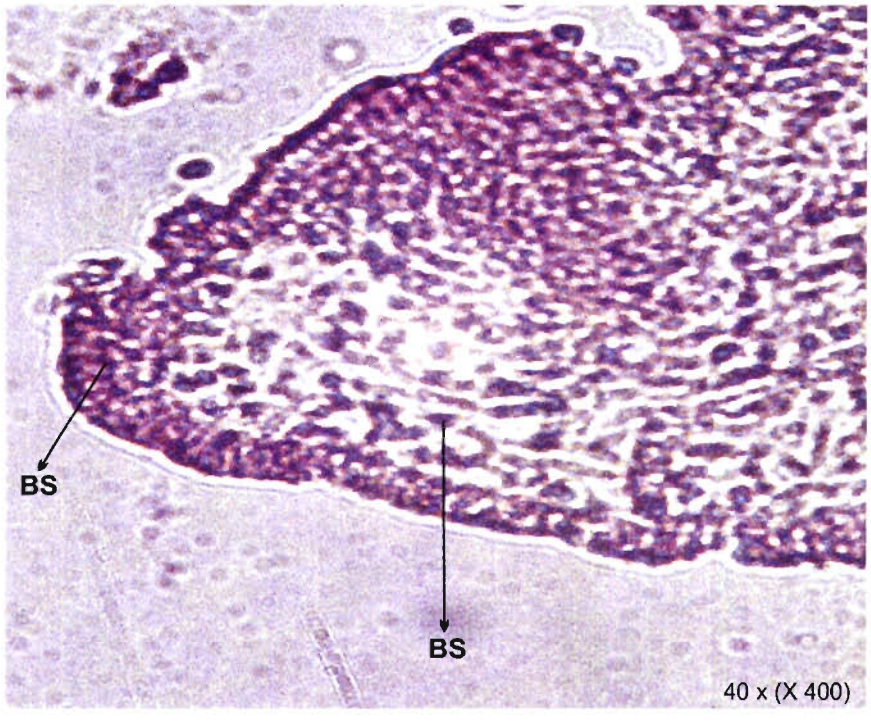
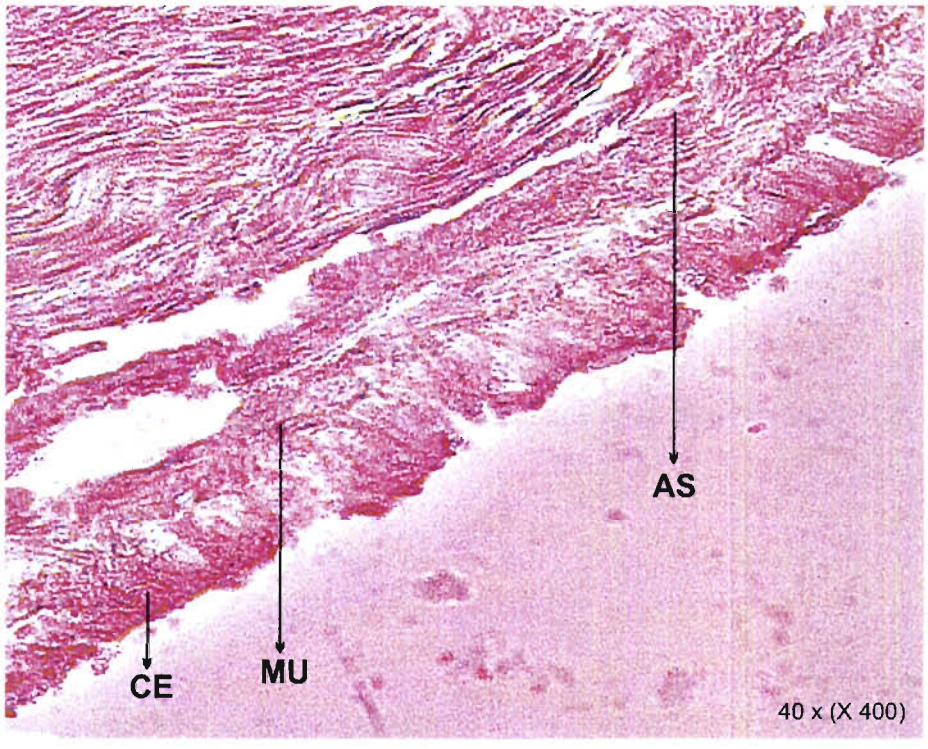


PLATE 4.7

A. *Pinctada fucata* (Gould) : Histological section of the outer margin of the central mantle

Concentration of mucous cells (MU) and acidophilic granular secretory cells (AS) below the columnar epithelium (CE).



B. Histological section of the inner margin of the central mantle

Inner non-pigmented low columnar epithelium (CC) and sub-epithelium secretory cells (SC).

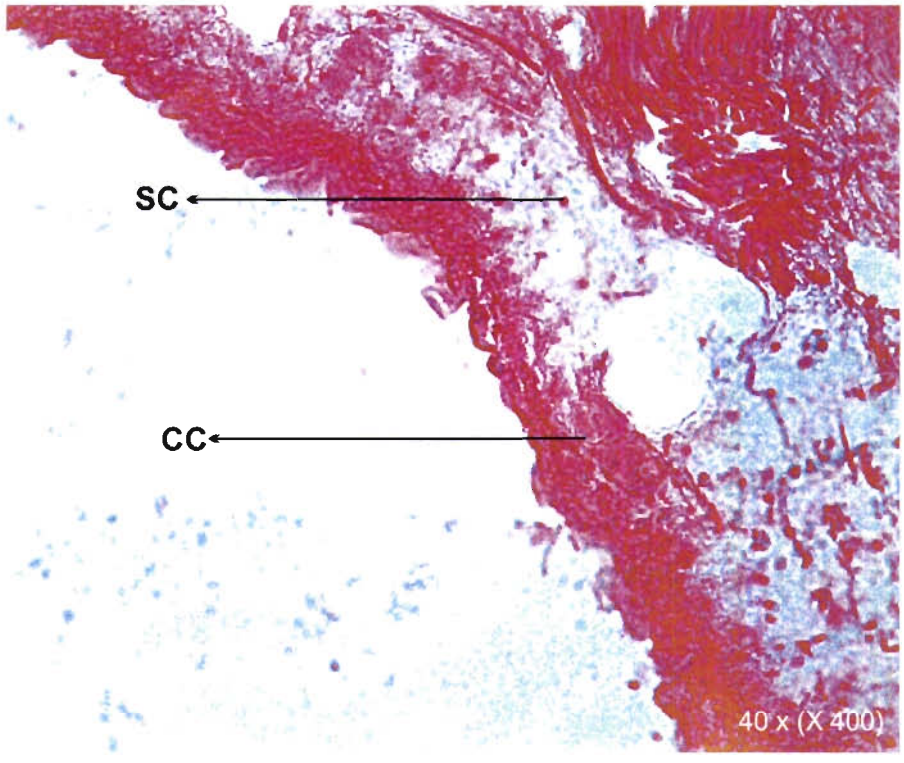
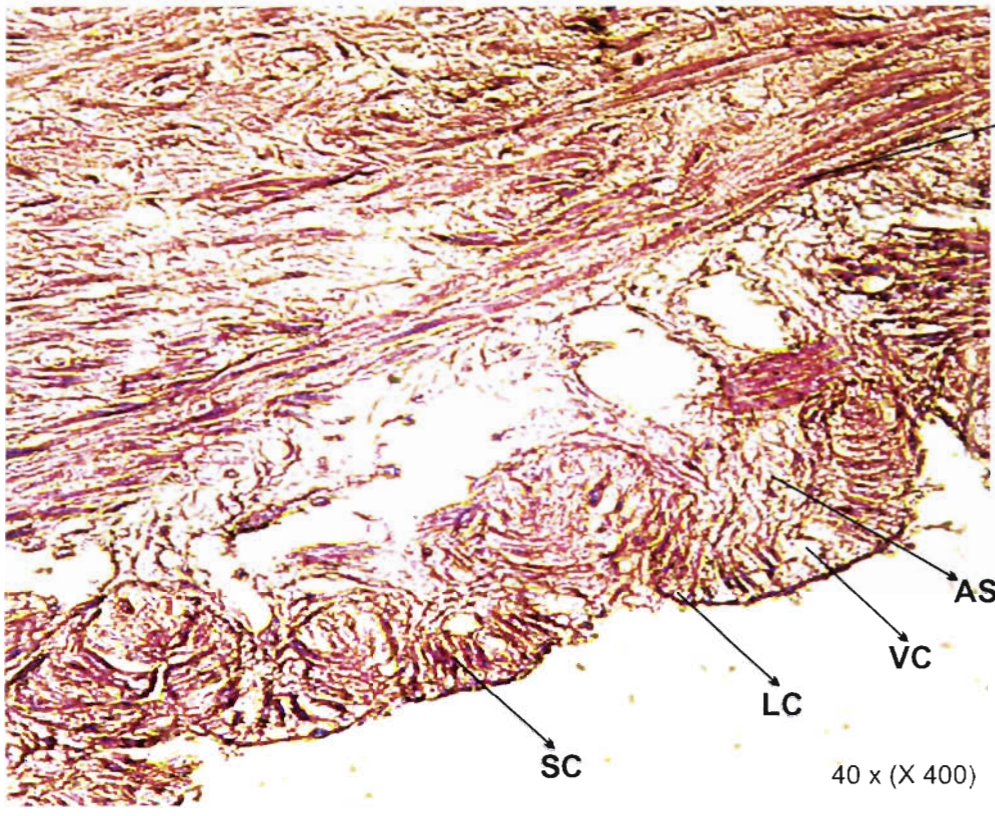


PLATE 4.8

A. *Pinctada fucata* (Gould) : Histological section of the pallial mantle

Outer non-ciliated low columnar epithelium (LC), sub-epithelial secretory cells (SC) vacuolated porous secretory cells (VC), muscular connective (MC) and (AS) acidophilic secretory cells.



B. Histological section of the pallial mantle

Inner ciliated pigmented low columnar epithelium (CE), sub-epithelial secretory cells (SC) and (AS) acidophilic secretory cells.

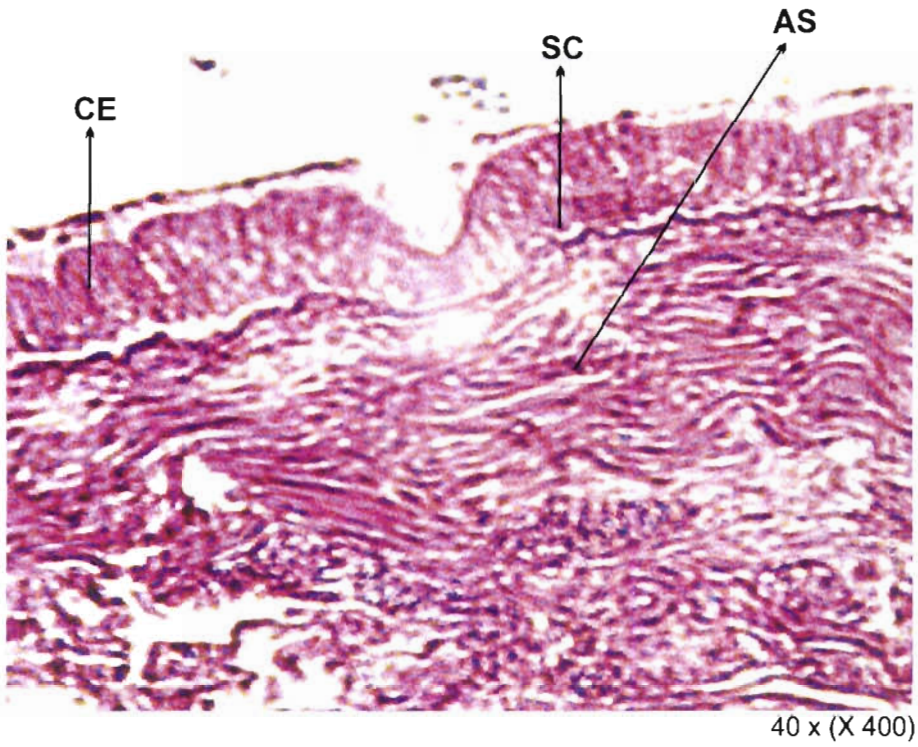
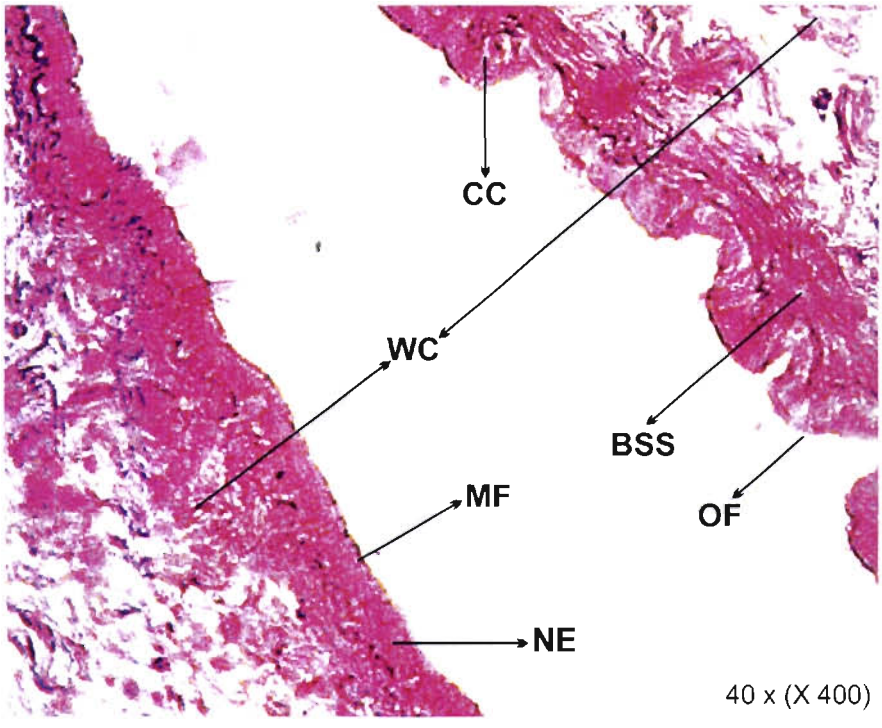


PLATE 4.9

A. *Pinctada fucata* (Gould) : Histological section of the middle and outer folds of the marginal mantle

Wandering secretory cells (WC) of the middle mantle fold, non-pigmented epithelium (NE) of the outer fold and coliated non-pigmented columnar epithelium (CC) and sub-epithelial mucous cells (BSS).



B. Histological section of the mantle isthmus.

Strongly basophilic non-ciliated columnar epithelium (NCC) and secretory cells (SC).

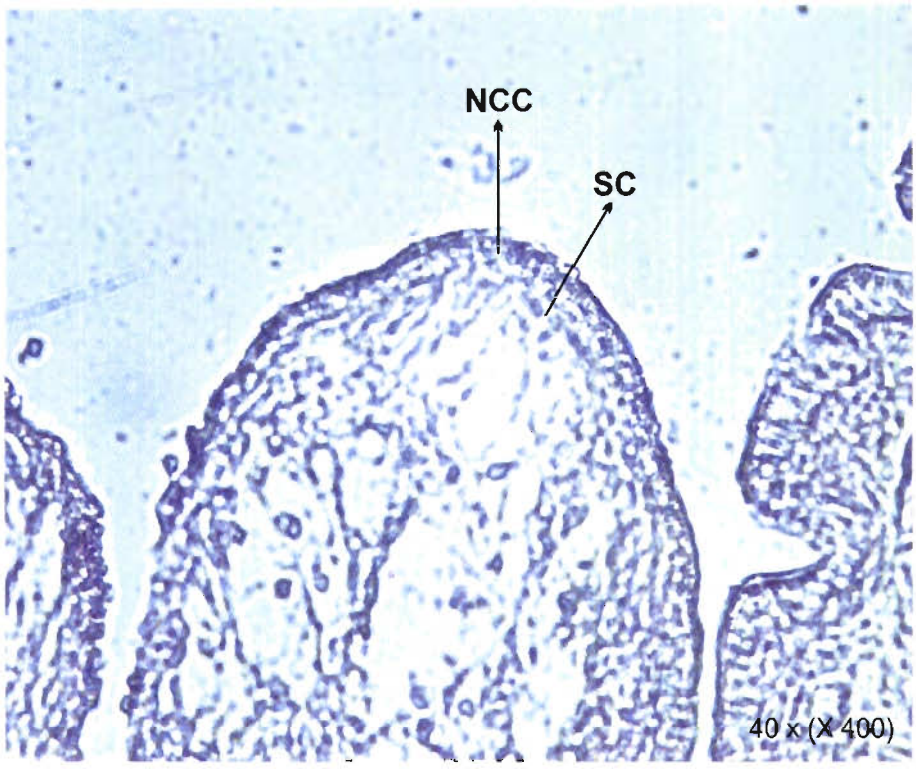
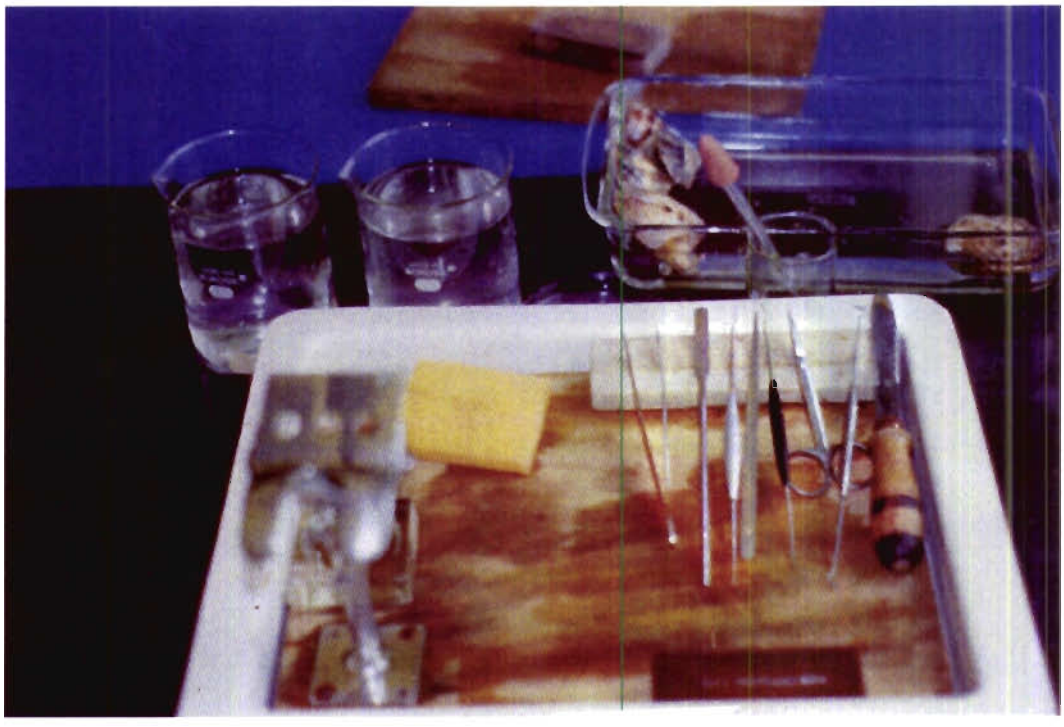


PLATE 4.10

A. *Pinctada fucata* (Gould) : Setup for pearl oyster surgery



B. Narcotization of pearl oyster surgery for pearl sac formation study

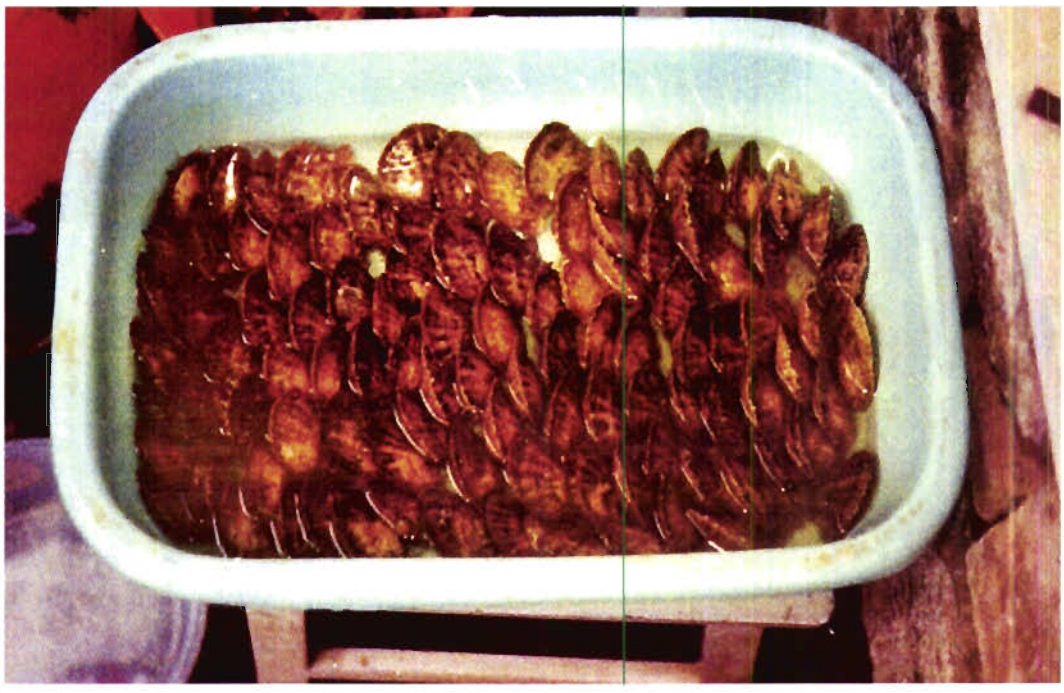


PLATE 4.11

A. *Pinctada fucata* (Gould) : Preparation of mantle graft tissue for pearl sac formation study

B. Implantation of graft tissue in to the gonad of the pearl oyster

PLATE 4.12

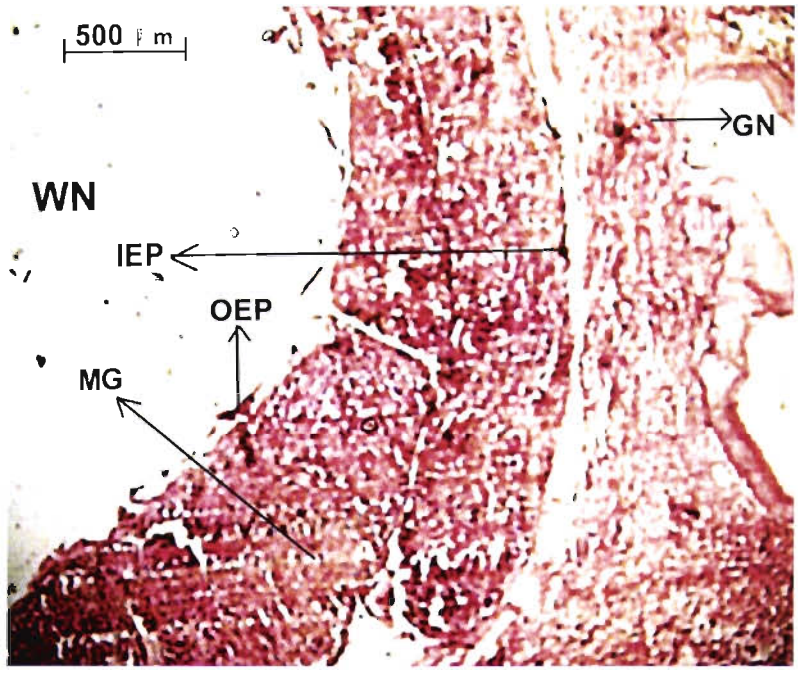
A. *Pinctada fucata* (Gould) : Insertion of wax nucleus in to the gonad of the pearl oyster for pearl sac formation study

B. Implanted oysters kept in controlled running water system for post-operative care and pearl sac formation study

PLATE 4.13

A. *Pinctada fucata* (Gould) : Studies on pearl sac formation using wax nucleus

Second day implanted graft tissue Spindle shaped secretory cells (SC) acidophilic and basophilic cells (BS) in the sub-epithelium and broken cells of vacuolated cells outer epithelial cells (OEP), inner epithelial cells (IEP)



B. Fourth day implanted graft tissue Spindle shaped secretory cells (SC) acidophilic and basophilic cells (BS) in the sub-epithelium and broken cells of vacuolated cells (VC) in the outer epithelium through which secretory cells come out towards wax nucleus (WN)

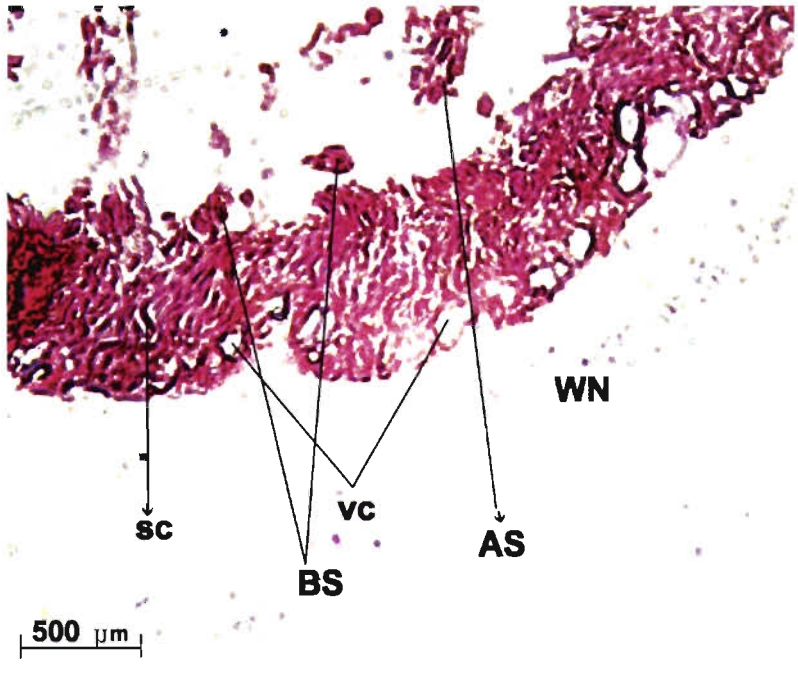
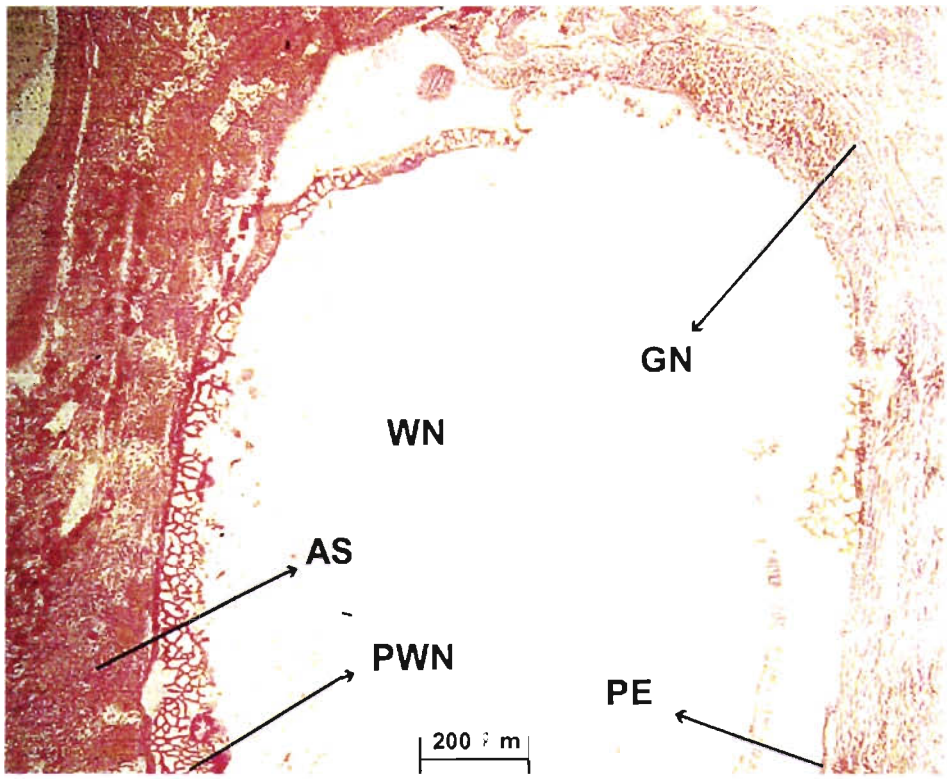


PLATE 4.14

A. *Pinctada fucata* (Gould) : Studies on pearl sac formation using wax nucleus

Pearl coating (PWN) formed on 18th day on 4mm wax nucleus (WN), pearl sac epithelium (PE) in the male gonad of pearl oyster



B. Structure of pearl secretion on wax nucleus

Crystalline secretion (CR) on the wax nucleus, acidophilic secretory cells (AS) in the pearl sac epithelium (EP) covering the wax nucleus.

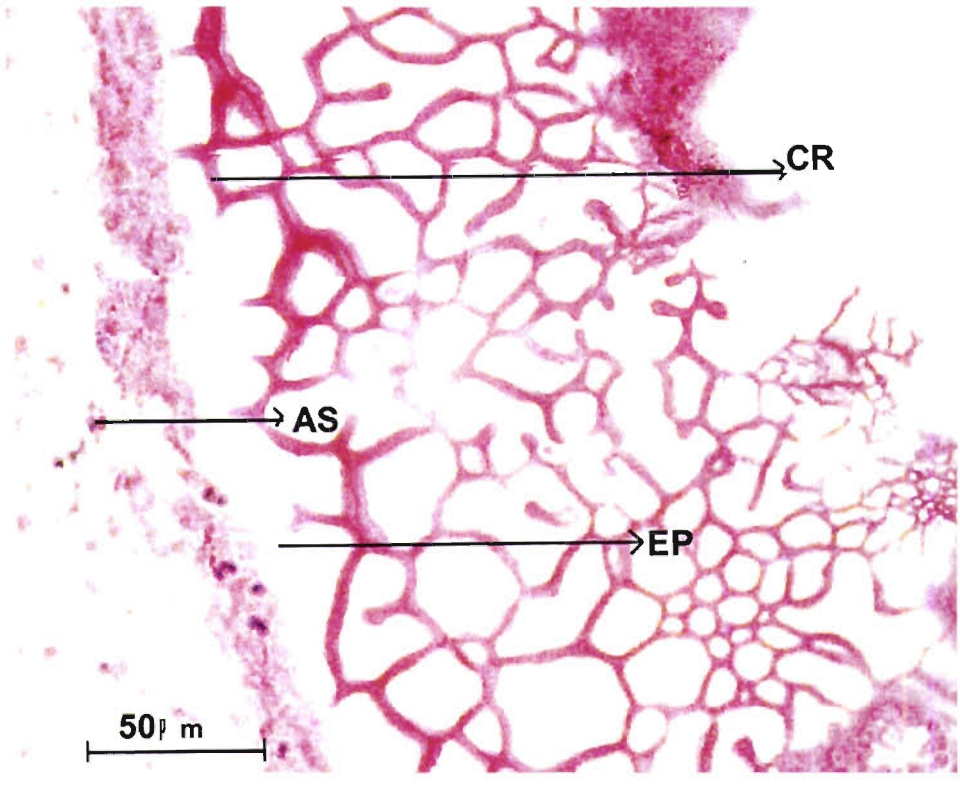
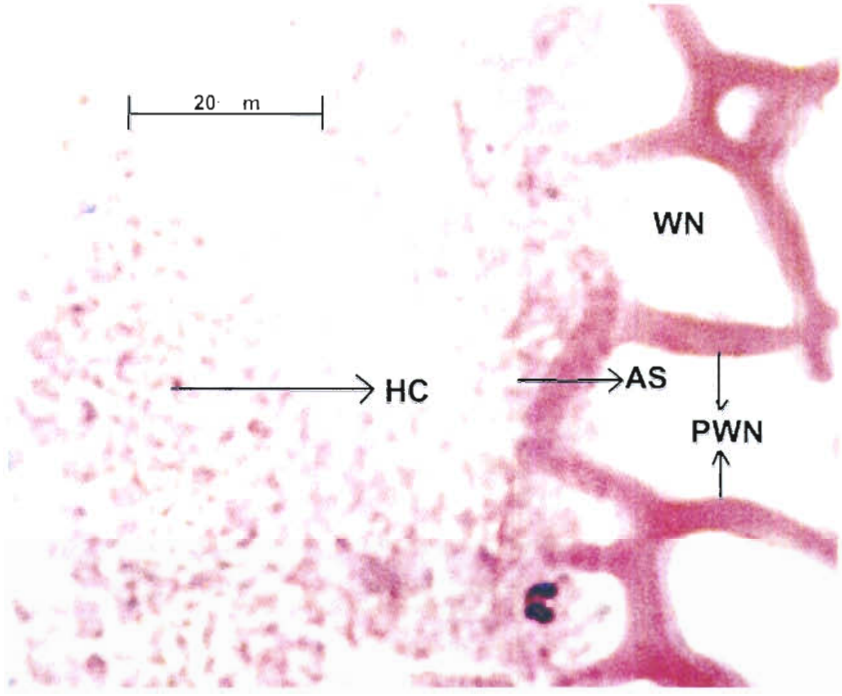


PLATE 4.15

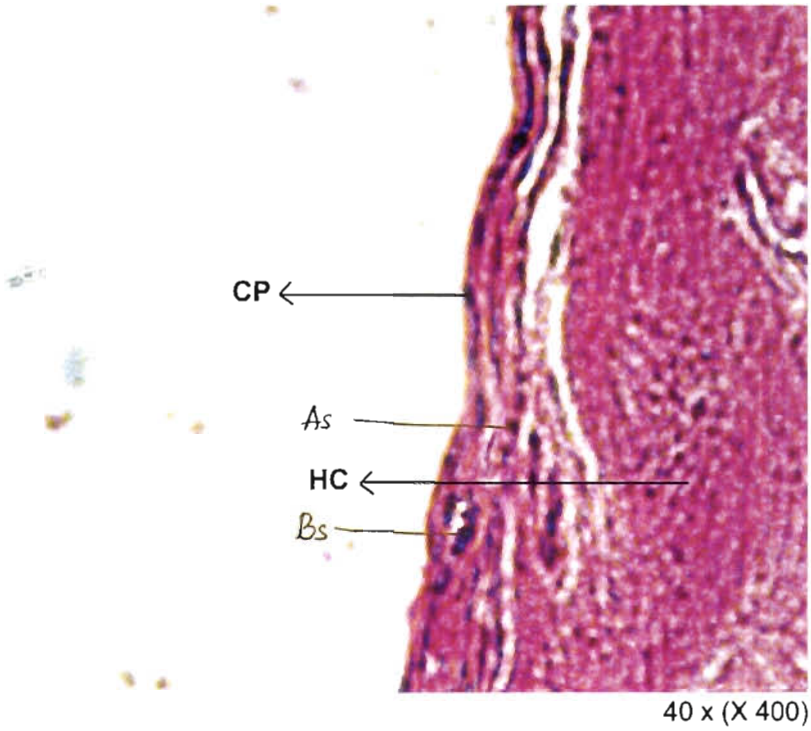
A. *Pinctada fucata* (Gould) : Histological section of the normal pearl sac

Concentration of granular acidophilic secretory cells (AS) in the sub-epithelium of nacreous perl sac formed on wax nucleus (WN), proliferated cells (haemocytes (HC) which have replaced part of the nacreous pearl sac epithelium and pearl coating (PWN).



B. Histological section of the normal pearl sac formed on shell bead nucleus

Cuboidal flattened epithelium (CP) of the normal pearl-sac formed on the shell bead nucleus implanted along with mantle graft (resulted in an "A" quality pearl). Haemocytes (HC) of the gonad tissue extended in to the pearl-sac epithelium, acidophilic secretory cells (AS) and (B) basophilic secretory cells.

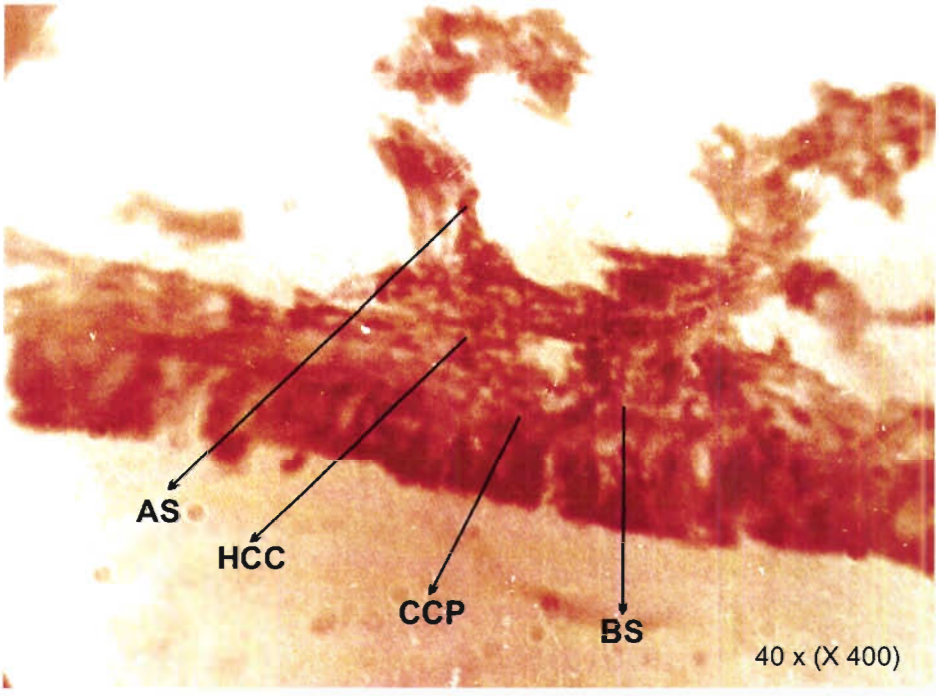


40 x (X 400)

PLATE 4.16

A. *Pinctada fucata* (Gould) : Histological section of the abnormal pearl sac

Tall columnar ciliated epithelium (CCP) of a periostracal pearl sac formed on wax nucleus, cells resembling haemocytes (HCC), basophilic mucous cells (BS), acidophilic secretory cells (AS) with large secretory granules.



B. Normal nacreous pearl formed by normal pearl sac on shell bead nucleus

Implanted oyster with quality pearl (P) coating on nacre in a spent oyster

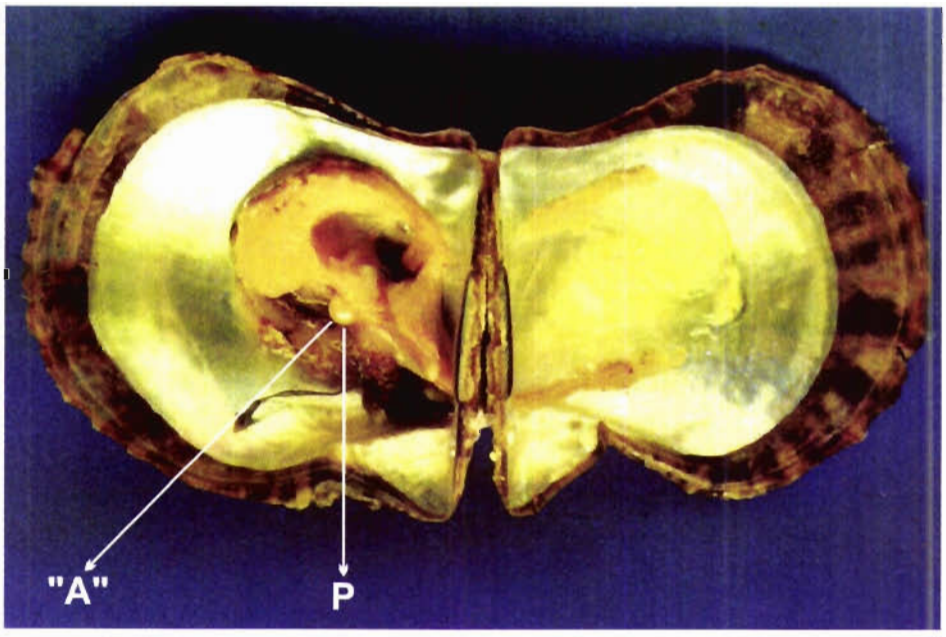


Table 4.1 *Pinctada fucata* (Gould): Duration of pearl sac formation

Size of wax nuclei	Duration of pearl sac formation	Day of pearl coating observed on wax nuclei
3.12 ± 0.11	3 - 7 days	Not observed
4.09 ± 0.10	4 - 10 days	Day 18
5.09 ± 0.06	6 - 12 days	Not observed

SUMMARY

SUMMARY

- Taxonomic studies using morphology, anatomy, and shell characteristics have revealed clearly that the taxonomic position that *Pinctada fucata* (Gould) is in the family Pteriidae.
- The distribution of the species is mainly in the coralline patches of the coasts of India and the two Oceanic Islands of Lashadweep and Andaman and Nicobar islands.
- Morphometric characters of pearl oysters collected from different regions of India showed that Gujarat oysters had more depth and thickness though not statistically significant.
- Ecology of the pearl farm was studied for one year from August 1998 to July 1999 by sampling water both surface and bottom. The results showed that there was no major fluctuation in the water quality except for certain parameters. Gross productivity was correlated with the season with high rates of productivity being shown in the months from November to February in low saline conditions.
- Seasonal (August 1998 to July 1999), depth wise pattern of fouling on farm reared pearl oysters and farm structures were studied by hanging glass panels in shaded and non shaded portions of a pearl oyster raft. The results showed that there was a definite seasonal and depth wise pattern and also shade had an effect. It was observed that barnacles dominated among the fouling organisms. *Balanus amphitrite variegates* being the dominant species of barnacle. *B. amphitrite vinustus* and *B. tintinnabulum tintinnabulum* were occasionally seen. Barnacles peaked in the month of June 1999 and showed a secondary peak in October 1998 and least in the month of May 1999. The fouling was significantly more in the non shaded panels and inversely proportional to the depth.
- Correlation coefficient values (r^2) of the barnacle fouling on panels showed that very high negative correlation existed between barnacle numbers, and weight for both shaded and non shaded panels and depth. A positive correlation existed between, months and barnacle fouling

- Among the other fouling organisms, the dominant species was hydrozoans. Here also, seasonal variations of total weight of fouling organisms revealed that peak settlement was in May 1999 and minimum in October 1998. Like barnacles, total hydrozoan fouling was inversely proportional to the depths.
- Correlation coefficient values (r^2) of the other fouling organisms on panels showed a negative correlation with depth, a positive correlation with month, clarity and salinity and a high positive correlation with silt.
- Silt also formed a minor fouling substance on both shaded and non shaded panels with two peaks, one in September 1998 and other in June 1999. The silt load was inversely proportional to depth.
- The percentage mortality of oysters at these different depths indicated that at 5 m the mortality was lowest. The mortality was directly proportional to the intensity of fouling with maximum mortality was seen in June 1999.
- The mortality of oysters due to fouling was highly positively correlated with month and fouling intensity, while the other variables such as depth and fouling and depth and mortality were highly negatively correlated.
- In the induced maturation experiments, 7.5 ± 3.54 %, 6.67 % and 15 ± 7.07 % of fully matured pearl oysters were obtained on day 43, 42 and 36 in oysters fed with mixed algae, (feed1) mixed algae and corn flour (feed2) mixed algae and rice flour (feed3) respectively in the laboratory conditions. Where it was 35 % ± 7.07 % of fully mature pearl oysters were obtained in the farm on 15^{th} day and none in the non-fed (control) conditions respectively. Among the treatments it was observed that the treatment of mixed algae with raw rice flour gave the best results. 62.5 ± 7.08 % pearl oysters matured on 29^{th} day itself in this treatment.
- Of the matured animals, 43.33 % of the matured pearl oysters (stage III) fed with mixed algae changed to stage IV within 19 days in the lab conditions, while 40 ± 14.14 % of pearl oysters changed to stage V within 26 days in without feeding conditions. Whereas maturity of 53.12 ± 11.97% of pearl oysters did not change within 24 days when maintained in the farm.

- Studies on the larval rearing of *P. fucata* showed that the larvae had the typical life history stages like Veliger, Umbo, Eyed, Pediveliger, Plantigrade and Spat stage. The total days required for to complete the life cycle was 20-25 days (usually 23 days). In the **Phase I** the growth rate of 12.1 ± 0.67 µm day $^{-1}$ in tank was significantly better than that of beaker (10.63 ± 0.85 µm day $^{-1}$). The regression analysis of larval growth showed that growth of experimental animals significantly differed from the control. The regression values for DVM x APM for the control and experiment was as follows. DVM = 48.82 x exp (0.073 x age), APM = 57.304 x exp (0.069 x age) r^2 = 0.9412; 0.9520; DVM = 46.65 x exp (0.079 x age), APM = 54.72 x exp (0.077 x age) r^2 = 0.9165; 0.9500 respectively.

- In the **Phase II** (from 23 day old spat to 60 day old spat) the growth rate was 120.83 ± 3.1 µm day $^{-1}$ in the tank. Whereas in the final phase, **Phase III** (day 60 to 2 years) the growth rate was 64.4 ± 2.1 µm day $^{-1}$. The spat growth regression analysis for DVM, Hinge length (HL) Depth (Dep) and Weight (Wt) with age was calculated. In the individual growth studies, the regression equations for dorso ventral measurement, DVM = 57.43 x (1-exp (-1.0576 x age), r^2 = 0.9863; for hinge length, HL = 53.291 x (1-exp (-1.1893 x age), r^2 = 0.9822; for depth, Dep = 42.663 x (1-exp (-0.3749 x age), r^2 = 0.9898; for weight, Wt = 17.9988 x age (-56005) and for the random measured spat, the regression equations were DVM = 64.247 (1-exp (-0.8942 x age), r^2 = 0.9755; HL = 53.0482 (1-exp (-1.1893 x age), r^2 =0.9792; Dep = 39.7754 (1-exp (-0.4466 x age), r^2 = 0.9820; Wt = exp (4.492 – 2.1313/age)

- The anatomy, histology of mantle and pearl sac formation was studied in *P. fucata*. The results showed that the animal had a typical anatomy for the family Pteriidae. However it was found that several anatomical structures could be of significant taxonomic importance like the anal pappila, typhlosole, and nervous system ganglions. Anatomical studies of the digestive system and nervous system showed that the anal papillae are trifold, the renogenital aperture was not prominent. In the case of the nervous system, there were four nerves, which enervate from the pedal ganglion confirming that the species under study was *P. fucata*.

- The mantle of pearl oyster consists of two identical lobes, right and left, united dorsally along the hinge line to form the mantle isthmus. The mantle lobe is divided into (i) marginal zone, (ii) pallial zone and (iii) central zone.
- Formation of pearl-sac was observed around the wax nucleus implanted in to the gonad of the oysters within 3 - 7 days of implantation in case of 3 mm nuclei, 4-10 days in the case of 4 mm nuclei and 6 -12 days in the case of 5 mm nuclei.
- The histological studies of the implanted graft on day 2 showed that the inner epithelial cells were not fully disintegrated while the outer epithelial cells facing the wax nucleus were seen to be slightly proliferating. The graft tissue on 4^{th} day showed proliferation of basophilic secretory cells (roundish, acidophilic, larger sickle and spindle shaped) in the sub epithelium.
- A thin film of nacreous coating was found deposited on the nucleus within 18 days on a 4 mm wax nucleus. Histological studies of the nacreous pearl-sac epithelium in the male gonad of the pearl oyster showed acidophilic cells in low magnification. In higher magnification the hexagonal crystalline secretion on the wax nucleus, acidophilic secretory cells in the pearl sac epithelium covering the wax nucleus was observed. In further higher magnification, this nacreous pearl sac with pearl coating on the wax nucleus, dense concentration of granular acidophilic secretory cells in the sub epithelium of the nacreous pearl sac was observed. The proliferated cells (haemocytes) have replaced part of the nacreous peal sac epithelium and pearl coating indicating that the pearl sac has become a part of the gonad of the host oyster.
- Histological studies of the nacreous pearl sac formed on the shell bead nucleus with good luster showed the presence of more cuboidal, flattened non-ciliated epithelial cells along with large secretory cells (4-6 µm). The cells were similar to the cells of the muscular tissues of the gonad. The haemocytes of the gonad tissue extended into the pearl-sac epithelium. The acidophilic secretory cells had large granules, and the basophilic mucous

cells were few; and these two types of secretory cells were located within and beneath the pearl-sac epithelium.

- In case of periostracal pearl sacs produced with the wax nuclei in the laboratory, tall, ciliated columnar epithelial cells (30-35 µm) were well distributed. The cells had basal nuclei and small granules. In some parts of the sac, projections of 10-15 µm were seen. Congregations of cells resembling haemocytes were also present in some areas of the epithelium. Basophilic mucous cells with granular inclusions were common. Acidophilic cells with large secretory granules were present in some parts of the periostracal pearl-sac.
- The pearl oyster implanted with 4 mm shell bead nucleus and 2 mm x 2 mm graft has produced good lustrous pearls after 4 months. Because of the abnormal pearl sac, the pearls were graded as "D" quality pearls.

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APPENDIX

APPENDIX TO THESIS

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