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STUDIES ON THE REPRODUCTION OF

MARCIA OPIMA (GMELIN)

THESIS SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
FISH AND FISHERIES SCIENCE
(MARICULTURE)

OF THE
CENTRAL INSTITUTE OF FISHERIES EDUCATION
(DEEMED UNIVERSITY)
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सारांश

टूटिकोरिन और अष्टमुडी इन दोनों स्थानों में पाई गई सीपी *मार्सिया ओपिमा* की जीव संख्या के जनन - चक्र का अध्ययन किया गया. अध्ययन से यह देखा गया कि दोनों स्थानों में *एम. ओपिमा* वर्ष में दो बार अंडजनन करता है. लेकिन टूटिकोरिन में अंडजनन काल मई से जून है और अष्टमुडी में यह नवंबर से जनवरी है. सीपी की पूरी तरह परिपक्वन स्थिति में जनन ग्रंथि सूचक के मूल्यों की बढ़ती और पाचक ग्रंथि सूचक की घटती दिखाई पडी. दोनों स्थानों में, तापमान में थोडी वृद्धि *एम. ओपिमा* के अंडजनन के लिए प्रेरित घटक दिखाया पडा. अष्टमुडी में परिपक्वन के लिए प्रेरणा देने केलिए किए गए प्रयासों द्वारा यह देखा गया कि खिलाई गई सीपियों में नहीं खिलाई गई सीपियों की अपेक्षा परिपक्वन शीघ्र हो गया. 28 °c तापमान में आवश्यक खाद्य के साथ रखी गई सीपियों में 23°c तापमान में रखी गई सिपियों की अपेक्षा जनन ग्रंथि सूचक, अवस्था घटक तथा अंडक - व्यास अधिक देखा गया. *एम. ओपिमा* का अंडजनन और डिंभक पालन स्फुटनशाला में सफल रूप से किया गया. ग्यारहवां दिन डिंभकों का जमाव हुआ. 75 वां दिन स्पाटों का आकार 2.81 x 2.37 मि मी हो गया. 25%o लवणता में स्पाटों में उच्चतम बढ़ती दर और 100% अतिजीविता देखी गई. जैव रासायनिक विश्लेषण से यह देखा गया कि पूरी तरह प्रौढ़ सीपियों में प्रोटीन का स्तर बढ़ते हुए अधिकतम हो जाता है. युग्मकजनन (गमीटोजनसिस) के दौरान कार्बोहाइड्रेट निम्न स्तर में हो जाता है. मादा सीपियों में लिपिड का स्तर नर सीपियों की अपेक्षा अधिक था. अध्ययनों से यह व्यक्त हो गया कि सीपी के आर्द्र मांस का भार ओक्सिजन उपभोग, अमोणिया उत्सर्जन और प्रोटीन अपचय पर आधारित है. टूटिकोरिन उपसागर की सीपी मात्स्यिकी में कई जाति जैसे *मार्सिया ओपिमा, सेमले स्ट्रयाटा, मीसोडेस्मा ग्लाब्राटम, गफ्रारियम ट्रयुमिडम, माक्ट्रा क्युनीटा* और *पैफिया* जाति सम्मिलित है. दोनों स्थानों से संकलित सीपी *एम. ओपिमा* के सापेक्षमितीय संबंध से विभिन्न आकृतिक विशेषताओं के बीच एक के निकट का सहसंबंध गुणांक दिखाया पडा. दोनों जीवसंख्याओं में प्रतिचयन अवधि के कुछ महीनों के दौरान परजीवी ग्रसन देखा गया.

ABSTRACT

The annual reproductive cycle of the clam, *Marcia opima* (Gmelin) from two geographically separated populations at Tuticorin and Ashtamudi was studied. It was observed that *M. opima* spawns twice a year at both the places. But the spawning periods are May to June and November to December at Tuticorin and March to May and November to January at Ashtamudi. Fully matured condition of the clam was associated with an increase in the values of gonad index and condition factor and a decrease in the digestive gland index. A slight increase in temperature initiated the spawning activity of *M. opima* at both the places. The sex ratio at Ashtamudi showed a male dominance. Attempts to induce the maturation of clams showed that fed clams matured faster than the unfed. The gonad index, condition factor and oocyte diameter of clams, kept at 28°C with proper feed was more than that kept at 23°C. Spawning and larval rearing of *M. opima* were successfully carried out in the hatchery. The settlement of larvae took place on 11th day. On day 75, the spats attained an average size of 2.81 x 2.37mm. The growth and survival of spats at varying salinities was studied. Maximum growth rate and 100% survival of spat was reported at salinity 25 ‰. During biochemical analysis, it was noticed that protein level increases to maximum in the fully matured clams. A low level of carbohydrate was observed during gametogenesis. Lipid level was more in the female gonad than the male gonad. Studies revealed that wet meat weight of clam is directly related to oxygen consumption, ammonia excretion and protein catabolism. The clam fishery at Tuticorin bay constituted a number of species like *Marcia opima, Semele striata, Mesodesma glabratum, Gafrarium tumidum, Mactra cuneata* and *Paphia sp.* Allometric relationships of *M. opima* from both the sites showed a correlation coefficient near to unity between various morphological characteristics. Parasitic infestation was observed in the clams during some months of the sampling period in both the populations.

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CHAPTER 1

GENERAL INTRODUCTION

Molluscs form 1% of the marine fish landings, mainly constituted by the catches of cephalopods (Narasimham, 1997). Among the other renewable molluscan resources in India, edible bivalves, including mussels, edible oysters and clams form an important fishery. They are distributed widely in the estuaries, backwaters and the littoral region of the coasts. Rao (1941) mentioned that clams, cockles and mussels are perhaps more important in the shellfish populations of our coasts. The word 'clam' is popularly used to the members of several bivalve families, which burrow into the substratum with the help of a well-developed foot. A few clam species are also known to attach to hard substrates with byssus threads.

The major molluscan producers in the world include China, Japan, Korean Republic, France, Spain, USA, Italy, Malaysia, Netherlands and other Asian countries. The major clam producing countries among them are China, Japan, Malaysia, Korean Republic and Thailand. The world landings of molluscs in 1998 were 9,143,000 t. About 47% of marine production is contributed by molluscs (FAO, 2000). On global basis, the production of clams by aquaculture was estimated at 901,374 t in 1993, forming 21.8% of the total mollusc production by farming. The surveys conducted at important production centres in India showed that an estimated 45,000 t of clams are landed annually (Narasimham, 1997). Clam fishery in India is at subsistence level. Out of 2.6 mt of clam production, India contributes 0.76 mt forming 29% of the world production (Appukuttan, 1996). Frozen clam meat exported in 1991 was 1232 t (Narasimham, 1993).

Clams belonging to the families Arcidae, Veneridae, Corbiculidae and Tridacnidae are commercially important and cultivable along the Indian coast. Main species of clams like *Villorita cyprinoides*, *Meretrix casta*, *M. meretrix*, *Paphia malabarica*, *Marcia opima* and *Anadara granosa* had total

landings to about 36,172 t during 1999 to 2000 (Ramadoss *et al.*, 2001). Information on the distribution and exploitation of clams from India was provided by Rao (1963), Jones (1968), Alagarswami and Narasimham (1973), Nayar and Mahadevan (1974), Rasalam and Sebastian (1976), and Narasimham *et al.* (1984 and 1986).

Among the arcid clams or blood clams a single species, *A. granosa* is important. It occurs all along the Indian coast in soft muddy substratum and forms a fishery of considerable magnitude in the Kakinada bay with annual landings of 2000 t/year (Narasimham, 1997).

The venerid clams are most sought after in the clam fisheries in India and three genera such as *Meretrix, Paphia* and *Marcia* are important. *Meretrix meretrix*, *Marcia opima* and *P. laterisulca* are the dominant species along the Maharashtra coast. In Goa, *Meretrix casta* forms a fishery. Fourteen estuaries along the Karnataka coast have abundance of clams, with *M. casta* found in all the estuaries, *M. meretrix* in Kalinadi and Coondapur estuaries, *P. malabarica* in the Mulky, Gurpur, Udyavara and Coondapur estuaries. *Marcia opima* is abundant in Coondapur, Uppunda and Sita Estuaries. Along the Kerala coast, *P. malabarica* forms a fishery in Koduvally, Azhikkal, Karyamgod and Chittari estuaries and Ashtamudi Lake. Along the east coast, *Meretrix casta* occurs at several places and forms a fishery at Vellar estuary and Pulicat Lake in Tamil Nadu and Bhimunipatnam backwaters in Andhra Pradesh. *Marcia opima*, *P. malabarica* and *Meretrix meretrix* contribute to the clam fisheries in the Kakinada bay. Along the Orissa coast, *Meretrix sp.* occurs in Chilka Lake and Sonapur backwaters.

In the Corbiculidae family, *V. cyprinoides* is the most important species. It is the major resource in Vembanad Lake and is also exploited in several backwaters, lakes and estuaries of Kerala. It also contributes to the

fisheries in Goa and in the Nethravathi, Gurpur, Udyavara, Swarna and Coondapur estuaries in Karnataka.

Family Tridacnidae is represented by *Tridacna maxima, T. crocea, T. squamosa* and *Hippopus hippopus*. These clams mainly occur in the Andaman and Nicobar and Lakshadweep Islands.

The venerid clams with an estimated annual production of 14,000 t form 30.9% of the total landings. These clams occur widely in all maritime states where clams are currently exploited. In this group, *Marcia opima* is the most important with a production of 5,552 t, followed by *Meretrix casta* (4,642 t), *P. malabarica* (1,793 t) and *M. meretrix* (965 t).

Among these clams *V. cyprinoides, M. Meretrix, P. malabarica* and *Marcia opima* are being exported. Of all the maritime states, Kerala leads in the clam production with a catch of 32,927 t, which accounts for 72.5% of the total clam landings. The annual clam landings of Karnataka state was estimated at 6,592 t (Rao and Rao, 1989), although considerable fluctuations in the landings have been recorded. The clam production in Goa has been estimated at 887 t / year and that of Maharashtra at 1100 t / year.

Along the east coast of India, the clam resources are limited. In Tamil Nadu, the Vellar estuary and Pulicat Lake together contribute to 1,087 t, while in Andhra Pradesh; the clam production was estimated at 2,816 t. There is only meagre information from Orissa and West Bengal. It may be due to the absence of organised exploitation rather than non-availability of the resources.

The clam meat, being rich in polyunsaturated fatty acids (PUFA) and low in saturated lipids, is relished much. Clams provide food for a large section of the poor people of the coastal tracts. In recent days frozen clam meat

is increasingly in demand in the export market. The clam meat is either frozen as blocks or individually quick frozen, canned and smoked. Clam juice, clam stripes, clam streaks, stuffed clams, clam pickle and chowder are the other products. In recent times clam meat is also used as shrimp feed. The clamshells, consisting of calcium carbonate have various industrial uses. The clamshells are used in the manufacture of cement, calcium carbide, sand-lime bricks and lime. Hornell (1916) made a detailed study of utilization of shells for making lime. The shells of several clams are used for making curios.

TAXONOMY AND IDENTIFICATION OF *Marcia opima*

The venerid clam *Marcia opima* (Gmelin) has, so far, been indicated as *Katelysia opima* (Gmelin) (John Taylor, Per. commu.) in the previous studies.

Phylum	:	Mollusca
Class	:	Pelecypoda
Order	:	Eulamellibranchiata
Sub-order	:	Heterodonta
Series	:	Veneracea
Family	:	Veneridae
Genus	:	*Marcia*
Species	:	*opima*

SHELL CHARACTERESTICS OF *Marcia opima*

The shell is thick, solid and inflated with a smooth and more or less glossy surface, somewhat resembling that of *M. casta* in general appearance, but may be readily distinguished from the latter by its richer and deeper

colouration. The shell is slightly longer than high, with an evenly rounded margin. The hinge bears three strong cardinal teeth, the tooth in front of the cardinals in the left valve and the hollow in the right being absent. The lunule is distinct, flattened and greatly elongated reaching almost up to the hind margin of the shell. The muscular impressions are very well marked and even slightly depressed, that of the anterior adductor being more strongly so. The pallial line is deeply sinuate, the apex of the sinus being bluntly angular. The inner surface is smooth, white and polished and devoid of any trace of denticulation. The outer surface is also highly polished and is pale yellowish brown or straw coloured, variously clouded, mottled and rayed with purplish grey markings. The pattern most frequently observed is a number of concentric, undulating lines, running closely parallel with three or four broad, radially widening, brownish or purplish grey bands diverging downwards from the umbo (Plate I and II).

Even under the existing conditions of sustenance fishing, little attention has been paid to systematic studies on clams and clam fisheries. Clam culture experiments in India were mainly concentrated on *Meretrix casta* (Silas *et al.*, 1982 Sreenivasan, 1983; Rao and Rao, 1989) and *A. granosa* (Narasimham, 1980). Species like *P. malabarica, Marcia opima* and *Meretrix meretrix,* which command high market potential in India as well as in the export market are found in limited quantities over restricted areas. Sufficient biological and ecological information is essential for culturing the clams. Clam *M. lusoria* is cultured in coastal ponds in Taiwan and Vietnam (Narasimham, 1997). Narasimham (1980) gave a production range from 38 to 42 t / ha by farming *A. granosa* in pen enclosures. In Thailand, *A. granosa* is grown along with the shrimp culture ponds. In India, *P. malabarica* and *A. granosa* offer opportunities for integrating clam farming with shrimp culture. Apart from generating additional income, clams purify the water by reducing the sediment load (Narasimham, 1997).

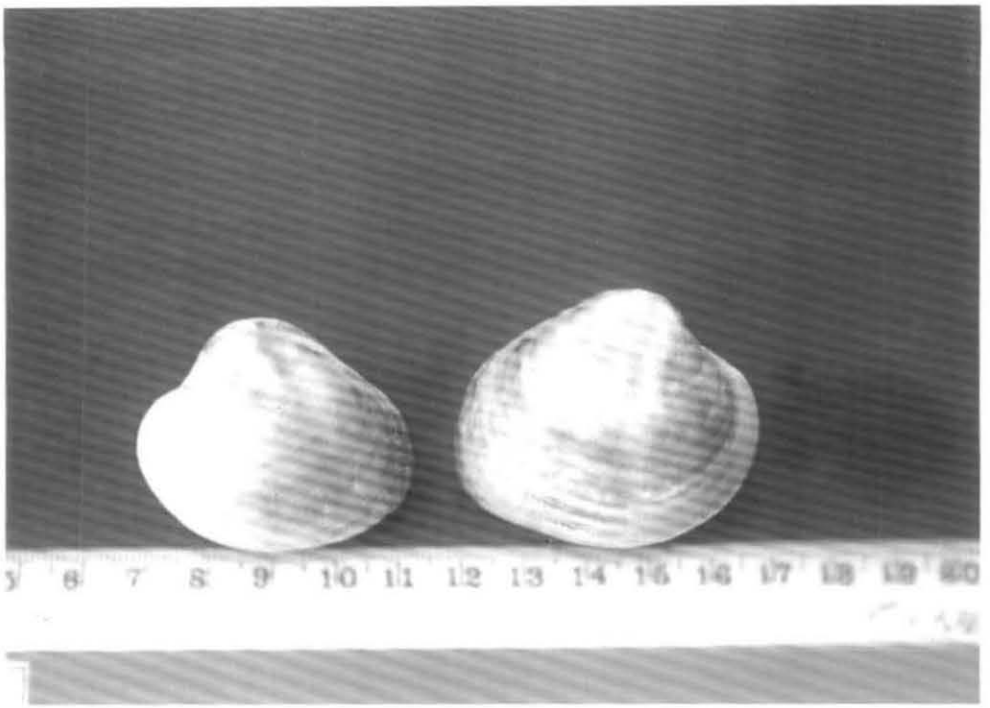


PLATE I. *MARCIA OPIMA* (Gmelin)

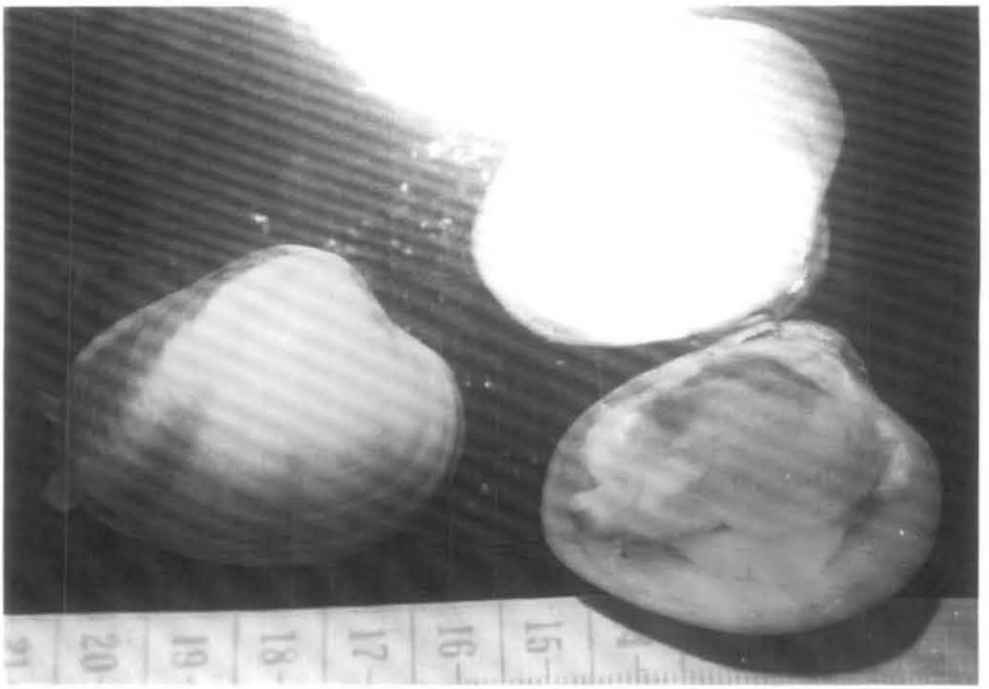


PLATE II . ***Marcia opima*** - Shell open

The prospects of clam culture are:

(1) Clams feed low in the food web on detritus and phytoplankton and give high production per unit area. They are efficient converters of primary production into nutritious seafood, suitable for human consumption.

(2) Clam culture is essentially a relaying practice of collecting the seed from high-density areas and stocking them in suitable grow out areas. The technology is simple and easy for adoption by the farmers.

(3) On bottom culture does not involve high labour or cost input.

(4) Clam culture can easily be blended with capture fisheries and can be taken up as an income and employment generation programme in rural areas.

(5) In clam culture, fertilizers and feed are not used and it is eco-friendly. Clams are good biological filters and the introduction of clams in areas of high eutrophication such as shrimp ponds helps to reduce the pollution due to high load of suspended matter.

The constraints of clam culture are:

(1) The major constraint for the large-scale production of clam is the absence of laws to allot water bodies to prospective farmers.

(2) Mapping of sites suitable for clam culture, based on species site interaction is needed for developing cultures.

(3) Consumption of clams is still localized; close to the production centres and only a small segment of the population take them as food.

They still remain as non-conventional food. Various extension drives are needed in popularization of the clam meat as a source of protein.

(4) Developing suitable hatchery techniques for large-scale seed production.

For promoting aquaculture of clams, the hatchery production of clams is an important criterion. Presently, the clam culture attempts in India subsist on seed from the wild. When more and more entrepreneurs enter this field, the wild seed resources may not be able to satisfy the demand. The hatchery production of clam seeds gains importance in this context. India, with its lengthy coastline, estuaries, bays and backwaters offers great potential for clam culture activities. The clam, *Marcia opima* is one of the widely distributed and mostly exploited species. It is a potential species for aquaculture and for the culture of this species hatchery seed production is a necessity. Seed production in hatchery is possible only if we could know about the reproductive biology of that particular species. With these factors in mind, the present investigation has been undertaken to study the annual reproductive cycle and reproductive behaviour between geographically separated populations of *M. opima*. Further attempts have been made in the induced maturation, spawning and larval rearing that are essential for mass production of seed of *M. opima* through hatchery techniques.

Objectives of the present study are:

(1) To study the annual reproductive cycle with environmental conditions

(2) Reproductive behaviour between geographically separated populations.

CHAPTER 2

REPRODUCTIVE CYCLE OF *M. OPIMA*

2.1 INTRODUCTION

The knowledge on gametogenesis, maturation and spawning of clams is essential for seed collection from natural beds as well as in the management of brood stock in the hatchery for large scale seed production. The reproductive strategy of commercially important marine bivalves has been studied extensively (Tranter, 1958; Giese, 1959; Fretter and Graham, 1964; Quayle and Bourne, 1972; Giese and Pearse, 1974; Bayne, 1976; Sastry, 1979). These studies led to the development of hatchery techniques for mass production of spat in the laboratories. Loosanoff (1937) explained the development of primary gonad and sexual phases in *Venus mercenaria*. Ansell (1961) and Ansell *et al.* (1964) conducted extensive work on the reproduction, spawning and growth of the hard clam *V. mercenaria*. Porter (1964) explained the gonadal cycles of hard clams in Long Island Sound and North Carolina. Hesselman *et al.* (1989) studied the reproductive behaviour of adult hard clams, *Mercenaria sp.* in the Indian River lagoon, Florida.

Reproductive studies on the soft clam, *Mya arenaria* was conducted by Rogers (1959), Pfitzenmeyer (1962 and 1965), Shaw (1962 and 1965) and Ropes and Stickney (1965). The reproductive cycle of *Cyprina islandica* was studied by Loosanoff (1953). Ropes (1968) explained the seasonal gonad development to determine the annual frequency and duration of spawning, larval occurrence and time of settlement of juvenile clams of *Spisula solidissima*. The gametogenic cycle of the southern surf clam, *S. s. similis* was reported from Georgia by Kanti *et al.* (1993). Calabrese (1970) described the development cycle in the coot clam, *Mulinia lateralis* in Long Island Sound. Peddichord (1977) studied the salinity and substratum effects on condition index of the bivalve *Rangia cuneata*. Broom (1983) observed the gonad development and spawning in *A. granosa*. The reproductive cycles of the molluscs, *A. trapezia*, *Venurupis crenata* and *Anemia descripta* in the Sydney region was explained by Hadfield and Anderson (1988).

Reproductive studies of *Ruditapes decussatus* have been conducted by Shafee and Daouadi (1991) on the Atlantic coast of Morocco. Robert *et al.* (1993) explained the reproductive behaviour of *R. philippinarum* in France. The reproductive pattern of the clams, *R. decussatus* and *R. philippinarum*, on intertidal flats in Brittany was reported by Laruelle *et al.* (1994). Gasper and Monteiro (1998) studied the reproductive behaviour of the razor clam, *Ensis siliqua* and the clam, *V. striatula* in the Southern Portugal. Marsden (1999) described the reproductive pattern of the surf beach clam, *Paphies donacina* from New Zealand.

Chipperfield (1953) explained the breeding and settlement of *Mytilus edulis* in British waters. Dix and Ferguson (1984) studied the reproductive cycle and condition in Tansmanian blue mussels, *M. edulis planulatus*. Cheung (1991) explained the energetics in relation to growth, condition and reproduction in green-lipped mussel, *Perna viridis* in Hong Kong. Lubet and Allarakh (1994) studied the variation in oocyte diameter in relation to the reproductive cycle of *Saccostrea cucullata*. Lango *et al.* (2000) proposed that oocyte diameter is a means to evaluate the gametogenic development of the pacific oyster *Crassostrea gigas*.

Reproduction of marine invertebrates has been influenced by exogenous factors such as food, water, temperature, salinity and tides and also by endogenous factors (Runnstorm, 1927; Loosanoff and Davis, 1952; Giese, 1959; Carriker, 1961; Galtsoff, 1964; Wilson and Hodgkin, 1967; Wilson, 1969; Hancock and Franklin, 1972; Bayne, 1975; Seed 1976; Peddichord, 1977; Sastry, 1968 and 1979). Populations of marine animals exposed to different environments within their geographical range may vary in physiological properties (Prosser, 1955). Sastry (1970) reported the physiological variation in the reproduction of geographically separated populations of the bay scallop, *Acquipecten irradians*. Keck *et al.* (1975) performed a comparative study of the reproductive cycles of clam, *Mercenaria mercenaria* from different geographical

regions in Delaware Bay. The changes in the gonad index and digestive gland index were studied in *A. irradians* by Sastry (1979). Sbrenna and Campioni (1994) explained the gametogenic and spawning patterns of Manila clams, *Tapes philippinarum* in two lagoons of the river Po Delta. The intraspecific variation in growth and reproduction in latitudinally separated populations of the giant scallop, *Placopecten magellanicus* was studied by Mac Donald and Thompson (1988).

The reproductive cycle of bivalves has been studied by various researchers in Indian waters. Rao (1951 a) studied the growth of *K. opima* at east coast. Nayar (1955) studied the growth, sexual maturity, spawning and effect of temperature and salinity of the inshore waters on breeding and growth of the wedge clam, *Donax cuneatus*. Abraham (1953) studied the reproductive aspects of *Meretrix casta* from Adyar estuary. Preliminary observations on the seasonal gonadal changes and spawning in the clam *M. casta* in the marine fish farm were reported by Durve (1964). Alagarswami (1966) studied the influence of environment on age and growth and the reproductive cycle of the clam *D. faba*. The annual reproductive cycle of *D. cuneatus* of the Madras coast has been studied, based on the seasonal gonadal changes of the adult clams by Rao (1967). Mane (1974) detailed the growth and breeding habits of the clam *K. opima* in the Kalbadevi estuary of Ratnagiri, west coast of India. Nagabhushanam and Mane (1975) studied reproduction and breeding of the clam *K. opima* in the Kalbadevi estuary of Ratnagiri. John (1980) discussed the reproductive cycle of *A. rhombea*. Maturity, spawning and sex ratio of *A. rhombea* from Port Novo backwaters was explained by Natarajan and John (1983). Sreenivasan (1983) reported the biology, distribution and spawning of *M. casta*. Thangavelu and Sanjeevaraj (1985) made extensive study on the fishery and biology of the clam *M. casta* in Pulicat Lake. Reproductive cycle of three commercially important clams, *M. meretrix*, *M. ca[illegible]* and *K. opima* was studied by Jayabal and Kalyani (1986). The spawning of edible oyster *C. madrasensis* at

Tuticorin was detailed by Rajapandian and Rajan (1987). Jayabal and Kalyani (1987 a) detailed the reproductive cycle of the estuarine bivalve *M. meretrix* of the Vellar estuary. Narasimham (1988) studied the biology of the wedge clam *A. rhombea* in Kakinada bay. The biology of great clam *M. meretrix*, in the Korampallam creek, Tuticorin was studied by Narasimham *et al.* (1988). Thangavelu and Poovannan (1994) studied some aspects of biology of the clam *M. casta* in Muttukadu Backwater. Abraham (1996) conducted studies on the gonad index and maturity of edible oyster, *C. madrasensis*. Appukkuttan (unpublished) studied the reproduction of *P. malabarica* in Astamudi Lake.

This chapter analyses the annual reproductive cycles of two geographically separated populations of *M. opima*.

2.2 MATERIALS AND METHODS

2.2.1 Sampling

Clams, *M. opima* were collected monthly from December 1998 to January 2000 from the sampling site in the Tuticorin bay (8° 45' N and 78° 12' E). From March 1999 onwards, clams were collected in every alternate month from Ashtamudi Lake, Quilon (9° 28' N and 76 ° 28' E) (Plate III). Generally, in natural conditions, the gametogenesis in bivalves is not completed within a month and hence there is no monthly occurrence of spawning. So bimonthly sampling was done from Ashtamudi. For the comparison of gonad maturation studies monthly sampling was carried out from Tuticorin. Samples of clams were collected from the intertidal zones of the sampling sites at Tuticorin and Ashtamudi. A wooden frame of 50 sq. m. area placed in the exposed area at low tide and clams were handpicked (Plate IV). The water temperature of the sampling sites on the collection date was noted. Water samples from the sampling area were taken for estimating salinity. Salinity was determined by Mohr-Kundson titration method as given by Strickland and Parsons (1968). Dissolved oxygen was estimated by following 'Modified Winkler method', as given by Strickland and Parsons (1968).

PLATE III. Collection areas of ***Marcia opima***

PLATE IV. A view of Sampling.

The collected clams were blotted dry with tissue paper. The shell-on weight of 20 clams was noted using a Sartorius microbalance to the nearest to 0.01g. The shell was then opened by severing adductor muscles and the soft tissues were removed. These were blotted to remove the excess moisture and weighed immediately in a pre weighed petridish for wet flesh weight. The gonadal maturity stage of the clam was assessed by examination of gonadal smears microscopically. The bimonthly samples from Ashtamudi were studied for the reproductive aspects, as carried out for the clams collected from Tuticorin.

Ten individuals from the collected clams were studied separately for gonadal and digestive gland indices and also for their gonadal maturity stages. The gonad and digestive gland were carefully cut from the visceral mass and their weight was taken separately on a Sartorius microbalance to the nearest of 0.01 gm. From the respective weight, the Gonad Index (GI) and Digestive Gland Index (DGI) were calculated by the formula as followed by Giese (1959) and Sastry (1966):

$$\text{Gonad index} = \frac{\text{Gonad weight} \times 100}{\text{Wet weight of meat}}$$

$$\text{Digestive Gland Index} = \frac{\text{Digestive gland weight} \times 100}{\text{Wet weight of meat}}$$

The average value of GI and DGI was calculated and has been taken for description. Twenty clams from samples were taken for the determination of condition factor (CF). The whole clam was put in a volume displacement jar filled with water up to the spout. Volume of water displaced was noted by collecting in a measuring cylinder. Then the clam was opened and the meat was removed. The water in the volume displacement jar was refilled to

the spout level and the pair of empty shell was placed in the jar. The volume of water displaced gave the volume of shells. The shell cavity volume was found by the difference of volume of whole clam to the volume of shell valves. The meat removed from the shell was weighed in a pre-weighed Petridish. The gonad condition was assessed by microscopic examination of fresh gonad smears. The meat of the clams was dried in an oven at 80°C for 24 hours. The dried tissues were weighed till constant weight was observed. Then the condition factor was calculated by using the formula:

$$\text{Condition Factor} = \frac{\text{(Dry meat weight X 1000)}}{\text{Volume of shell cavity}}$$

The average value of condition factor was calculated and used for statistical analysis to determine its relationship with the gonadal maturity stages.

2.2.2 Histology

For the purpose of histological studies, the clams were shucked and the meat was taken out carefully. The sex of the individual clam and the gonad condition were ascertained by examining fresh smears of gonad under the microscope. Gonad tissues were fixed in Bouin's fixative and 10% neutral buffered formaldehyde for a period of 24 – 48 hours. For dehydration of fixed tissues, they were put in ascending grades of alcohol series (30 -100%). Later they were kept in a mixture of alcohol and xylene (1:1 ratio). After that the tissues were cleared in xylene. Cold impregnation was done by putting the tissues in a mixture of xylene and paraffin wax (1:1 ratio). The tissues were then transferred to molten wax for hot impregnation (paraffin wax with ceresin, Ranbaxy, melting point 58 - 60°C). The tissues were put in fresh molten wax for two more changes of 15 minutes duration. The tissue blocks were prepared by using L-blocks (Clark, 1981).

Sections of thickness 5 – 7 μ were taken from the prepared blocks by using a rotary microtome. The sections were fixed on clean glass slides using fresh Mayer's albumen. Flattening was done by keeping the slides on a slide warmer with a few drops of distilled water. After flattening the water was drained off and the slides were dried. The slides were segregated according to their quality. The best slides were taken for staining. Before staining, the sections were de-paraffinised by two changes in xylene and then passed through a descending series of alcohol grades (100 - 70%) for dehydration. Staining was done with Harry's Haematoxylin. The stained tissues were put in tap water, where they turned to blue colour. Then they were counter stained with eosin. These slides were then put in 70% alcohol and again staining was done with alcoholic eosin. The sections were then dehydrated in two changes of absolute alcohol. They were cleared in xylene and mounted by using DPX mountant with 0.1 mm cover slips. The slides were air dried and used for microscopic examination of the gonad stage and for taking microphotographs. The gonadal stages have been indicated following the description given by Nagabhushanam and Mane (1975) for *K. opima*.

2.2.3 OOCYTE DIAMETER

Increase in the size of oocytes is a function of oogenesis and hence micrometric measurements of oocytes in different stages of gonadal maturation will provide information regarding the sexual maturity. For measuring the oocyte diameter, a drop of female gonadal smear was taken by using a micropipette and observed under the microscope. The diameter of at least 50 eggs was measured along the largest and smallest axis passing through the nucleus, and the average value was taken for further estimation. The measurement was taken using an ocular micrometer, pre-calibrated using a stage micrometer. The measurements were classified into 10 μm class intervals. The values of oocyte diameter were measured for the maturing and matured gonadal stages.

2.2.4 STATISTICAL ANALYSIS

Correlation analysis was done to relate the environmental parameters with gonad index. Attempts were also made to correlate the indices of gonad, digestive gland and condition factor at both stations. Chi – square test was performed to test the homogeneity of the sex ratio observed in the population over the sampled months.

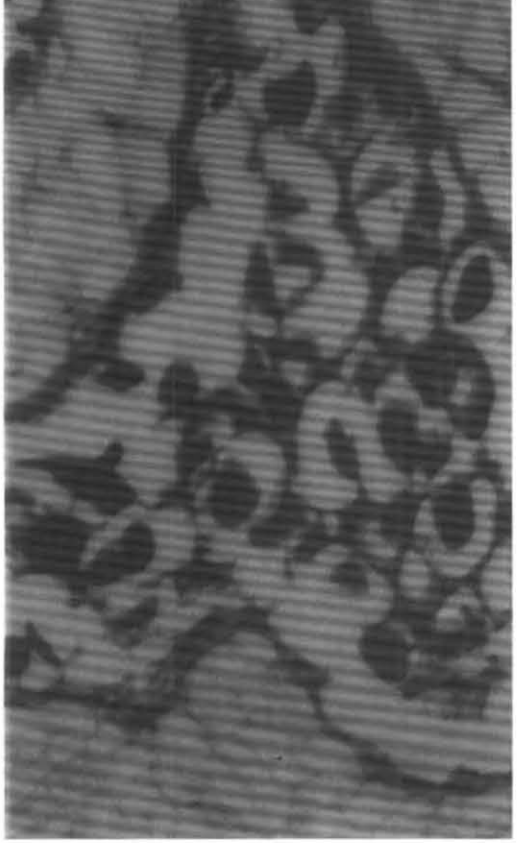
2.2.5 PHOTOGRAPHY

The photographs of the animals were taken using a Nikon FG 20 camera. The film used was Kodak Gold colour. Histological sections were photographed using a Nikon AFX-DX II microscope fitted with a Nikon FX-35 camera, with photo micrographic attachment. Kodak Gold colour film was used for filming the histological sections.

2.3 RESULTS

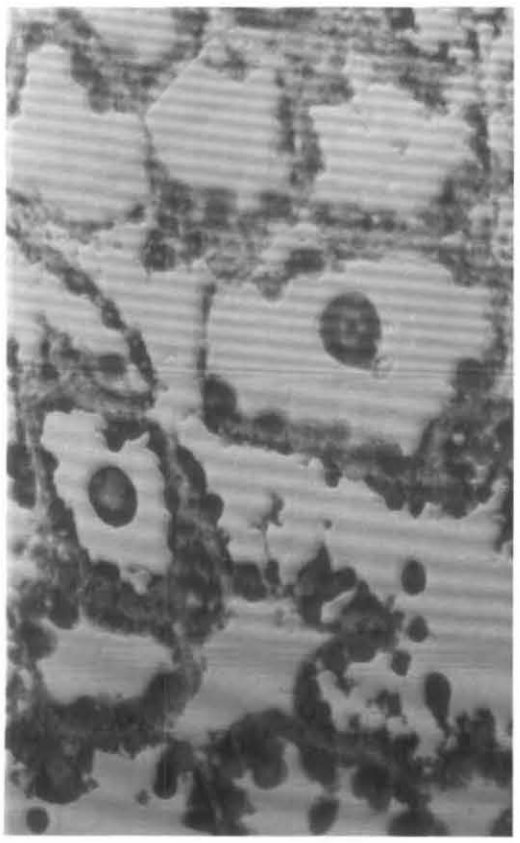
2.3.1 Classification of gonadal stages

The gonad morphology as well as histological characteristics varies along with the progression of maturity stages. In *M. opima*, five stages of gonad were identified based on the histological observations on gonad in both the sexes, as the morphology could not unfold much information (Table 2a). The cross sectional views of gonad at different stages of maturity are shown in Plates V and VI. A stage-wise description on the features of gonad is given below:

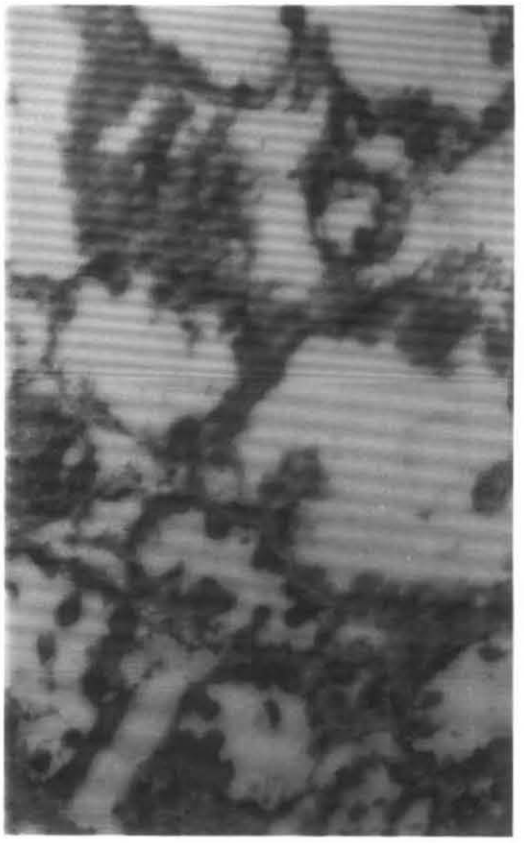


MATURING FEMALE GONAD

MATURE FEMALE GONAD

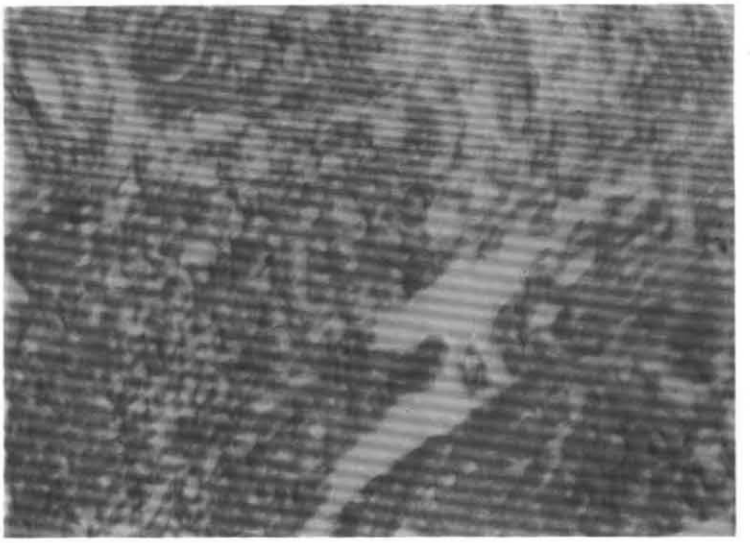


PARTIALLY SPAWNED
FEMALE GONAD

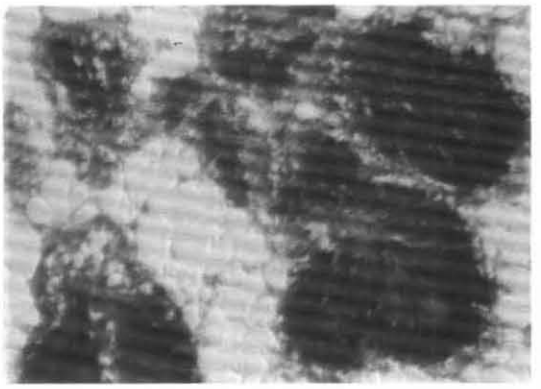


SPENT FEMALE GONAD

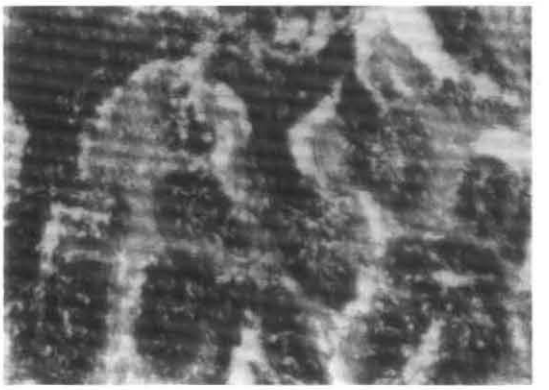
PLATE VI. CROSS SECTION OF DIFFERENT GONADAL STAGES - FEMALE GONAD



INDETERMINATE GONAD



MATURING MALE GONAD



MATURE MALE GONAD

PARTIALLY SPAWNED MALE GONAD

SPENT MALE GONAD

PLATE V. CROSS SECTION OF DIFFERENT GONADAL STAGES - INDETERMINATE AND MALE GONAD

Table 2a. Characteristics of gonad at different maturity stages

Stage	Male	Female
Maturing	Gonad is small, inconspicuous and colourless. Secondary spermatocytes appear in large numbers along with the primary spermatocytes	Gonad is small, inconspicuous and colourless. The oogonia with indistinct nucleus, small and spherical in shape and the size ranges from 22–33 µm diameters
Mature	Gonad attains maximum size, cream coloured; entire lumen is filled with bunches of spermatozoa	Gonad attains maximum size, cream coloured; Nucleated oocytes of size range 33–66 µm fill up the lumen and get detached from the stalks.
Partially spawned	Gonad becomes flabby and loose in consistency. Colour of the gonad slightly changes to grey. Few unreleased sperms are seen in the lumen.	Gonad becomes flabby and loose in consistency. Colour of the gonad slightly changes to grey. Lumen shows the presence of unreleased oocytes.
Spent	Follicular walls collapse and shrink further; unreleased sperms undergo degeneration. The vesicular connective tissue increases.	Follicular walls collapse and shrink further. Phagocytes appear. The vesicular connective tissue increases.
Indeterminate	Gonad is small and translucent, with much connective tissue. No traces of gametes are seen, so the sex is indistinguishable.	

2.3.1.1 Stage I (Maturing)

In the maturing stage, the gonad is small, inconspicuous and colour less, but highly active. On the onset of active phase, the follicles increase in size in male gonad and their periphery contains numerous spermatogonia and a few spermatids radiating towards the lumen of the follicle. The spermatogonia are easily recognized by their large nuclei with the cytoplasm enveloping them. As the maturing phase advances, the secondary spermatocytes appear in large numbers along with the primary spermatocytes. The primary and secondary spermatocytes can be differentiated only by size and staining intensity.

In the female gonad, the primary germ cells under go mitotic division and give rise to oogonia in this stage. The onset of oogenesis is indicated by the appearance, growth and spreading of follicles and the occurrence of oogonia and oocytes in the pre-meiotic stage. The oogonia are small and spherical in shape and the size ranges from 22 – 33 µm diameters. The cytoplasm is small and the nucleus is not distinctly visible. In the late maturing phase, a rapid increase in the size of follicles is seen, along with secondary oocytes. The follicles occupy more area among the connective tissue.

2.3.1.2 Stage II (Mature)

As the stage advances, the gonad becomes larger in size, thick and firm. In matured stage, the gonad is full and plump and attains a maximum size. The colour of the gonad becomes creamy with very little connective tissue.

As the male gonad attains the ripe condition, the spermatids differentiate into spermatozoa and lie as a core in the lumen of the follicle. A ripe gonad is characterized by bunches of spermatozoa arranged more or less radially with their tails facing towards the centre of the follicular lumen. In a fully ripe gonad, the entire lumen is filled with bunches of spermatozoa.

In the female gonad, the oocytes grow further, enlarge in size, and become nucleated and irregular in shape. Most of the large oocytes are attached to the follicular walls by slender stalks. The stalk is broad at the base and stumpy or pointed at the tip. Oocytes in the size range 33 – 66 µm are found in more numbers in this stage. As the growth of the oocytes advance, the size of the stalk reduces and it becomes more or less round. A fully ripe female gonad is characterized by the occurrence of more detached oocytes in the lumen of the follicle.

2.3.1.3 Stage III (Partially Spawned)

In the partially spawned stage, the gonad becomes flabby and loose in consistency. Colour of the gonad slightly changes to grey. Follicle wall ruptures and become empty with a few residual gametes. A few phagocytes may be present. A number of disorganized cells including spermatids and secondary spermatocytes are found in the lumen of a partially spawned male gonad. The vesicular connective tissue increases.

The follicle shows varying degrees of emptiness in a female gonad. The vesicular tissue, the connective tissue cells and free oocytes are found scattered in the lumen. The residual oocytes in the lumen are few. Phagocytes may appear in the inter-follicular space in some cases.

2.3.1.4 Stage IV (Spent)

In this stage, the follicular walls collapse and shrink further. Phagocytes appear both inside and outside the follicles. The vesicular connective tissue increases. Phagocytes present in the lumen engulf the left out residual pockets of gametes. Vesicular and connective tissues occupy the spaces between the follicles.

2.3.1.5 Stage V (Indeterminate)

In the indeterminate stage, the gonad shrinks further and becomes translucent. The wall of the gonad appears with much connective tissue. The follicles are completely shrunk and collapsed. During this stage, the gonad is quiescent without the trace of any germinal cells. Differentiation of sex is difficult at this stage.

2.3.2 Monthwise frequency of different gonadal stages

Monthwise percentage of each stage for the entire fourteen months period at Tuticorin and the bimonthly samples from Ashtamudi indicate seasonal changes in the annual reproductive cycle of *M. opima*.

2.3. 2.1 December 1998

Clams with spent gonad were dominant, forming about 76.92% of the sampled population. Another 15.38% were in the partially spawned condition and the rest had mature gonads.

The partially spawned and spent gonads were flabby and slightly loose in consistency, showing a dull cream colour. Later on, the follicles shrunk further resulting in marked reduction in the number of gametes within the lumen. The unreleased oocytes remained attached to the follicular wall but were smaller in size. In some of the follicles of the male gonad, the lumen was empty due to the complete discharge of the spermatozoa.

2.3. 2. 2 January 1999

The percentage of maturing gonads was 76.47%. Matured clams constituted 5.88% of the population. Partially spawned individuals represented 5.88%. Spent clams were 10.77%. There were no indeterminate specimens.

In the active maturing phase the pronounced enlargement of follicles was due to the rapid increase of their size in the gonads. In male gonads, the reproductive follicles contained large number of early spermatogonia with spermatocytes and few spermatids radiating into the lumen of the follicles. The gametogenic activity in the females proceeded rapidly. The number of follicles in the ovary increased and small oocytes were observed with round distal end protruding into the lumen and the other end attached to the follicular wall by slender stalk. A few follicles were still in the process of proliferation with young oocytes in the follicular wall.

2.3. 2.3 February 1999

Maturing individuals represented 76.67% of the population. 20% of the clams were in the indeterminate resting phase. Rest, 3.3% of the population were spent males.

2.3. 2.4 March 1999

Maturing specimens were 43.33% of the *M. opima population* at Tuticorin. The gametogenic activity initiated during the previous month became intense and, as a result, the percentage of matured gonads increased to 56.67%. There were no indeterminate or spent clams during this month. As a result of the rapid growth of the follicles in the gonad, the gonads were full and plump and formed a major part of the visceral mass. The follicles were enlarged and packed with reproductive elements. The external appearance of the gonad was smooth.

At Ashtamudi, the frequency of spent clams was 63.33%, of which 36.67% was spent females. Spent males were 26.66%. 10% of the population comprised partially spawned females. Indeterminate specimens started to appear in this month, but with a lesser percentage of 3.33. Mature animals

constituted 23.33% of the total population, out of which 16.66% were mature females and 6.67% were males.

2.3. 2.5 April 1999

The population was highly dominated by mature clams, which constituted 83.33%. Maturing clams were 16.67%. As observed in the previous month, indeterminate and spent clams were not present in the population. The follicles in the gonads of males and females were closely packed with ripe gametes without any inter space. The vesicular connective tissue was completely obliterated. In ripe females, the follicles filled the entire gonad area with little inter space in between. Large numbers of spherical, free oocytes were found in the lumen. They had distinct nucleus and nucleolus.

2.3. 2.6 May 1999

At Tuticorin, spawning had occurred in the clams as evidenced by the presence of spent gonad phase in 53.33% and partially spawned ones in 30% of the population. The gonads in the matured phase were decreased to 6.66% in female and 10% in male populations.

Majority of the sampled population were constituted by indeterminate specimens that formed 65.38%, in the Ashtamudi population during this month. Spent clams were less, which constituted 26.92%. Partially spawned ones represented 3.8%. Samples had 3.8% mature males.

2.3. 2.7 June 1999

As spawning had advanced further, majority of the clams were in the spent phase. This was indicated by the short fall of the partially spawned gonads in both the sexes. The spent gonads represented 83.33%, of which spent males represented 46.66%. Partially spawned ones were only 3.33%.

Matured clams formed 13.33% of the population. The fully spent gonads appeared very loose and transparent. The lumen contained few numbers of residual oocytes and spermatozoa. The follicles were collapsed in some cases, while in others the follicular walls were indicated by faint lines. Phagocytes in large number appeared both inside and outside the follicles, which cytolysed the remnants and unreleased residual gametes.

2.3. 2.8 July 1999

Gametogenesis had commenced at Tuticorin. As a result, 37.14% of the population were in the maturing stage and 14.28% were in the matured condition. In the indeterminate specimens, which were 31.42%, the follicles were completely ruptured leaving a hieroglyphic appearance. Spent clams represented 17.14% of the samples. The gametes, which were left as residue in the lumen were undergoing resorption and were covered by connective tissue *around* the follicles. During the time, the nucleus disappeared, the cytoplasm oozed out, the oocytes were disintegrated and became transparent and thus the contents of the gametes were completely resorbed. When the process of cytolysis and resorption was completed, the follicle became empty and the gonad became translucent.

Sampled population showed the commencement of gametogenesis during July at Ashtamudi. 25% were maturing clams. Due to the gametogenic activity, 25% of the clams were in mature stage, with more male specimens than females. The indeterminate specimens and spent ones had a frequency of 31.25% and 18.75% respectively.

2.3. 2.9 August 1999

In August, the percentage frequency of maturing phase increased further to 46.15%. Due to active gametogenesis 41.02% had entered into the matured phase. 7.69% of the population were indeterminate individuals. Spent animals constituted 5.13%.

2.3. 2.10 September 1999

During this month at Tuticorin, the gonads of majority of the collected specimens have attained full maturity. Matured specimens represented 55.26% of the total sampled population. Maturing and spent clams represented 34.21% and 10.53% of the samples respectively.

Only 10% female population was noted at Ashtamudi during this month. Samples of clams represented all the five stages of gonadal maturity. Percentage of mature animals increased to 26.66%, when compared to July. 40% of the sampled animals were maturing, 17.77% were spent and partially spawned specimens were 6.66%. Indeterminate clams were 8.88%.

2.3. 2.11 October 1999

The presence of partially spawned and spent clams indicated that spawning had progressed in this month. Partially spawned and spent specimens formed 15.74% and 5.27% of the sampled population respectively. Matured individuals represented 52.03%. Maturing clams formed 21.05% and indeterminate specimens were 5.27%.

2.3. 2.12 November 1999

The percentage of partially spawned and spent animals gradually increased in the Tuticorin population. The gonad appeared to be loose and translucent in most of the animals. The percentage frequency of partially spawned clams was 34.78%. Spent clams were 17.39%, while 13.04% were mature. The percentage frequency of indeterminate was 21.74% and 13.04% of the clams were still in the maturing phase.

Sampled specimens of Ashtamudi showed active gametogenesis. Mature and maturing animals were 13.04% each. Spent ones formed 39.13% of the population. Indeterminate specimens had a frequency of 34.78%.

2.3. 2.13 December 1999

The percentage frequency of the spent animals was on the increase. Of the total clams sampled 55.76% were spent animals. Partially spawned individuals formed 3.70%. Indeterminate clams were 18.52% of the population. Maturing specimens represented 22.22%.

2.3. 2.14 January 2000

The percentage of indeterminate clams increased rapidly to 56.67% of the collected samples at Tuticorin. The residual gametes in the gonad were completely resorbed and became translucent. In some gonads, undifferentiated germ cells began to appear along the lining of the follicular walls. There was no trace of follicle was observed in the indeterminate gonads and it was completely resorbed. In this month, maturing clams represented only 26.66%. Spent animals constituted 16.67% of the sampled population. There were no matured animals.

A higher percentage of spent clam population during this month indicated that spawning had occurred at Ashtamudi. Partially spawned and spent ones together formed 60.0% of the population. 10% of the collected samples were mature, 25% were maturing and 5.0% were indeterminate.

Monthly percentage occurrence according to the maturity stages of *M. opima* population at Tuticorin and Ashtamudi is shown in Figs. 1 and 2.

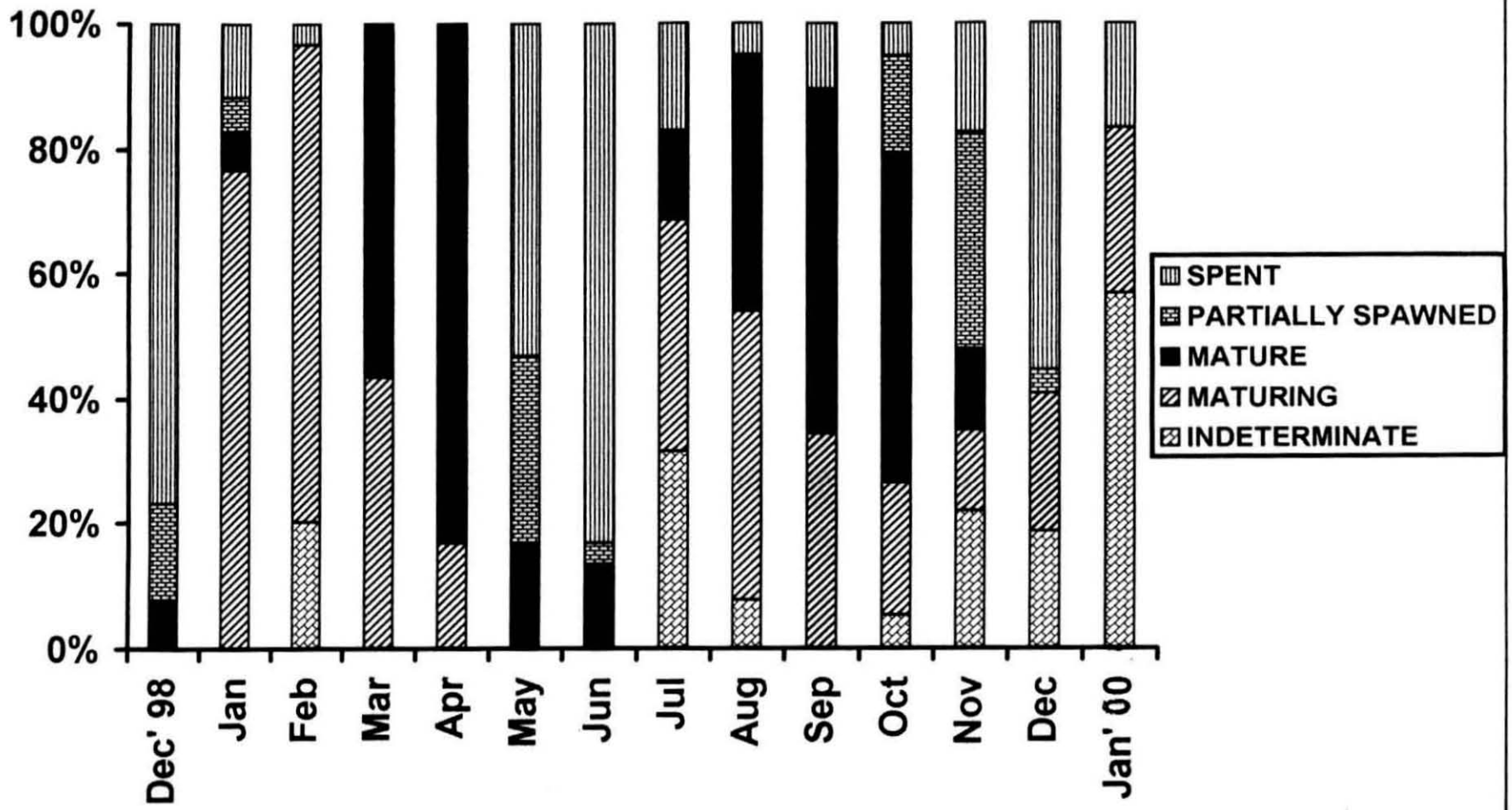


Fig. 1. Percentage occurrence according to the maturity stages of *M. opima* at Tuticorin

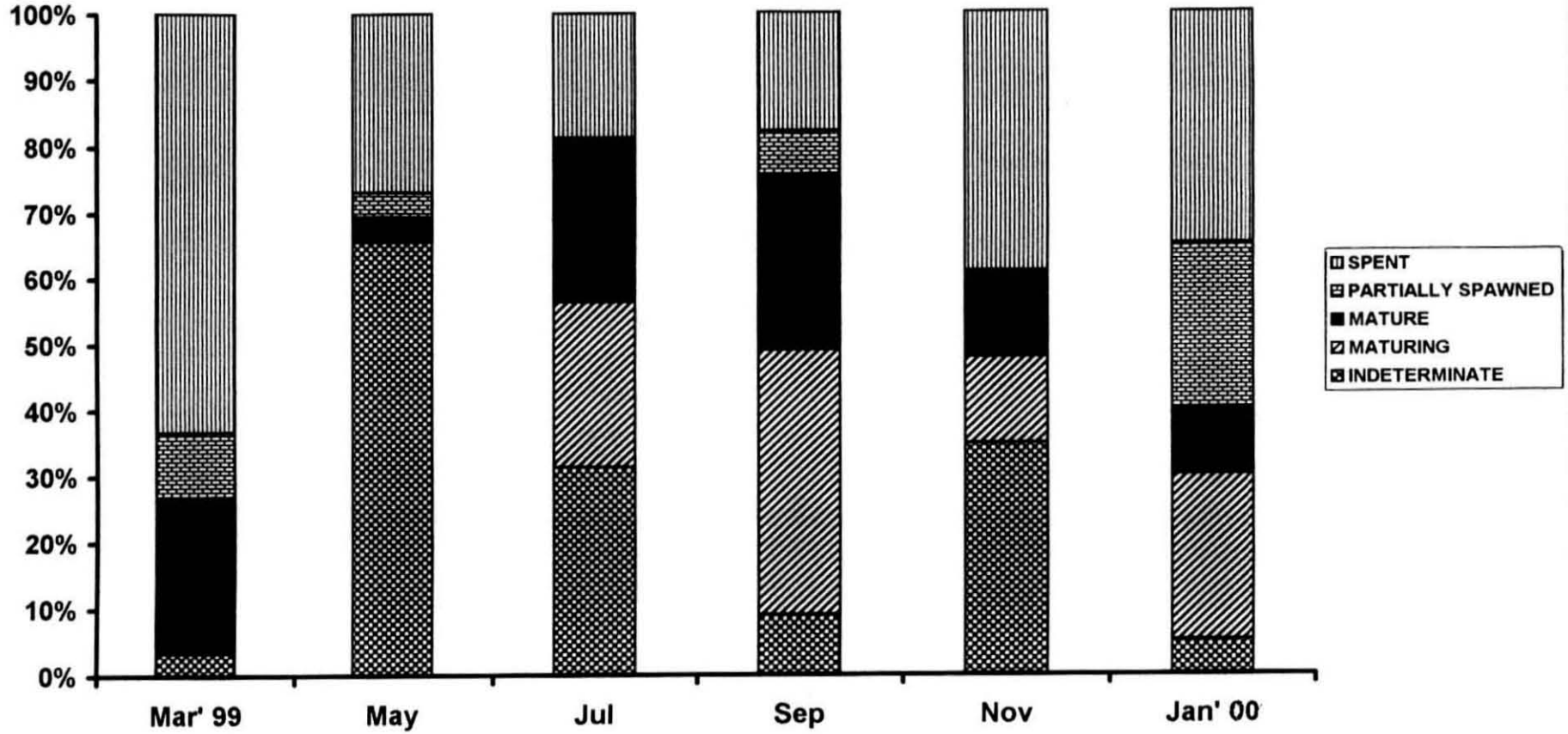


Fig. 2. Percentage occurrence according to the maturity stages of *M. opima* at Ashtamudi

2.3. 3 Gonad Index

The mean gonad index of *M. opima* showed variations according to the reproductive activity at Tuticorin. Mean gonad index was 11.69 ± 2.3 in December 1998, when 80% of the clams were in spent condition. The values tend to increase to 11.85 ± 2.1 in January 1999 and 13.38 ± 1.7 in February 1999 as 80% of the clams had maturing gonad. The gonad indices rose to 17.57 ± 2.4 in March 1999 and to a peak of 17.16 ± 3.4 in April 1999 as 60 to 70% of the clams were in matured condition. As spawning occurred during May, the indices for May and June decreased to 10.44 ± 3.2 and 9.78 ± 2.5 respectively. During these two months, the spent class predominated (80% - 90%) the samples. The value slightly increased to 11.38 ± 1.3 in July, and to13.87 ± 2.4 in August as the gonads switched over to maturing condition. In September, the gonad index went up to 15.45 ± 2.4 as all the clams were having maturing and matured gonads. Spent condition was found in 20% of the samples in October and in 40% of the samples in November1999. This brought down the index to 13.5 ± 2.6 in October and to 13.26 ± 3.3 in November1999. The gonad index in December was 11.78 ± 1.6. The onset of maturation of gonad in January 2000 increased the index to 13.0 ± 2.2.

At Ashtamudi, gonad index of *M. opima* varied with the reproductive stages in a similar pattern as that of Tuticorin. It varied from 5.72 to 11.74. In March 1999, the gonad index was 7.11 ± 2.2 when 70% of clams were in spent stage. In May the value decreased to 5.72 ± 1.7. In July 1999, 86.7% clams were in maturing stage and the gonad index value was increased to 8.4 ± 1.9. In September, 90% of the clams were mature and the gonad index further increased to 11.74 ± 1.3. The gonad index was 7.29 ± 1.7 and 6.68 ± 2.3 in November 1999 and January 2000 respectively.

2.3.4 Digestive gland index

The mean digestive gland index of *M. opima* at Tuticorin varied between 1.94 and 3.2 during the study period. Digestive gland index was 2.18 ± 0.6 in December 1998 and 2.48 ± 0.9 in January 1999. It increased to 3.21 ± 0.9 in February 1999. During March and April 1999 the value decreased to 2.52 ± 0.8 and 2.41 ± 0.8 respectively. A low value of digestive gland index, 1.94 ± 0.4 was observed in May 1999. In June 1999, digestive gland index showed a mean value of 2.23 ± 0.5 and in July, the value was 2.37 ± 0.8. During August and September 1999, digestive gland index increased to 3.0 ± 0.9. In October 1999 onwards, digestive gland index again decreased to 2.8 ± 0.5 and in November it was 2.5 ± 0.2. During December, the value further decreased to 2.0 ± 0.2. But in January 2000, digestive gland index showed a gradual increase and had a value of 2.31 ± 0.3.

The digestive gland index in March and May 1999 for *M. opima* at Ashtamudi was 3.11 ± 0.3 and 3.24 ± 0.4 respectively. In July, the digestive gland index was 2.94 ± 0.6 and in September 1999, it was 2.75 ± 0.7. In November, digestive gland index further decreased to 2.32 ± 0.5. A low value of digestive gland index, 1.97 ± 0.4 was observed during January 2000.

2.3.5 Condition factor

Condition factor of *M. opima* showed variations along with the gonad maturation process. At Tuticorin, the value of condition factor ranged from 33.19 in December 1998 for spent clams whereas matured clam had a high value of 103.3. The average value of condition factor was 74 ± 11.9. In January 1999, the value was more or less same at 73.44 ± 13.6. Thereafter as gonad started maturing, the average condition factor increased to 88.67 ± 16.7 in March 1999 and reached high value of 93.61 ± 9.4 in April 1999, indicating the matured gonadal condition. During this period maximum value of 114.4 was noted in a ripe male of size 31.4mm. During May to July 1999, moderate value of condition

factor, 66 ± 8.9 to 69 ± 11.5 was observed and the clams were in spent stage after a peak spawning during March to April. In August, when 50% of clams were having maturing gonad, the condition factor ranged from 42.86 to a maximum of 112.20 with an average value of 76.62 ± 18.4. Thereafter during September to October 1999, the condition factor remained between 86.04 ± 20.4 and 90.35 ± 8.5. In November 1999, the condition factor was 86.88 ± 17.9. During these three months the matured clams were more and spent clams comprised 20 to 40% of the population. Maximum value of condition factor, 126.87 for an individual clam was observed in a ripe male during October 1999. As the gonad were mostly in spent stage in December 1999 the condition factor dropped to 73.66 ± 20.2 with a minimum value of 44.21 for an indeterminate and 124.12 for male ripe clam. In January 2000, the condition factor of maturing clam population was 57.3 ± 13.7.

Although the pattern of variation was the same, the condition factor showed a lesser value for the Ashtamudi population of *M. opima* than those collected from Tuticorin. In March 1999, condition factor ranged from 31 in spent clams to 60 in clams with matured gonads. The average value was 41.68 ± 8.9. A similar value of condition factor was observed in May 1999 also (41.7 ± 5.9). In July 1999, when the gonad started maturing the average value increased to 48.63 ± 7.7. A high value of condition factor, 62.8 was observed for a matured male clam. In September 1999, for the partially spawned and spent clams, the value was low ranging from 32.56 to 42.13, where as 61.84 was noted in matured female clams. The average condition factor was 43.86 ± 10.7. It decreased to 43.36 ± 7.9 in November 1999 as the spent and indeterminate stages was predominant in the samples. In January 2000, a high condition factor of 72.5 was noted in clams with matured gonad stages. Since majority of the clams were with spent and indeterminate gonadal stages, average value of condition factor was 43.78 ± 10.6 (Tables 2b and 2c; Figs. 3 and 4).

Table 2b. Mean variations of GI, DGI and CF of *M. opima* at Tuticorin

Months	GI	DGI	CF
Dec-98	11.69±2.3	2.18±0.6	74.00±11.9
Jan-99	11.85±2.1	2.48±0.9	73.44±13.6
Feb-99	13.38±1.7	3.21±0.9	84.56±11.4
Mar-99	17.57±2.4	2.52±0.8	88.67±16.7
Apr-99	17.16±3.4	2.41±0.8	93.61±9.4
May-99	10.44±3.2	1.94±0.4	69.28±11.5
Jun-99	9.78±2.4	2.23±0.5	66.18±8.9
Jul-99	11.38±1.3	2.37±0.8	68.95±7.3
Aug-99	13.87±2.4	3.00±0.9	76.62±18.4
Sep-99	15.45±2.4	2.97±1.0.	86.04±20.4
Oct-99	13.5±2.6	2.80±0.5	90.35±8.5
Nov-99	13.26±3.3	2.50±0.2	86.88±17.9
Dec-99	11.78±1.6	2.00±0.2	73.37±20.2
Jan-00	13.00±2.2	2.30±0.3	57.30±13.7

Table 2c. Mean variations of GI, DGI and CF of *M. opima* at Ashtamudi

Months	GI	DGI	CF
Mar-99	7.11±2.2	3.11±0.3	41.68±8.9
May-99	5.72±1.7	3.24±0.4	41.7±5.9
Jul-99	8.40±1.9	2.94±0.6	48.63±7.7
Sep-99	11.74±1.3	2.75±0.7	48.36±10.7
Nov-99	7.29±1.7	2.32±0.5	43.36±7.9
Jan-00	6.68±2.3	1.97±0.4	43.78±10.6

GI - Gonad Index
DGI - Digestive Gland Index
CF - Condition Factor

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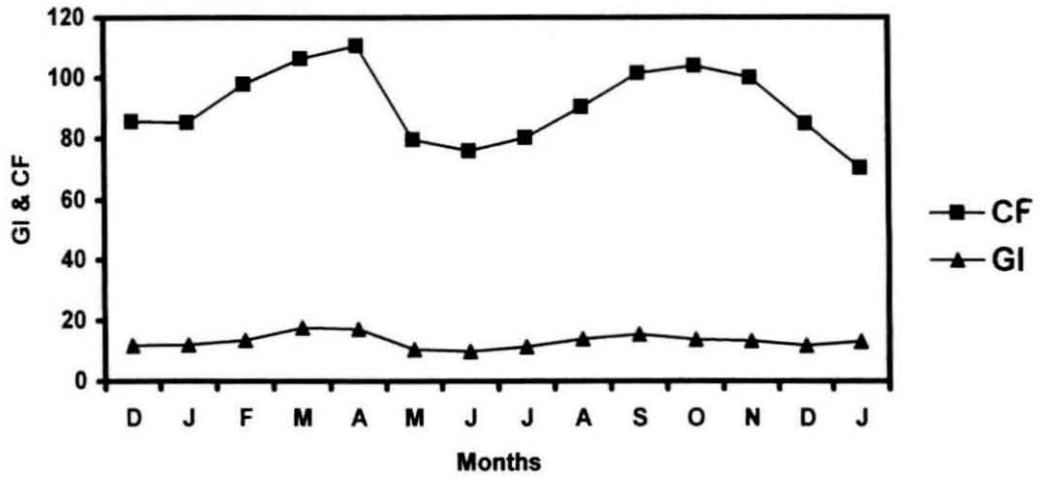


Fig. 3. Mean variations in GI and CF of *M. opima* at Tuticorin

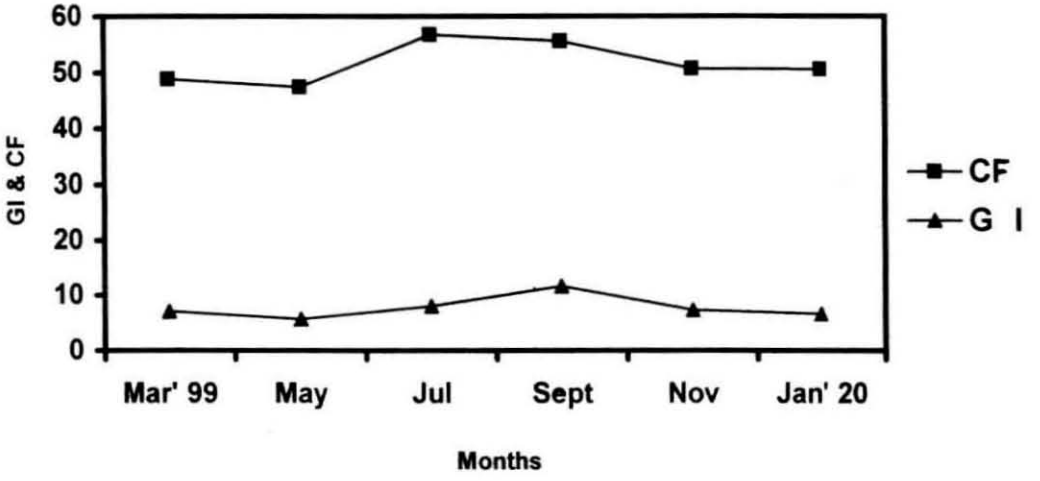


Fig. 4. Mean variations in GI and CF of *M. opima* at Ashtamudi

2.3.6 Dry / wet weight ratio of flesh

During the study period, the dry/wet flesh ratio for the clams sampled at Tuticorin varied from 0.16 to 0.32. There were two distinct depressed values, 0.18 in May 1999, and 0.16 in December 1999.

The variations in the dry/wet meat weight ratio for the Ashtamudi clams ranged from 0.14 to 0.20. The value was lowest in November 1999.

2.3.7 Sex ratio

During the samplings made, a slight female dominance was noticed at Tuticorin. Out of the 420 specimens of *M. opima* collected at Tuticorin, 163 were males and 195 were females. The rest were indeterminate. The observed male: female ratio at Tuticorin was 1:1.3. To test the significance of the observed sex ratio with that to the expected one, Chi-square test was performed and it revealed that during the sampled months, it did not deviate from the expected 1:1 ratio. To test the homogeneity of the sex ratio over the months sampled, Chi-square test was performed again. It was found that the Chi-square value at 13 degrees of freedom (N=14) at 5% level of significance was 22.36, which is significantly higher than the observed value of 10.72. It could be inferred that the population of *M. opima* at Tuticorin follow the sex ratio of 1:1 although the samples showed a female dominance.

At Ashtamudi, the observed sex ratio was 1.27:1, showing a male dominance. Out of 180 samples collected there, 80 were males and 63 were females. Chi-square test revealed a deviation of sex ratio from the expected one during the months of July 1999 and September 1999. The test also showed that the population of *M. opima* at Ashtamudi had a dominance of male clams over females, as indicated by a higher Chi-square value of 13.55 at 5 degrees of freedom (N=6) at 5% level of significance than the table value of 11.07.

2. 3. 8 Oocyte diameter

The monthly mean diameter of oocytes was 26.4 ± 5.5 µm in January 1999 for *M. opima* collected at Tuticorin. As the gonad activity increased during February, March and April 1999, the month wise average diameter of oocyte in the maturing clams was 30.8 ± 6.8 µm, 38.4 ± 6.9 µm and 47.3 ± 6.3 µm respectively. In May, an average diameter of 48.95 ± 5.61 µm was noted in ripe females. Average oocyte diameter was 51.7 ± 6.3 µm in the matured clams for the month of June. During these periods gonadal activity was reached at its peak. After spawning, gametogenic activity restarted with a very less number of maturing population. So the average diameter of oocytes in July 1999 was 24.75 ± 4.9 µm. Due to the rapid gonad growth, oocyte diameter increased gradually during August, September and October 1999 with values of 26.4 ± 5.5 µm, 35.75 ± 5.5 µm and 40.15 ± 8.0 µm respectively. The diameter of oocyte recorded in December had a value of 24.75 ± 4.9 µm. Average oocyte diameter was 57.33 ± 5.2 µm in January 2000 (Table 2d).

The mean oocyte diameter of Ashtamudi clams was 47.3 ± 6.3 µm in March 1999. After the spawning season, the oocyte development was observed in the maturing clams during May 1999 and the mean diameter in that month was 24.8 ± 4.9 µm. The oocyte diameter increased to 30.3 ± 7.0 µm in July 1999. In September1999, when 60% of clams were in matured stage, the mean oocyte diameter was 49.5 ± 7.6 µm. Oocyte diameter was 27.6 ± 7.5 µm in November. In January 2000, the mean oocyte diameter was 43.8 ± 6.3µm (Table 2e).

When the monthly oocyte diameter variations and the maturity stages were compared it was observed that the maturing clams of *M. opima* had an average oocyte diameter of 28.24 µm with a range of 22 – 33 µm and the matured clams had an average oocyte diameter of 48.28 µm with a range of 33 – 66 µm.

Table 2d. Mean variations of oocyte diameter of *M. opima* at Tuticorin

Months	Oocyte Diameter (µm)
Jan-99	26.4± 5.5
Feb-99	30.8± 6.8
Mar-99	38.4± 6.9
Apr-99	47.3± 6.3
May-99	48.9± 5.6
Jun-99	51.7± 6.3
Jul-99	24.8± 4.9
Aug-99	26.4± 5.5
Sep-99	35.8± 5.5
Oct-99	40.2± 8.0
Nov-99	42.4± 6.4
Dec-99	24.8± 4.9
Jan-00	57.3± 5.2

Table 2e. Mean variations of oocyte diameter of *M. opima* at Ashtamudi

Months	Oocyte Diameter (µm)
Mar' 99	47.3± 6.3
May	24.8± 4.9
Jul	30.3± 7.0
Sept	49.5± 7.6
Nov	27.6± 7.5
Jan' 00	43.8± 6.3

2.3.9 Environmental parameters

During the sampling period the temperature ranged between 26°C and 32.5°C at Tuticorin. In December 1998, the water temperature was 29°C, which increased to 29.5°C in January 1999 and 31.5°C in February 1999. In March and April 1999, the temperature remained at 30°C and in May, it rose to 31°C. Thereafter up to September 1999, it ranged from 28 to 29°C. In October 1999, a low value of temperature, 26.5°C was noticed. In November, the temperature recorded was 32.5°C and in December 1999, the temperature was 30°C. Temperature recorded in January 2000 was 26°C. The pattern of oscillation seen in the atmospheric temperature was bimodal with two peaks in February and November 1999 and two depressions in the months of October 1999 and January 2000.

The salinity ranged from 32 to 37 ‰. The salinity showed a gradual increase from 34 ‰ in December 1998, to 36.5 in April 1999. In May 1999, a maximum value of salinity, 37 ‰ was observed. From June 1999 onwards, salinity showed a decreasing trend and in October 1999, a minimum salinity of 32 ‰ was recorded. During November to December 1999, the average salinity was 34 ‰. The salinity again decreased to the minimal value of 32 ‰ in January 2000. The pattern of oscillation seen in the salinity values was also bimodal with two peaks in May and November 1999 and two depressions in October 1999 and January 2000 (Table 2f).

At Ashtamudi, the temperature recorded ranged between 24°C and 34°C. The highest temperature, 34°C was recorded in March 1999. It decreased to 29°C in May and in July 1999 the lowest temperature 24°C was recorded. During September, the temperature was 28°C and in November 1999, it was 27°C. In January 2000, the temperature further decreased to 26°C. The pattern of oscillation was bimodal with two peaks in the months of March and September 1999 and two depressions in July 1999 and January 2000.

Table 2f. Environmental parameters at Tuticorin

MONTHS	SALINITY (‰)	TEMPERATURE (°C)
Dec-98	34	29
Jan-99	34	29.5
Feb-99	35	31.5
Mar-99	36	30
Apr-99	36.5	30
May-99	37	31
Jun-99	36.5	29
Jul-99	34	29
Aug-99	33	28
Sep-99	33	29
Oct-99	32	26.5
Nov-99	34	32.5
Dec-99	34	30
Jan-00	32	26

2g. Environmental parameters at Ashtamudi

MONTHS	SALINITY (‰)	TEMPERATURE (°C)
Mar-99	30	34
May-99	29	29
Jul-99	27	24
Sep-99	27	28
Nov-99	26	27
Jan-00	26	26

The salinity was highest at 30 ‰ in March 1999 when the temperature was at a peak. In May, the salinity was 29 ‰. During the period July to September, salinity recorded was 27 ‰. A lower salinity of 26 ‰ was observed from November 1999 to January 2000 (Table 2g).

Correlation analysis between salinity, temperature and gonad index revealed that at Tuticorin, salinity is negatively correlated (r= -0.0224) with gonad index. Temperature also followed a similar trend. Same was the case with Ashtamudi population of *M. opima* with both salinity (r= -0.269) and temperature (r= -0.1354) showing a negative correlation to gonad index.

2.4 DISCUSSION

It was observed that both the populations of *M. opima* have two spawning seasons in a year. Dominance of ripe males and females in April 1999 and also in October 1999 was prior information regarding the arrival of two spawning periods for *M. opima* at Tuticorin. In the present investigation, it is understood by the gonadal studies that *M. opima* at Tuticorin spawns twice in a year with a major spawning period during May to June and a minor spawning during November to December. At Ashtamudi, dominance of matured clams in March and in November was an indication of two spawning periods in a year. *M. opima* at Ashtamudi also spawned twice in a year. The major spawning period at Ashtamudi was March to May and minor spawning period was November to January. In some bivalves gametogenesis occurs immediately after the completion of spawning (Loosanoff, 1953; Nagabhushanam and Mane, 1975 and Jayabal and Kalyani, 1987 a). In the present study, it was observed that *M. opima* at both the stations enter into a resting indeterminate stage after the spawning. Indeterminate stage is followed by the maturing stage in which active gametogenesis starts. This agrees with the observations of Rao (1967), Natarajan and John (1983) and Narasimham (1988).

The same species from two geographically separated places may exhibit a difference in the frequency of annual spawning. At one place it may spawns twice in a year but at another place it may show single spawning season. Chipperfield (1953) suggested that timing and duration of the spawning and also the number of spawning each year is dependent on the species and is interrelated with different environmental factors. Rao (1951 a) reported a single spawning season for *K. opima* from Adyar estuary, Madras at the east coast. Nagabhushanam and Mane (1975) observed a biannual spawning for *K. opima* in Kalbadevi estuary, Ratnagiri at west coast. In the present study also a biannual spawning for *Marcia opima* was observed at both the stations. Abraham (1953) reported that *Meretrix casta* in Adyar estuary spawns twice in a year with peak spawning activities in March to May and October to November. Whereas Durve (1964) opined that *M. casta*, in the marine fish farm at Mandapam is a continuous breeder with a break of few months in the late summer due to unfavourable environmental conditions. According to Nayar (1955) *D. cuneatus* of Palk Bay, spawns from January to April. Rao (1967) observed that *D. cuneatus* of the Madras coast showed a single reproductive cycle. *D. faba* from Mandapam have a prolonged breeding period from November to June with two spawning peaks, one in November to December and another in May to June (Alagarswami, 1966). A biannual reproductive cycle was observed by Ropes (1968) in *S. solidissima* gonads from offshore New Jersey. But *S. s. similis* from St. Catherines Sound, Georgia showed a single gametogenic cycle (Kanti *et al.*, 1993). Appukkuttan (unpublished) observed spawning from October to January with peaks during October and November for *P. malabarica* in Ashtamudi Lake.

The changes in the breeding season with respect to latitude and an extension of the season as one goes south has been observed in many temperate marine invertebrates (Giese, 1959). The duration of the spawning period varies in species occurring in different parts of its geographic range (Ropes and Stickney, 1965 and Seed, 1976). Breeding period of widely distributed marine animals is earlier in the southern part of their range, but at about the same temperature as in the north (Runnstorm, 1927).

Although, *M. opima* at Tuticorin and Ashtamudi show biannual reproductive cycles, there exists only a slight difference in the spawning periods at both the stations. Due to the similarity that exists in the latitudinal positioning of the two populations, some sort of resemblance was observed in the reproductive behaviour. Site-specific differences in the reproduction of bivalves have been noted previously (Abraham, 1953; Ansell *et al.*, 1964; Keck *et al.*, 1975; Laruelle *et al.*, 1994). Observation of varying periods of reproductive activity was noted in bay scallop, *A. irradians* in latitudinally separated areas, at Woods Hole and Beaufort (Sastry, 1970). Abraham (1953) suggested that the period of active spawning does not remain constant from year to year in the same environment nor does it coincide in the different environments. Inconsistency in the onset and duration of the rainy season, which controls the salinity of the water accounts for this. Mac Donald and Thompson (1988) suggested that local variability in gonadal development could be ascribed to local variations in environmental factors, among which food availability is of major importance. Difference in the gonad development may be the result of local differences in temperature, food availability, salinity or other factors regulating gametogenesis (Hesselman *et al.*, 1989; Sbrenna and Campioni, 1994). Physiological variation in population of a species exposed to different environments could be either a phenotypic response of a single genotype or could be truly genetic (Prosser, 1955). From these observations it can be concluded that the difference in the reproductive activity of *M. opima*, from two geographically separated areas of Tuticorin and Ashtamudi may have been favoured through selection as an adaptive response to the geographical differences in temperature, salinity or food availability.

The monthly mean gonad indices showed a cyclic pattern of gonad activity for both the populations. The gonad growth for *M. opima* at Tuticorin takes place from January to April, and also during July to September. It was observed that the gonad index showed an increasing trend during these months. In the months of May to June and November to December, when the spawning

activity was taking place the mean gonad index value was minimum. Maximum value of gonad index was always associated with the gonads of mature clams. Hence in the months when matured clams were more average value of gonad index was also more. Same trend was observed in the clams of Ashtamudi also. From the observations of the present study, it is understood that the gonad index value and reproductive cycle are highly related to each other. The reduction in the gonad index value during the spawning period is due to the reduction in the gonad weight after releasing the gametes.

The changes in the digestive gland index of *M. opima* from both the populations also showed the same trend. It was less during the active gametogenesis. In the annual cycle, gonad growth seems to occur by an accumulation of nutrient reserves in the tissue, which are apparently utilized by the developing gametes for synthesis of various biochemical constituents (Giese, 1959; Sastry, 1968). It was observed that the digestive gland index was higher during vegetative and rearing stages (Sastry, 1970). The results of the present study agree with this. During the months when indeterminate resting clams were more, digestive gland index was also high. Since there was no gonad development in these months, the accumulation of nutrient reserves takes place. As a result, the weight as well as the index of digestive gland was increased. The changes in the monthly digestive gland index probably indicate the amounts of nutrient reserves in the tissue, which vary with gonad activity and seasonal changes in the environment.

The changes in the monthly gonad and digestive gland indices in some marine invertebrates show a reciprocal relationship (Giese, 1959; Sastry, 1968). A negative correlation was observed between the gonad index and the digestive gland index of scallops (Sastry, 1970). No significant correlation was observed between gonad index and digestive gland index of *M. opima* at Tuticorin ($r= 0.4338$) and Ashtamudi ($r= 0.019$) in the present study. The

regression equation showing the relation between gonad index and digestive gland index of *M. opima* at Tuticorin was,

$$DGI = 1.4969 + 0.0735\ X$$

The same at Ashtamudi was

$$DGI = 2.6832 + 0.0043\ X$$

The condition factor of bivalves can act as an indicator of reproductive activity and the condition of clam meat is dependent on the gametogenic activity (Dix and Ferguson, 1984; Cheung, 1991). In the present study, clams of both the populations with a high condition factor value indicated matured gonadal condition and it coincided with high gonad index. A low value of condition factor during the months, when spawning activity was taking place coincided with a reduced value of gonad index. A low value of condition factor corresponding to the time of resting and spent stages was found in *Mercenaria sp.* by Hesselman *et al.* (1989). Narasimham (1988) suggested that a high value of condition factor in the post spawning periods in *A. rhombea* was probably due to the accumulation of body reserves. Condition factor and gonad index tend to be directly related in many bivalves (Ansell *et al.*, 1964; Durve, 1964; Alagarswami, 1966; Abraham, 1996). A direct relationship and positive correlation between the condition factor and the gonad index was noted in *M. opima* at Tuticorin ($r= 0.7373$) whereas, the correlation was insignificant ($r= 0.3008$) in the case of Ashtamudi population in the present study. This may be due to a lesser sampling frequency at the latter site. The regression equation showing the relation between gonad index and conditions factor of *M. opima* at Tuticorin was,

$$CF = 32.857 + 3.4152\ X$$

The same relation for Ashtamudi population was,

$$CF = 41.009 + 0.3658\ X$$

It was observed that the ratios of dry/wet meat weight of *M. opima* were low during the months of May and December at Tuticorin and it was low during March and November at Ashtamudi. It could be seen that the two

spawning periods at Tuticorin was May to June and November to December and at Ashtamudi the spawning activities occurred during March to May and November to January. The reduction in the ratio of dry/wet meat weight was observed during the same months at both the sampled population. In bivalves, spawning causes considerable changes in water content of meat. The spawning of *A. granosa* in October to November and May to July, based on depressed ratio of dry/wet meat weight was explained by Broom (1983). Hancock and Franklin (1972) reported a possible 40% reduction in dry weight of European cockle, *Cerstoderma edule*, after spawning. In edible oyster, *Crassostrea madrasensis* the meat became watery after spawning (Rajapandian and Rajan, 1987). Peddichord (1977) observed a similar phenomenon in other bivalves also. The ratio of dry weight to wet weight of Ashtamudi population was found to be lower than that of Tuticorin samples. It may be due to a lesser meat weight or higher water content of the samples collected from Ashtamudi.

Sex ratio study in *M. opima* indicated a male-female ratio with female dominance (1:1.3) at Tuticorin and male dominance (1.27:1) at Ashtamudi. Statistically Tuticorin population showed no deviation from the expected sex ratio of 1:1. But the same is not the case with Ashtamudi population. Statistical analysis proves a male dominance in the *M. opima* population at this site rather than the expected one. Several possible explanations have been forwarded to explain the inequalities in the sex ratio of bivalves. These include a small sample size, differential rates of development and differences in the maximal shell lengths for male and female bivalves (Marsden, 1999). The statistical approval of male dominance at Ashtamudi may be an after effect of a smaller sample size. This point is stressed by the fact that the observed ratio at Tuticorin is in favour of females, but still justified statistically. Nagabhushanam and Mane (1975) observed female dominance for *K. opima* in Kalbadevi estuary. Jayabal and Kalyani (1987 a) reported that males are slightly dominant in the *Meretrix meretrix* population of Vellar estuary with a male-female ratio of 1:0.98. Narasimham *et al.* (1988) observed that the male-

female ratio was 1:1.36 in *M.meretrix* of Korampallam creek, Tuticorin with slight dominance of female clams. This ratio lies very close to the sex ratio observed for *Marcia opima* at Tuticorin in the present study. Male-female sex ratio was 1.27:1 in *A. rhombea* in the Port Novo waters with male dominance (Natarajan and John, 1983). According to Narasimham (1988), females outnumbered males of *A. rhombea* in the Kakinada bay. From this observation it is evident that the sex ratio of the same species from two geographically separated areas differs from each other. The present study, which showed female dominance at Tuticorin and male dominance at Ashtamudi, agrees with the findings of Natarajan and John (1983) and also with that of Narasimham (1988). Broom (1983) suggested that the sex ratio was 1:1 in *A. granosa*. Hesselman *et al.* (1989) observed a 1:1 sex ratio in *Mercenaria sp.* at Florida. In *S. s. similis*, the observed sex ratio was 1:1 (Kanti *et al.*, 1993). A female dominance was reported in the *M. casta* population in Muttukadu backwater by Thangavelu and Poovannan (1994). A sex ratio of 1:1 was observed in *V. striatula* by Gasper and Monteiro (1998). In *E. siliqua*, there were significantly more females in smaller size classes and significantly more males in larger size classes because of the higher mortality rate in adult females than in adult males (Gasper and Monteiro, 1998). Marsden (1999) reported a sex ratio of 1.7 females to 1 male in *P. donacina*, which differs from the more usual 1:1 sex ratio in some other bivalves.

A maximum value of average oocyte diameter was observed in conjunction with the period of maturation and spawning. Minimum oocyte diameter corresponding to early development stage was observed. By reporting a same result in *Mercenaria sp.*, Hesselman *et al.* (1989) suggested that variation in oocyte diameter was the result of variability between individuals and within individuals. Sastry (1979) proposed that the average oocyte diameter and the stages of gametogenic cycles are related to each other. Lubet and allarakh (1994) reported that the young oocytes of *S. cucullata* had a diameter below 50 µm and old oocytes were generally larger than 50 µm. Lango *et al.* (2000) suggested that the larger oocytes in *C. gigas* were probably due to the

environmental and genetic differences between population which could have resulted in variations of gametogenic process for the same species.

Temperature is an important factor affecting the regulation of the gonadal cycle in a variety of marine bivalves in temperate regions (Loosanoff, 1937; Carriker, 1961; Galtsoff, 1964 and Calabrese, 1970). Loosanoff (1937) observed that temperature plays a very important role in the development of sex cells and spawning in *V. mercenaria*. Loosanoff and Davis (1952) suggested that the developing gonads show no dependency on seasonal changes in factors like light, tidal rhythms, precipitation, small variations in salinity and certain planktonic organisms. *C. islandica* in the Atlantic coast spawns when the water temperature is 13.5°C (Loosanoff, 1953). In *Mercenaria*, spawning is induced at a temperature of 20 to 25°C through out its range (Keck *et al.*, 1975). In the Indian River Lagoon, Florida, *Mercenaria sp.* spawned at a temperature range of 18.5 to 28.3°C. The incidence of gonadal neoplasia increased during periods of high water temperature, which is an indication of stressful environmental conditions (Hesselman *et al.*, 1989). Sastry (1970) reviewed that spawning was related to either rise or fall in water temperature or salinity. Broom (1983) suggested that spawning in *A. granosa* is initiated by the salinity depression. Hadfield and Anderson (1988) reported that spawning in *A. trapezia* is triggered by high water temperature and high phytoplankton density. According to Carriker (1961) the depth of water and circulation together with temperature greatly affect the onset of spawning activity in hard clams. Low temperature can delay the gametogenesis and the periodicity of spawning in *S. solidissima* (Ropes, 1968; Kanti *et al.*, 1993) and also in *P. donacina* (Marsden, 1999).

In the present study, at Tuticorin, first spawning period of *M. opima* was initiated in May when the water temperature increased by 1°C from the previous month. An increase of salinity was also observed during this period. But during peak spawning period, the temperature decreased and the salinity also decreased. During the second spawning period also the same trend was

observed. The initiation of spawning was reported with an increase of temperature and salinity and the drop in these parameters was associated with peak spawning activity. At Ashtamudi also lowering of salinity and temperature was observed during peak spawning period. In both the stations, during gametogenesis, the salinity and temperature did not show much variation. Loosanoff and Davis (1952) reported that the maturation of gonads in case of oysters is dependent on the temperature level which is different from that required for spawning.

In tropical waters, the changes in temperature during summer and winter are negligible. Salinity has been found to be the most important factor to influence the growth in marine and estuarine bivalves (Abraham, 1953; Nayar, 1955). Although the ranges in the monthly mean salinity and temperature values are not markedly high, there appear to be a distinct seasonal trend in their fluctuations, which has got a bearing on the breeding behaviour of *D. cuneatus* (Nayar, 1955). In *D. cuneatus*, initiation of spawning was preceded by a sudden drop in salinity and the peak spawning period was marked by a steady increase in temperature (Nayar, 1955). Alagarswami (1966) reported that the peak spawning in *D. faba* is probably induced by the lowering of salinity and temperature from the previous months and as the salinity values increased after the monsoon, gametogenesis and maturation takes place. These studies are in agreement with the present study. Contradictory to this, Rao (1967) opined that the active gametogenesis in *D. cuneatus* of Madras coast takes place when the temperature and salinity are low and spawning takes place when the temperature and salinity are high. Similar observation was showed in*A. rhombea* by Narasimham (1988). In *M. meretrix*, at Korampallam, Tuticorin, spawning occurred when the temperature varied over a narrow range from 25°C to 31°C and the salinity showed marked variation from 11.9 to 42.5 ‰. The absence of spawning in November to December when the salinity was less than 10 ‰ indicates that the salinity requirement for successful spawning in *M. meretrix* was above 10 ‰ (Narasimham *et al.*, 1988).

In the present study, spawning occurred at Tuticorin, when the temperature varied between 29 to 32.5°C and the salinity varied within a narrow

range from 34 to 37 ‰. Rajapandian and Rajan (1987) described that at Tuticorin, peak period of spawning in edible oyster, *C. madrasensis* may be attributed to the high diurnal temperature variation during April to May which is considered as a reason for spawning. The peak period of spawning at Ashtamudi during November to January for *M. opima* might be due to favourable salinity after the monsoon season as noted for *K. opima* of Kalbadevi estuary by Nagabhushanam and Mane (1975). The range of temperature and salinity preferred for the spawning activity at Ashtamudi was greater than that of Tuticorin. During the spawning period, at Ashtamudi, the temperature ranged between 26 to 34°C and the salinity varied between 26 to 30 ‰. Below this range of salinity and temperature no spawning activity was reported at both the stations. This may be due to the retardation of metabolic and sexual activities at higher or lower salinity and temperature. It can be concluded that an optimum range of temperature and salinity is required by *M. opima* of both the populations for successful reproductive activity. Retardation of growth rate in bivalves due to low salinity is known in Indian waters (Mane, 1974; Narasimham, 1988). In the present study, when the environmental parameters such as temperature and salinity were tried to correlate with the gonad index, a negative correlation was observed for the clams of both the populations. This shows that a high temperature and hypersaline condition may inhibit the growth and maturation in the clams, *M. opima*. Many authors have reported similar results. Water with high salinity and high temperature caused mortality of adult clams of *D. cuneatus* (Nayar, 1955). Durve (1964) also reported that the hypersaline water in the marine fish farm may perhaps a reason for the retardation of sexual activity in *M. Casta*. Mane (1974) suggested that low salinity during monsoon season is causing retardation of growth in *K. opima* in the Kalbadevi estuary at Ratnagiri and gradual rise in salinity accelerated growth whereas the high salinity and high temperature resulted in moderate growth. Nagabhushanam and Mane (1975) reported that the feeding process in *K. opima* at Kalbadevi estuary slows down, because, for the majority of the period, valves remained closed in the very low salinity of the estuary resulting in very slow metabolic rate. Jayabal and Kalyani (1987 a) explained that the absence of spawning in bivalves during monsoon time is due to the influence of salinity.

CHAPTER 3

INDUCED MATURATION

3.1 INTRODUCTION

Induced maturation of clams plays a vital role in viable seed production in the hatchery. The study of gonad growth and dynamics of its index with relation to gametogenic stages and its influence on meat condition of the clam is very useful both for natural seed collection and harvesting the clams.

Temperature has been considered to be the most important factor in regulating the breeding period in marine invertebrates (Runnstorm 1927). Experimentally, the gonads of *Crassostrea virginica* and *V. mercenaria* were ripened in the winter and the larvae were reared in the laboratory (Loosanoff and Davis, 1950 and 1952). Sastry (1966) established the role of nutrient reserves in gonad growth and gametogenesis. Sastry (1968) reported that gonad growth and gametogenesis are initiated in bay scallops, *Acquipecten irradians* when the animals are exposed to a minimum threshold temperature with food. The same study also reported that exposure of bivalves to threshold temperature combined with proper supply of food were essential for attaining optimum gonadal growth and gametogenesis. Effect of temperature on physiological processes in marine animals was reviewed by Kinne (1971), Newell (1980) and Newell and Branch (1980). Nayar *et al.* (1987) stated that the oysters when intensively fed with mixed algae tend to have mature gonads and they served as brood stock. Laing *et al.* (1987) studied the effect of diet and temperature on the growth of juvenile clam species such as *Tapes decussata, T. semidecussata* and *M. mercenaria*.

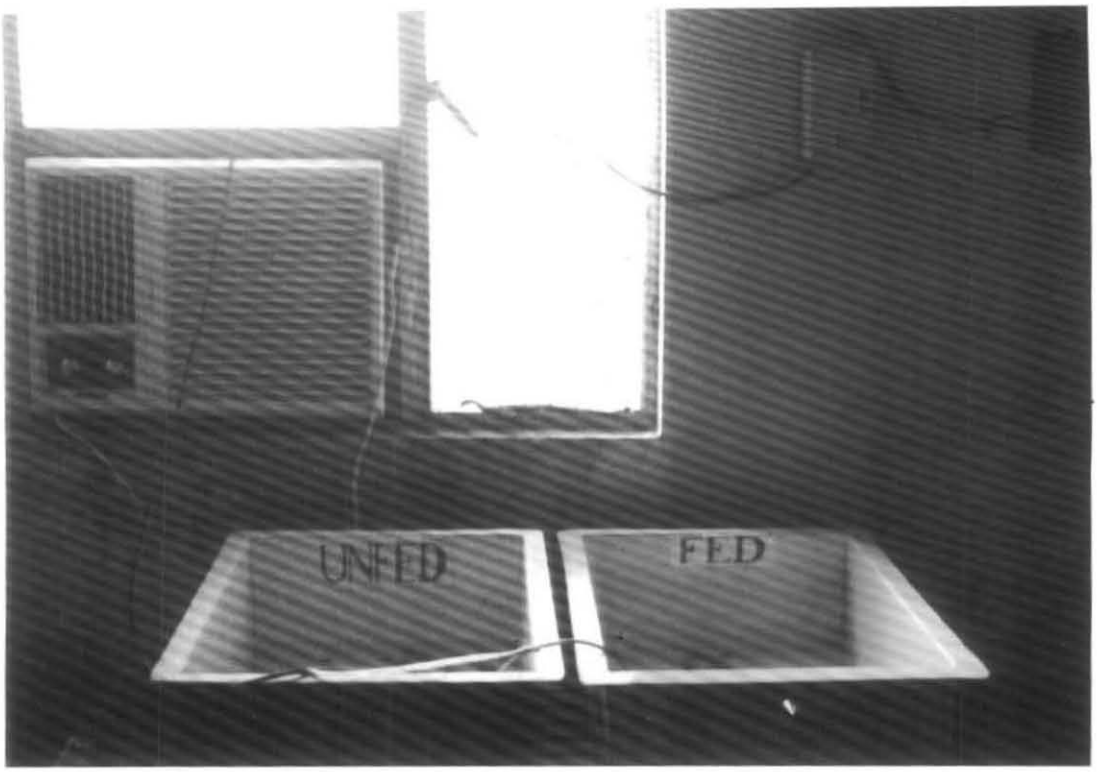
Sastry (1979) listed out various factors affecting the gametogenesis in bivalve molluscs. Gonad development had been induced in temperate molluscs outside their normal breeding period by exposing them to appropriate temperature and feeding regimes (Loosanoff and Davis, 1952; Sastry and Blake, 1971). They also suggested that the nutrient reserves were transferred apparently from the digestive gland to the gonad and utilized for developing gametes in the synthesis of various biochemical constituents. Effect

of temperature and ration on gametogenesis and growth in the tropical mussel *Perna perna* was studied by Velez and Epifanio (1981). Albentosa *et al.* (1994) suggested the optimal thermal conditions for growth of the clam seeds of *Venurupis pullastra*. Abraham (1996) explained the relationship between gonad index, digestive gland index and condition factor in an experiment conducted to induce the maturation of edible oyster *C. madrasensis*.

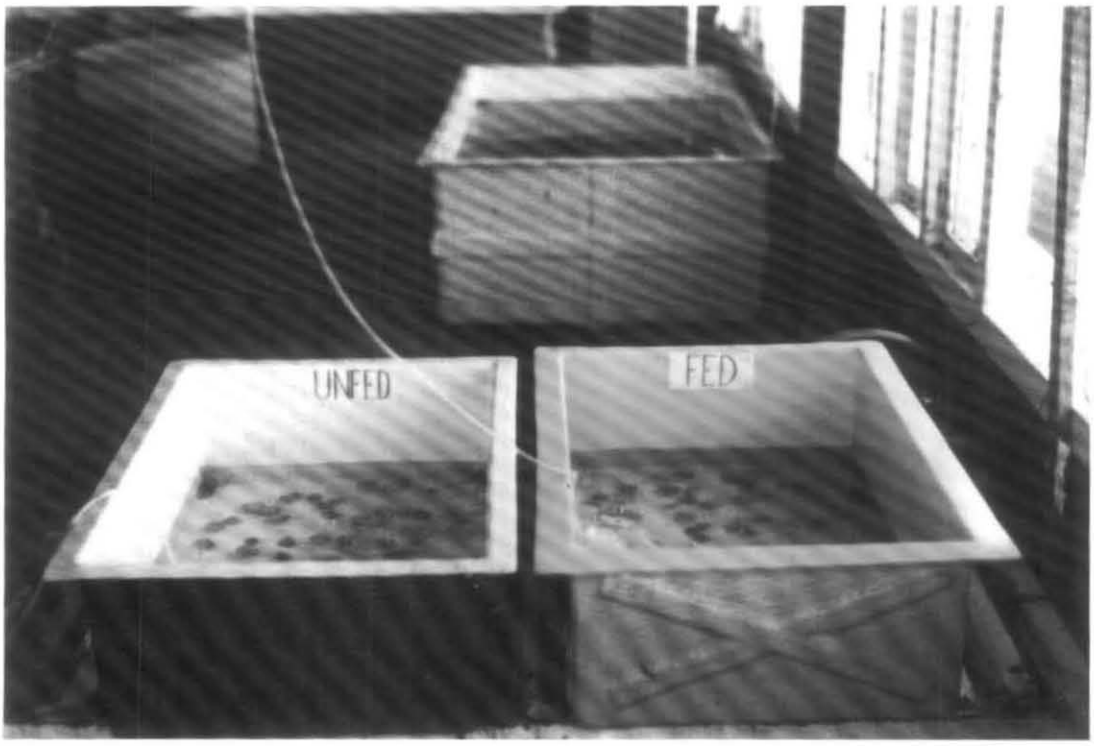
The effect of temperature on the gonadal maturity of clams has not been attempted in tropical countries, whereas in temperate regions considerable studies have been made on the induced maturation of clams. Since no information is available on the role of temperature and food on gonadal growth and gametogenesis of *M. opima*, certain studies were taken up to induce the maturation activity in the species with temperature and feeding regime as stimulating factors. This information will be useful for effective induced maturation of gonad in conditioning the brood stock in clam hatcheries.

3.2 MATERIALS AND METHODS

For studying the combined influence of feed and temperature on gonad growth and maturity, experiments were conducted for a period of 45 days at temperatures of 23°C and 28°C (Plate VII). Brood stock size clams were selected and made four sets of 20 clams each. Clams were kept in rectangular FRP tanks of 100 L capacity. Two sets were taken to the temperature of 23°C, maintained in a room of 4.5x3.25 m size with the help of an air conditioner. Out of these, one treatment was left unfed. The 28°C treatments were kept at the room temperature in the wet laboratory. There also one treatment was left unfed. Other sets were fed with cultures of *Isochrysis galbana* and *Chaetoceros sp.* Gentle aeration was provided for all the set of clams. Water was exchanged daily. Seawater, filtered through charcoal and sand filter was used. After water change the clams were given *Isochrysis* and *Chaetoceros together* at the rate of one m cells/ animal/ day. For the unfed clams, the seawater only changed. Once in 15 days interval, five clams from each tank were taken at random and their gonad index, digestive gland index, condition factor and oocyte diameter were studied and mean values were calculated.



TREATMENTS AT 23^0 C



TREATMENTS AT 28^0 C

PLATE VII. EXPERIMENTAL SET - UP FOR INDUCED MATURATION

Algae were cultured in separate FRP tanks of 150 L capacity as per the culture procedure followed by Gopinathan (1982). The average cell concentration varied from 1.5 - 1.8 m cells / ml. Daily, required quantity of algae were harvested and supplied at the rate of 1.5 L / clam as feed. The culture was continued using subcultures.

To compare the experimental trials, every fortnight five clams from each experimental set up were studied for their average values of indices of gonad and digestive gland and also for their condition factor. The average oocyte diameters of the maturing and matured clams of each trial were observed. The experiment was conducted for a time period of forty-five days. A comparative analysis of these parameters was done between the experimental trials to estimate the best conditions required for inducing the gonad maturation of *M. opima*.

3.2.1 STATISTICAL ANALYSIS

Two way Analysis of variance (ANOVA) was done on the effect of the two experimental temperature settings (23°C and 28°C) on the gonads of fed and unfed clams using Excel computer software. Two - way ANOVA was also done to analyze the effect of feed on the gonad index values of *M. opima* at 23°C and 28°C.

3.3 RESULTS

Before the start of feeding experiment at different temperatures, five clams from the stock were analysed for their gonad index, digestive gland index and condition factor. The mean value of gonad index was 13.7 ± 1.6 and digestive gland index was 3.0 ± 0.7. The average condition factor was 83.2 ± 6.9. All the clams were in maturing stage with mean oocyte diameter of 22.0 µm.

3.3.1 Condition of clams at 23°C

Every 15 days the condition of five fed and unfed clams were studied, up to 45 days. The study on condition showed marked difference in the fed and unfed treatments. The gonad index of fed clams showed tremendous progression, while that of unfed clams showed a declining trend. The gonad index of fed clams increased to 14.8 ± 1.0 by 15th day and then to 16.1 ± 1.2 after 30 days and finally it reached 17.1 ± 1.0 after 45 days. On the contrary, the unfed treatment showed lower gonad index values of 13.8 ± 0.6, 12.8 ± 2.5 and 12.6 ± 1.9 after 15th, 30th and 45th days respectively.

The average values of condition factor also followed the same progression in fed clams. After 15 days of experiment, the condition factor was 83.3 ± 5.71 and it rose to 89.2 ± 3.7 by 30th day and then to 92.1 ± 5.7 by 45th day. For the unfed treatment, the condition factor declined steadily from the initial value to 75.4 ± 5.8 after 15 days and then to 60.2 ± 13.0 and 58.7 ± 7.7 after 30th and 45th day respectively.

The digestive gland index of fed and unfed clams showed great difference. For the fed clams, the digestive gland index showed constancy at a value of 3.1 ± 0.8 after 15th, 30th and 45th days' observations, throughout the experimental period. The digestive gland index of unfed clams lowered to 2.7 ± 0.23 by 15th day and to 2.3 ± 0.2 after 30 days and finally to 2.1 ± 0.1 after 45 days.

The oocyte diameter remained at the same value as that of the initial value even after 15 days, which rose to 28.6 ± 6.0 µm after 30 days and then to 46.2 ± 9.2 µm after 45 days in the fed clams, showing proximity to ripeness. In the unfed clams, the oocyte diameter was 24.2 ± 4.9 µm after 30 days of conditioning. It did not show any increase even after 45 days in the unfed treatment.

3.3.2 Condition of clams at 28°C

The condition of clams at 28°C also was tracked at similar intervals as in the case of 23°C treatment. The condition of fed clams showed marked improvement, while that of unfed clams showed much decrease at 28°C. The average gonad index was 14.6 ± 1.5 and 15.5 ± 0.9 at 30th and 45th days respectively for the fed group of clams. The gonad index of unfed clams decreased to 11.8 ± 1.2, 11.5 ± 1.9 and to 11.0 ± 1.9 at the end of 15th, 30th and 45th days period respectively. The condition factor also showed improvement in fed clams by a raise from the initial value to 88.8 ± 4.2 at 15th day, 89.8 ± 5.7 at 30th day and 92.3 ± 3.0 at 45th day. But the condition factor of unfed clams showed a decreasing trend with the values, 65.9 ± 3.0, 63.9 ± 13.2 and 65.2 ± 5.9 respectively for the same time periods. The digestive gland index at the end of 30th day for the fed clams was 2.9 ± 0.3, but the value was 2.2 ± 0.2 for the unfed ones. It rose to 3.1 ± 0.4 in fed clams by 45th day but declined in unfed clams to 2.0 ± 0.4 at the same time.

The oocyte diameter of fed clams at 28 °C on 15th day was at 26.4 ± 4.9 whereas that of unfed clams was 24.2 ± 4.9 µm. The oocyte diameter started signs of maturity and it rose to 37.4 ± 6 µm on the 30th day in fed clams. But in the unfed ones it was 26.4 ± 4.9 µm. By 45th day, the oocyte diameter of the fed clams reached a peak value of 46.2 ± 9.2 µm showing maturity. The unfed clams were represented by an oocyte diameter of 26.4 ± 6 µm on 45th day. Variations of gonad index, condition factor and digestive gland index between fed and unfed treatments at 23°C and 28°C for a time period of forty five days is given in Table 3a.

Statistical analysis showed significant difference between unfed treatments at 23°C and 28°C (P=0.026) (Table 3b). No significance was noted on the effect of feed on both the treatments (Tables 3c and 3d). Similarly, no significant difference was noted between fed treatments at 23°C and 28°C (Table 3e).

Table 3a. Variations of GI, CF and DGI between fed and unfed treatments at 23°C and 28°C

Temperature	Days	Unfed			Fed		
		GI	CF	DGI	GI	CF	DGI
23° C	0	13.7±1.6	83.2±6.9	3.0±0.7	13.7±1.6	83.2±6.9	3.0±0.7
	15	13.8±0.6	75.4±5.8	2.7±0.2	14.8±1.0	83.3±5.7	3.1±0.3
	30	12.8±2.5	60.2±13.0	2.3±0.2	16.1±1.2	89.2±3.7	3.1±0.5
	45	12.6±1.9	58.7±7.7	2.1±0.1	17.1±1.0	92.1±5.7	3.1±0.8
28° C	0	13.7±1.6	83.2±6.9	3.0±0.7	13.7±1.6	83.2±6.9	3.0±0.7
	15	11.8±1.2	65.9±3.0	2.5±0.4	14.6±1.5	88.8±4.2	3.2±0.4
	30	11.5±1.9	63.9±13.2	2.2±0.2	15.5±0.9	89.8±5.7	2.9±0.3
	45	11.0±1.9	65.2±5.9	2.0±0.4	18.1±0.9	92.3±3.0	3.1±0.4

GI - Gonad Index

DGI - Digestive Gland Index

CF - Condition Factor

Table 3b. Two way ANOVA on the effect of temperatures (23° and 28° C) on GI for unfed treatments

Source of Variation	*SS*	*df*	*MS*	*P-value*	*F*
Between days	0.60	2.00	0.30	0.32	2.15
Between Temperature	5.06	1.00	5.06	0.026*	36.61

Table 3c. Two way ANOVA on the effect of feed on GI at 23° C

Source of Variation	*SS*	*df*	*MS*	*P-value*	*F*
Between days	0.871316	2	0.435658	0.729181	0.371402
Between feed	11.41812	1	11.41812	0.089201	9.73404

Table 3d. Two way ANOVA on the effect of feed on GI at 28° C

Source of Variation	SS	df	MS	P-value	F
Between days	1.887489	2	0.943745	0.721365	0.386261
Between feed	32.36868	1	32.36868	0.067886	13.24804

Table 3e. Two way ANOVA on the effect of temperatures (23° and 28° C) on GI for fed treatments

Source of Variation	*SS*	*df*	*MS*	*P-value*	*F*
Between days	8.436101	2	4.218051	0.074914	12.34869
Between Temperature	0.003651	1	0.003651	0.927093	0.010688

* Significant at $P < 0.05$

GI - Gonad Index

3.4 DISCUSSION

Induced maturation and conditioning plays vital role for mass production of seed throughout the year. Comparative studies have taken place proving the effect of environmental parameters and feed on the maturation and spawning of molluscs. The present study also throws some light into the effect of feed and temperature in the induced maturation of gonads in *M. opima*.

From the results of the study, a comparison of the experimental trials was done to choose the best one for inducing the maturation of *M. opima*. It was observed that the indices of gonad and digestive gland, condition factor and oocyte diameter which are the indicators of reproductive activity showed variation in their values at 23°C and at 28°C and also between the fed and unfed trials. There was gradual increase in the indices and in the oocyte diameter of the fed clams at both the temperature whereas they showed a declining trend in the unfed clams. The fed clams always showed a tendency to mature faster than the unfed ones. As suggested by Sastry (1968), the nutrients from the feed are stored in the digestive gland and utilized for the gonadal growth. This shows that proper quantity of food with optimum nutrient value is essential for maturation and gonad development. When diets of good to moderate food value were fed to *T. semidecussata* juveniles, along with an increase of temperature to 25°C, the animals grew faster because of the availability of more energy. When food was absent, the clams suppressed their oxygen consumption in the conservation of energy and the maintenance of some growth (Laing *et al.*, 1987). Thus, in the unfed clams, the lack of sufficient nutrients could suppress the gonadal activities and as a result the maturation also.

Within limits, a temperature increase accelerates most physiological processes, including feeding activity, metabolism and growth (Kinne, 1971; Newell and Branch, 1980). Kinne (1971) suggested that individuals could modify the pattern of response to temperature by means of adaptation,

which ultimately results in an increase in its capacity to survive, reproduce or compete under the new conditions. According to Newell (1980), four potential mechanisms by which bivalves may respond to an increase in the temperature of habitat include an increase in food consumption, an increase in absorption efficiency, suppression of energy losses through respiration and a combination of these three. The optimum temperature required by any animal for its maturation, changes among the species and also within the species (Albentosa *et al.*, 1994). In the present study, it was observed that the clams attained faster maturity at 28°C than that at 23°C. Since the maximum value of gonad index and condition factor is associated with the ripe stage of gonad, faster maturity was proved by comparing the values of gonad index, digestive gland index, condition factor and oocyte diameter of the clams kept at 23°C and 28°C. Although there was increase in the indices values for the fed clams at 23°C, maximum value was reported in the fed clams at 28°C. According to Loosanoff and Davis (1950), the clams *V. mercenaria* were induced to mature by increasing the temperature to 20 - 22°C at Long Island Sound.

When statistical analysis was done, it was observed that there was no significant difference between the fed treatments at 23°C and at 28°C. But the unfed treatments kept at two different temperatures showed significant difference. It could be inferred that in unfed treatments, temperature plays a major role in deciding the gonad development of animals.

It is well known that bivalve feeding rates are greatly influenced by temperature. Growth efficiency of clams can be modified by diet as well as temperature. An optimum temperature and good quality feed can enhance the growth and maturation in clams, since the chance for loss in percentage lipid content is less (Laing *et al.*, 1987). The attainment of ripe condition of clams at 30^{th} day in the present study was in agreement with induced maturation studies conducted by Nayar *et al.* (1987) in oysters.

CHAPTER 4

SPAWNING AND LARVAL REARING

4.1 INTRODUCTION

In the hatchery production of seeds, the induced spawning technique facilitates to have a continuous production of seed through out the year. Several methods to induce spawning in bivalves are increasing the temperature, altering the salinity, addition of gametes or phytoplankton and also by using chemicals like KCl, NaCl and NH_3 (Ino, 1972; Daniel *et al.*, 1977; John and Strehlow, 1983; Gibbons and Castagna, 1985; Honkoop *et al.*, 1999). The induced spawning technique in clams dates back to 1912, when Belding tried to raise the larvae of *V. mercenaria*, in the laboratory, prior to the settling stage. Wells (1927) succeeded in growing clam larvae to the settling stage. Jorgensen (1946) explained the reproduction and larval development of Danish marine bottom invertebrates. Loosanoff and Davis (1950) conducted induced spawning experiment of *V. mercenaria* in winter, by placing the conditioned clams in trays of running seawater having a temperature 5.0 to 7.0°C and then gradually raising the temperature up to 34°C, at intervals of 3 to 5 days. Loosanoff and Davis (1963) studied the rearing of bivalve molluscs. Walne (1974) detailed the culture of marine bivalve larvae. Chanley (1965) reported the larval development of the brackish water mactrid clam, *Rangia cuneata*. Loosanoff *et al.* (1966) reported the dimensions and shapes of larvae of marine bivalve molluscs. The larval rearing of clam *M. mercenaria* has been documented by Castagna and Kraeuter (1981). Alagarswami *et al.* (1983 and 1987) reported the larval rearing and spat production of pearl oyster *Pinctada fucata*. Bacterial diseases in bivalve larval culture and their control were studied by Brown (1983). Nayar *et al.* (1984 and 1987) studied the larval rearing and spat production of *C. madrasensis* in the hatchery system. Laing and Millican (1986) reported the relative growth and growth efficiency of *Ostrea edulis* spat fed with various algal diets. From Malaysia, Wong *et al.* (1986) gave an account on induced spawning, larval development and juvenile growth of *A. granosa*.

The clam *M. meretrix* was induced to spawn in the laboratory by rapid salinity changes and addition of sperm suspension. The clam eggs were fertilized and the larvae were reared up to metamorphosis (Kalyanasundaram and Ramamoorthi, 1987). Narasimham *et al.* (1988) successfully reared the larvae obtained through induced spawning of *M. meretrix* in the laboratory by giving thermal shock and the larvae were reared to spat. Castagna and Manzi (1989) developed the larvae of *K. rhytiphora* and *A. trapezia.* Utting and Spencer (1991) tried the hatchery culture of bivalve molluscs' larvae and juveniles. Larval rearing, spat production and juvenile growth of blood clam *A. granosa* were illustrated by Muthiah *et al.* (1992). Nell *et al.* (1994) attempted rearing of *K. rhytiphora* in Australia. Azcona *et al.* (1996) established the induced spawning and larval rearing of *D. trunculus* by thermal induction at 25°C and additional stimulation by adding gametes stripped from one of the conditioned clams. Shafee *et al.* (1998) conducted culture of carpet shell clam, *R. decussatus* on the Atlantic coast of Morocco. The present study report is the first on the larval rearing and spat production of *M. opima.*

Salinity is one of the important factors that affect the distribution of a species in an ecosystem. Knowledge of salinity tolerance of commercially important bivalves is of prime importance for taking up culture experiments. With the development of the hatchery technology for the production of the clam seed, nursery management of the seed assumes significance as a prelude for understanding programmes connected with field culture of sea ranching of clams. Of the various factors, salinity plays a prominent role affecting growth and survival in the sea ranching of the post-set clams to stocking size.

Although the information on molluscan resources along the Indian coast is available (Abraham, 1953; Ranade and Kulkarni, 1972; Rao, 1951 a), investigations on salinity tolerance, which is an essential pre-requisite for aquaculture, are very few. Turner and George (1955) observed the changes in the early larvae of *V. mercenaria* when introduced to diminishing salinities.

Estuarine animals are subjected to various biotic changes, the chief factor being salinity. For the nursery management of any species, practical knowledge on the optimum salinity requirements is very much significant.

Davis (1958) studied the survival and growth of clam *V. mercenaria* and oyster *C. virginica* larvae at different salinities. The adaptation of different animals to different salinities was explained by Kinne (1967). Chen (1984) studied the recent innovations in molluscs in Taiwan, with special reference to the small abalone *Haliotis diversicolor* and the hard clam *M. lusoria*. The response and survival of blood cockle *A. granosa* at varying salinity ranges was explained by Davenport and Wong (1986). Namaguchi and Tanaka (1987) studied the effect of temperature and salinity on growth of early young clams of the hard clam *M. lusoria*. Salinity tolerance of the venerid clam *P. malabarica* was studied by Ram Mohan and Velayudhan (1993). Sundaram and Shafee (1994) investigated the salinity tolerance of the clam *M. meretrix*, the green mussel *Perna viridis* and the oyster *C. madrasensis* of Ennore estuary. Muthiah *et al.* (unpublished) conducted an experiment to determine the effect of salinity on growth and survival of hatchery produced juvenile clams of *M. meretrix* and *A. granosa*. The present investigation was taken up to determine optimum salinity requirements for the juveniles of *M. opima*.

4.2 MATERIALS AND METHODS

4.2.1 LARVAL REARING

The clams of length ranged between 30.7 - 50.8 mm were collected from Ashtamudi Lake, Quilon. The salinity of the collection site was 27 ‰ and the temperature was 27°C. The clams were brought to the shellfish hatchery at Tuticorin Research Centre of CMFRI. They were kept in a plastic trough containing water, having salinity 32 ‰ and temperature 31°C. Sufficient aeration was provided. Spawning occurred on the same day. After spawning the clams

were removed. The fertilized eggs were collected in 40 μm sieve to remove excess sperms and debris. Sand filtered seawater was supplied to the rearing tank through a hose, the delivery end of which was plugged with surgical cotton. The water was changed completely on alternate days and half the volume of water was replaced on the days preceding complete water change. Gentle aeration was provided to the rearing tank. Periodically 20 larvae/spat preserved in 1% formalin were measured for length in the anterior - posterior axis and for breadth in dorso - ventral axis. The averages of these measurements were given for different growth stages. *I. galbana* was given as food once a day after determining the cell concentration with haemocytometer. During the trial, the water temperature in the tank ranged between 28°C and 32.5°C and salinity from 34 - 36 ‰.

4.2.2 GROWTH AND SURVIVAL OF *M. opima* SPAT

Thirty-four days old, 240 juveniles from same brood of clam *M. opima* with a mean length of 2.09 mm reared in the hatchery, were divided equally into 12 groups. They were exposed to salinities from 10 to 40 ‰. Twenty seed clams for every salinity treatment were kept in 2 containers (Plate VIII). Thus the experiment was carried out in duplicate for every salinity treatment. The salinity of each water sample was checked by using an Atago refractometer. The lower salinities (10 to 25 ‰) were obtained by addition of sufficient quantity of freshwater to seawater of known salinity and the higher salinity of 40 ‰ was made by addition of required quantity of common salt to seawater. The salinity treatments selected for the study were 10, 15, 20, 25, 36 ± 1 (normal seawater) and 40 ‰. The juveniles were transferred straight to 20 L plastic containers with appropriate medium. Water was changed daily and the dead clams, if any, were removed. Daily 500 ml of *I. galbana* was given as feed. Gentle aeration was provided in each container. The duration of experiment was 30 days. On 15th day and 30th day the survived seed clams from each basin were measured for their length, nearest to 0.01 mm with ocular micrometer. The mean length was calculated for statistical analysis.

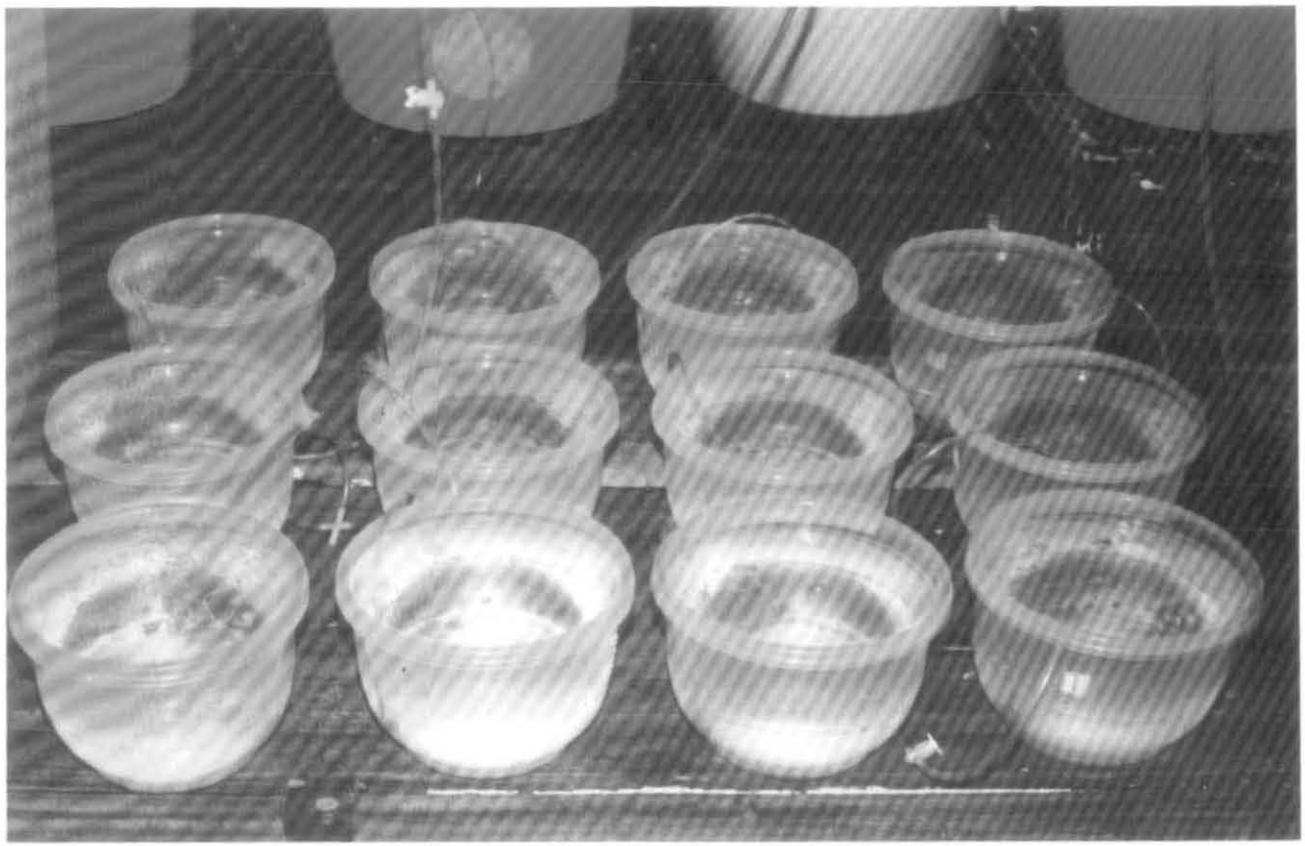


PLATE VIII. A view of experimental set-up on salinity tolerance of *M. opima* spat

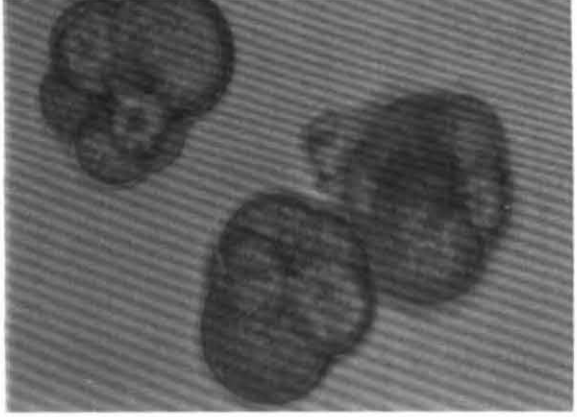
4.2.3 STATISTICAL ANALYSIS

Statistical analysis of the arcsine transformed percentage survival data of *M. opima* spat at different salinities was done through one-way Analysis of Variance (ANOVA) with Excel computer software. Two - way ANOVA was done to analyse the actual growth rate of *M. opima* spat in different salinity treatments using Excel computer software. In all the cases, when the F-value of the treatments was significantly different, the best treatment was found out through pair-wise comparison (Students t-test at $P < 0.05$) of treatment means.

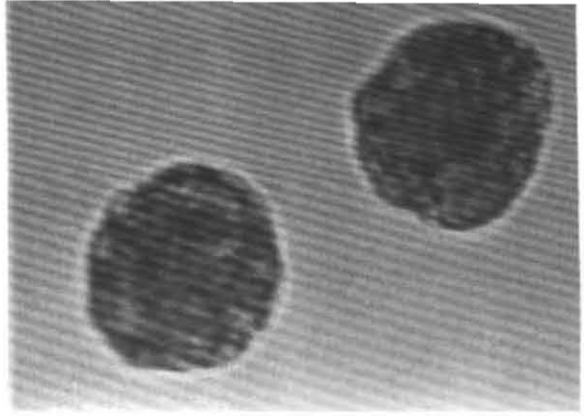
4.3 RESULTS

4.3.1 LARVAL REARING

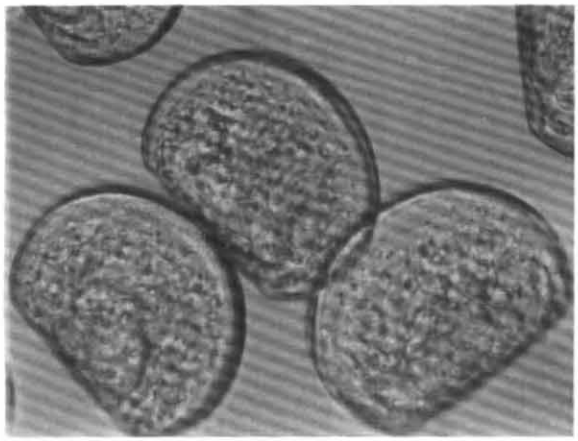
As the embryonic and larval stages of *M. opima* are similar to other bivalves (Alagarswami *et.al.* 1983; Nayar *et al.*, 1987; Narasimham *et al.*, 1988 a; Muthiah *et al.*, 1992) detailed description of the larval stages are not dealt with. The eggs were spherical and measured 50 - 60 µm in diameter. Fertilization occurred immediately and soon after the eggs became opaque. The fertilized eggs measured 65 µm on an average. The polar body extruded within 10 to 15 minutes afte fertilization followed by the first cleavage of the cell into two unequal halves in 30 minutes. During the second division, four blastomeres were formed in 10 minutes after the first cleavage and in the third division, 8 - celled stage resulted 10 minutes after the second division (Plate IX). Further divisions resulted in multi cellular stage and after passing through the blastula and gastrula stages, the morula larvae developed within 2 - 4 hours. Trochophore larvae of size 70.4 µm x 56.1 µm were attained in 7 hours and the larvae started moving by lashing the terminal flagellum. The larvae reached straight hinge stage with a well-developed ciliated velum in 20 - 24 hours. On the first day the D-shaped larvae with a minimum size of 77 µm in length and 55 µm in height, and maximum size of 110 µm x 99 µm with an average of 91.3 µm x 75.9 µm was observed. They were fed with *I. galbana* at 5,000 cells / larva/ day.



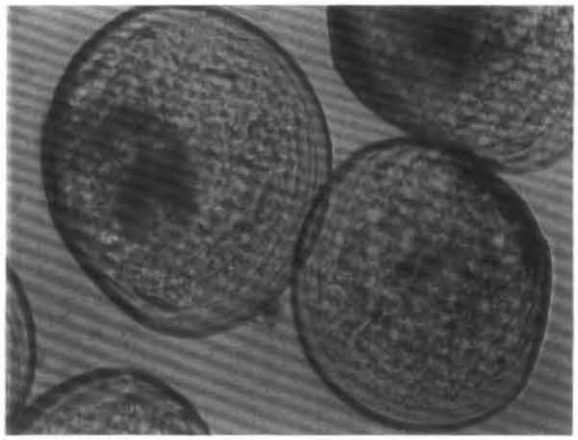
8 - CELLED STAGE



MORULA

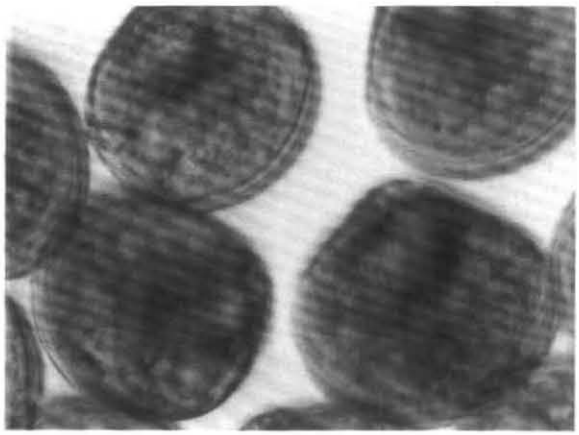


D - SHAPED LARVA

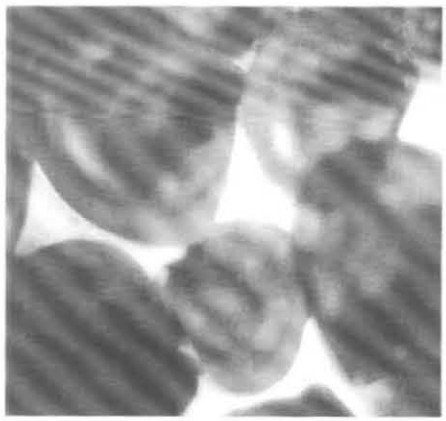


EARLY UMBO

PEDIVELIGER



SETTLED SPAT



34 DAYS OLD SPAT

PLATE IX. LARVAL STAGES OF *MARCIA OPIMA*

On 4th day, the straight hinge larvae attained an average size of 115.5 µm x 92.13 µm. Early umbo was observed on 5th day and the larvae measured 147.4 µm x 130.35 µm. The feeding was increased to 8,000 cells / larva/ day. On 8th day the larvae reached the size of 214.5 µm x 198.0 µm. On 9th day, some of the larvae developed foot and on day 12, majority developed foot, marking the advent of pediveliger stage. Settlement started on 9th and 10th day at a larval length of 225 µm. Settlement completed on 11th day and at this stage the average size of the larvae was 272.8 µm x 259.6 µm. The algal food was then increased to 10,000 cells / larva / day.

The relationship between length and breadth of the entire larvae is linear and is described by the following equation,

$$H = -11.997 + 0.9421\ L$$

The exponential form is described by the equation,

$$H = 35.279\ e^{0.0077L}$$

Where, 'L' and 'H' are length and breadth in microns. For these parameters, the correlation coefficient 'r' between these parameters showed significance with a value of 0.8817 (Fig. 5).

The average relationship between length and breadth of the larvae is also linear and is described by the following equation,

$$H = -10.333 + 0.9445\ L$$

The exponential form is described by the equation,

$$H = 37.175\ e^{0.0077L}$$

For these parameters, the correlation coefficient 'r' = 0.9970 which shows high degree of significance (Fig. 6).

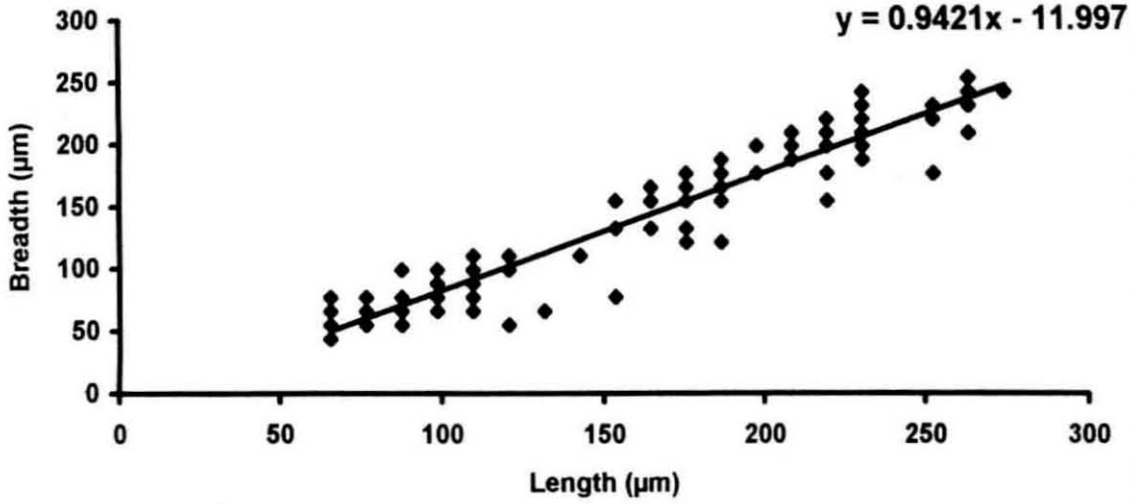


Fig. 5. The relationship between length and breadth in the larvae of *M. opima*

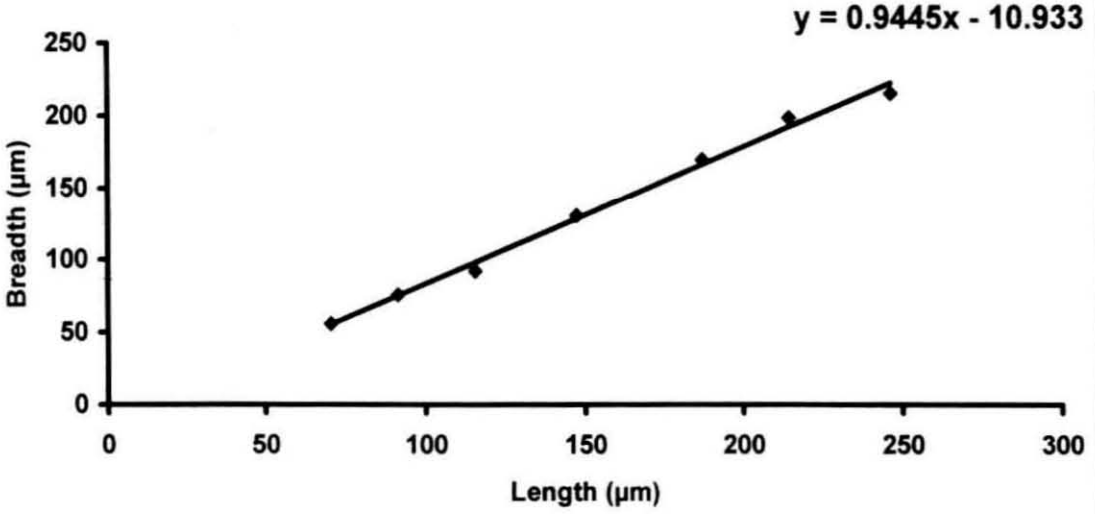


Fig. 6. Average length - breadth relationship of larvae of *M. opima*

On day 14, the size of the post set clams was 294.25 µm x 277.75 µm, on day 17 it grew to 404.3 µm x 375.1 µm and on day 20, it was 499.4 µm x 469.3 µm. The feed concentration was increased from 10,000 cells to 12,000 cells/spat from day 10 to 20. The food was increased to 15,000 cells / spat/ day on day 25 and it was further increased to 20,000 cells / spat/ day on day 40. On day 27, the average size of the spat was 0 .917 mm x 0.837 mm. On day 34, the maximum size of the spat was 1.62 mm x 1.45 mm, minimum 1.10 mm x .93 mm with an average of 1.39 mm x 1.29 mm. From day 40 onwards the food was increased to 25,000 cells / spat / day.

On day 41, the spat measured 2.01 mm x 1.71 mm, on day 48, 2.33 mm x 1.86 mm, on day 63, and 2.37 mm x 1.93 mm and on day 75, the clam seed attained a minimum size of 1.80 mm x 1.55 mm, maximum size of 3.65 mm x 3.15 mm with an average of 2.81 x 2.37 mm.

The growth of post-set clams in the hatchery was linear and could be described by the following equation.

$$Y = 0.0619 X - 0.9454$$

Where, 'Y' is the length in mm and 'X' is the breadth in mm after 82 days of spawning. The correlation coefficient value 'r' was 0.9698, which indicates high degree of significance (Fig. 7).

4.3.2 EFFECT OF SALINITY ON GROWTH AND SURVIVAL OF *M. opima* SPAT

The initial average length of juvenile clams used for experimental trials was 2.09 mm. Growth and survival was records on 15th and 30th day from the experimental trials showed that the juvenile clams attained highest growth

rate at 25 ‰, followed by 20 ‰. On 15th day, the clams had a maximum size of 6.36 mm and a minimum size of 5.06 mm with an average size of 5.80 mm in 25 ‰. On 30th day the same juvenile clams attained a minimum size of 5.27 mm, maximum of 7.00 mm with an average of 6.08 mm. At 20 ‰ salinity, the spats attained a size of 5.58 mm within thirty days. The slowest growth rate was recorded at 10 ‰ and 40 ‰, where the average size was 4.97 mm after thirty days. At 15 ‰ and in normal seawater salinity (36 ± 1 ‰) the growth rate was moderate, where the juvenile clams attained an average growth of 5.21 mm within thirty days.

On 15th day the survival rate was 100% in the treatments of 10,15,20,25 and 40 ‰ salinities. But it was 95% at normal seawater salinity (36 ± 1 ‰). The observation on 30th day showed that, at 20 ‰ salinity also the survival rate was decreased to 95%. In the other treatments survival rate remained at 100%. The growth rate and survival of spats for different salinities at which the experiment was conducted is given in Table 4a. Effect of salinity on growth and survival of *M. opima* spat is shown in Fig. 8.

Single factor Analysis of Variance on the effect of salinity on the survival of *M. opima* spat revealed no significance between the treatments (Table 4b). Whereas, significant difference between the treatments was observed on the effect of salinity on the growth rate of *M. opima* spat as done by two – way Analysis of Variance (Table 4c). When pair comparison of growth rate means were done by way of Students t-test, significant difference was noticed between salinity treatments 10 and 20 ‰, 10 and 25 ‰, 20 and 25 ‰, 20 and 40 ‰, 25 and 36 ‰, and 25 and 40 ‰ at the end of the study period. The best treatment selected was 25 ‰ with a growth rate of 0.13 mm / day.

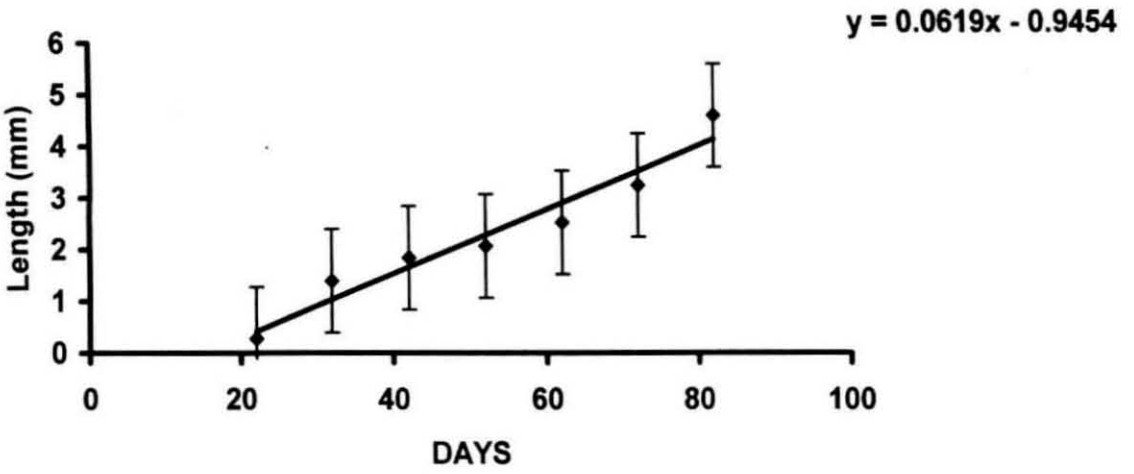


Fig. 7. Growth of the spat of *M. opima* in the hatchery. Vertical lines represents the length range and the solid circles represents the mean length

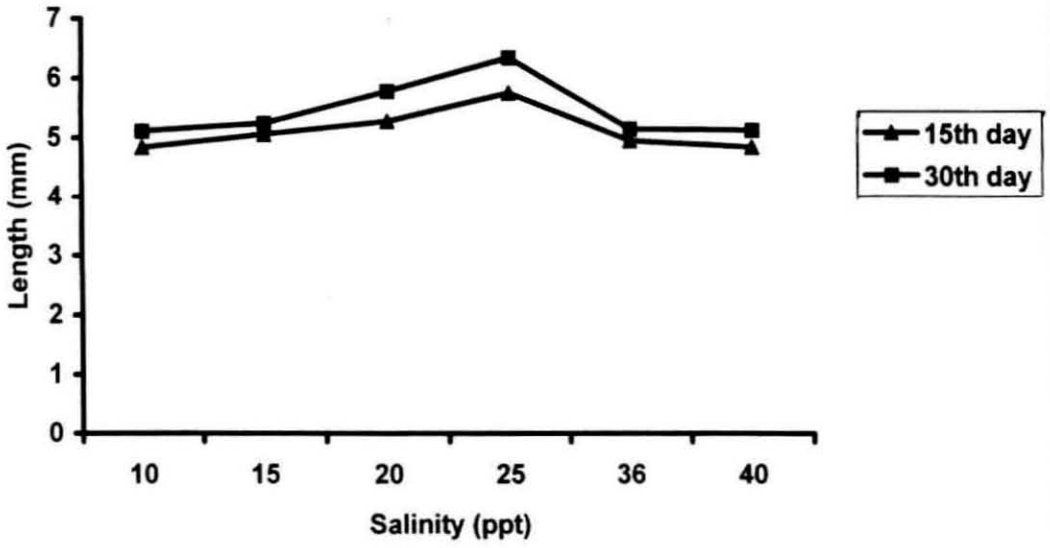


Fig. 8. Effect of salinity on the growth of *M. opima* spat.

Table 4a. Average growth rate and survival of *M. opima* spat at different salinity treatments

	Salinity	10	15	20	25	36±1	40
Growth rate	Day 15	0.18	0.2	0.21	0.24	0.19	0.18
	Day 30	0.10	0.10	0.12	0.13	0.10	0.10
Survival %	Day 15	100	100	100	100	95	100
	Day 30	100	100	95	100	95	100

Table 4b.Single factor ANOVA on the effect of salinity on the survival of *M. opima* spat

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	326.67	5.00	65.33	0.02	1.00	4.39
Within Groups	16828	6	2804.67			

Table 4c. Two- way ANOVA on the effect of different salinties on growth of *M. opima* spat

Source of Variation	*SS*	*df*	*MS*	*P-value*	*F*
Between days	0.05	1.00	0.05	0.00 *	304.62
Between salinity	0.01	5.00	0.00	0.00 *	8.79
Interaction	0.00	5.00	0.00	0.62	0.73
Within	0.00	12.00	0.00		

* Significant at $P < 0.05$

4.4 DISCUSSION

In this study, larval development was completed within 9 days. Nell *et al.* (1994) observed settlement on 19th day for *K. rhytiphora* larvae reared at 19°C. The earlier settlement for *M. opima* may be attributed due to the larval rearing at a higher temperature range of 28 - 32.5°C. Similarly Loosanoff and Davis (1963) observed that in the venerid clam *M. mercenaria*, the larvae undergone metamorphosis earliest on day 16 at 18°C and on day 7 at 30°C after fertilization. Wong *et al.* (1986) observed that the larval settlement *in A. granosa* happened between 21 and 23 days after fertilisation. Kalyanasundaram and Ramamoorthi (1987) observed the completion of larval life history of *M. meretrix* in 12 days. Narasimham *et al.* (1988) observed that in *M. meretrix* the duration of the larval life is short with earliest setting on day 7 and 10 respectively in two experiments at a temperature range of 30.5 and 32.5°C.

There was no eyed stage in the larval development of *M. opima*. Eyespot is usually present in the larvae of a number of bivalve molluscs, but there are many instances wherein eyed larval stage is absent. Chanley (1965) stated that eye spot was absent in the larvae of the mactrid clam *R. cuneata*. Loosanoff *et al.* (1966) mentioned about the absence of eyespot in the larvae of the dwarf surf clam *Mulinia lateralis*, venerid clam *Pitar morrhuana* and the cockle *Laevicardium mortoni*. Narasimham et *al.* (1988) reported the same for the clam *M. meretrix*.

In the present study, it was observed on 7th day that some of the largest larvae were approximately 231 µm in size and were ready to metamorphose, the other larvae of the same culture were only 198 µm. Regarding the growth of the larvae, Jorgensen (1946) opined that the size of the larvae at the time of setting may vary considerably according to the conditions of the environment and, therefore the measurements made at setting are only of

relative importance. Loosanoff and Davis (1950) suggested that some times, because of over crowding or a difference in temperature or other factors, the average size of the larvae of two parallel cultures carried in separate jars would also show significant differences. Loosanoff and Davis (1963) Alagarswami *et al.* (1983), Narasimham *et al.* (1988) in *M. meretrix* and Muthiah *et al.* (1992) in *A. granosa* observed that larvae from the same batch of spawning showed considerable disparity in the growth. In the present study also, significant variations in the size of the individual larvae of the same cultures were very often noticed.

Out of the 0.32 m larvae reared at a density of 0.6/ ml about 80,000 settled with a percentage of 25. The density factor at different growth stages of the species is very important to achieve optimum growth and survival rate (Narasimham *et al.,* 1988). Observations from a single spawning showed that the rearing density at the 'D' stage was 3, 20,000, in the umbo stage 1, 32,000 and in the pediveliger it was 80,000. Nell *et al.* (1994) in *K. rhytiphora* got a survival percentage of 42 at the pediveliger stage. Alagarswami *et al.* (1987) found that for larval rearing, a density of two larvae / ml is the optimum in the pearl oyster, *P. fucata* whereas Nayar *et al.* (1987) recommended 25/ ml for eggs and D-shaped larvae 5 / ml up to umbo stage and 2 / ml to the eyed larvae in the edible oyster *C. madrasensis*. Narasimham *et al.* (1988) used the larval densities 0.45 / ml and 2.98 / ml in *M. meretrix* and suggested that the lower density resulted in higher percentage of spat production.

Regarding the feeding protocol, Alagarswami *et al.* (1983) have given a daily ration of *I. galbana* cells at 12,000 – 25000/ larva/ day and Nayar *et al.* (1984) increased the cell concentration from 3000 to 12000/ larva/ day from 'D' shaped to early spat. According to Loosanoff and Davis (1950) clam larvae were not too selective in their food, surviving and growing rapidly on many microorganisms instead of being confined to a few forms. The exception was when the clam larvae were fed almost a pure culture of a certain species of

Chlorella. The larvae so fed grew more slowly and showed a heavier mortality than those, which were fed mixed plankton cultures. The food requirements of the clam larva are to be evaluated taking into account the intake of algal cells from the water during different larval stages for optimising growth and survival. Further work on standardization of feeding protocol and feeding frequency has to be attempted in future. Laing and Millican (1986) have shown that *O. edulis* spat, fed with mixed phytoplankton gave better growth rate than the single algal diet of *I. galbana*. Further studies have to be undertaken to evolve the growth of clam larvae fed with mixed algae to single algal diet.

Antibiotics are provided to control disease outbreaks in culture of bivalve larvae (Walne, 1974). In this rearing experiment, chloromphenicol was administered at the rate of 8 mg/ L to control bacterial diseases. Brown (1983) reported that chloromphenicol (2.5 ppm) inhibits growth of most marine bacteria commonly associated with the rearing of bivalves.

In the present study, it was observed that the juveniles of *Marcia opima* prefer a salinity of 25 ‰ for optimum growth. Other salinity levels results in either moderate or poor growth. In *Meretrix lusoria*, Namaguchi and Tanaka (1987) observed that for juvenile growth, a salinity range of 19.3 - 32.2 ‰ is favourable and complete mortality was observed below 6.4 ‰. In the same species, Chen (1984) reported that the seed grow best at 25 ‰ and poor growth was observed at 5 ‰. Muthiah *et al.* (unpublished) noted that the optimum salinity for juvenile growth of *M. meretrix* is 21 ‰ and this species prefers a slightly lower salinity than its congener. The juveniles of *A. granosa* grow well under a wide range of salinity from 12 to 26 ‰.

Davis (1958) reported that the optimum salinity for the development of straight - hinge larvae from eggs of *V. mercenaria* from Long Island Sound is about 27.5 ‰. The salinity range for development of eggs of these clams is from 20 ‰ at which only 16 percent to 21 percent of the eggs developed, to 35 ‰, at

which salinity only 1 percent or less of the eggs develop normally. The optimum salinity for growth of clam larvae after they reach the straight - hinge stage is 27.5 ‰ or higher, while 15.0 ‰ is the lowest at which clam larvae were reared to metamorphosis. Once clam larvae attained the straight-hinge stage, they grew quite well at 20 ‰. But contrary to Turner and George's (1955) results, there was no significant mortality in Davis' experiment in the same species. Turner and George (1955) observed that the larvae swam upward, until they came into the seawater at 15.0 ‰. The larvae appeared sluggish at both 17.5 and 15.0 ‰, grew slowly and suffered high mortality either prior to reach setting stage (15.0 ‰) or during metamorphosis (17.5 ‰).

In the present study, 100% survival rate was observed at 10, 15, 25 and 40 ‰. At 20 ‰ and normal seawater salinity survival rate was 95%. This shows that *M. opima* is a euryhaline animal, which can tolerate a wide range of salinity. Regarding the survival rate, Davenport and Wong (1986) observed that in *A. granosa* at 9.6 ‰ and below, there was total mortality in 7 days. The mortality exceeded 50% at 12.8 ‰ and there was no mortality at 22.4 ‰ and above. Muthiah *et al.* (unpublished) observed high mortality of *A. granosa* at about 6.5 ‰. Ranade and Kulkarni (1972) found that *A. granosa* survived in salinity as low as 10.5 ‰. Maximum survival rates for large size groups of *P. malabarica* was recorded between salinities 17 ‰ and 34 ‰, while for the small size groups maximum survival rates was recorded between 20 ‰ and 30 ‰ (Ram Mohan and Velayudhan, 1993). Sundaram and Shafee (1994) reported that *C. madrasensis* is more tolerant to low salinities as low as 7 ‰. *P. viridis* tolerate up to 16 ‰ salinity. The statements made by Kinne (1967) is that the animals acclimatized to low salinity have greater resistance to low salinities are significant and in agreement with the present study. Since the experimental juvenile clams were produced from the brood stock of Ashtamudi Lake, where salinity fluctuates widely due to the freshwater influx during monsoons, it is evident that they are always affected by wide range of salinity fluctuations and are tolerant to such changes.

In Taiwan, the farmers practice high density nursery rearing of the seed of *Meretrix lusoria* in coastal ponds, which are periodically fertilized. As this species grows well at 25 ‰ the salinity of the pond water is maintained close to this value by mixing fresh seawater with less saline underground water (Chen, 1984). Development of such a nursery system of rearing the seed of *Marcia opima* may be helpful to optimize the production of sufficient seeds from clam nurseries.

CHAPTER 5

BIOCHEMISTRY

5.1 INTRODUCTION

Information on the biochemistry of commercially important bivalves will be useful in cultural aspects. Various studies have been carried out on the biochemical constituents in relation to reproductive cycle of different bivalves. Variations in biochemical constituents seem to be mainly influenced by reproductive cycle and availability of food. Investigations on biochemical composition in different body parts of an animal would be more informative than estimation in entire body for studies related to seasonal variations (Giese, 1969). The biochemical information along with the available information on the ecology and biology of clams will be of importance in initiating mass culture practice and in the proper utilization of clam resources.

In the determination of body component indices, it is apparent that some indices such as gonad index, digestive gland index and foot index vary with the reproductive season. Gonadal maturity marks a change in the growth pattern of many molluscs, resulting from the reproductive drain of materials meant for somatic growth, to the gonads. During the reproductive cycle, nutritive energy is stored when gonads are not active and mobilized during periods of rapid gonad growth (Giese, 1966 and 1969). In oysters and clams nutrient storage occurs in the gonad itself and the metabolite is glycogen (Masumoto *et al.*, 1934).

The biochemical constituents such as carbohydrate, protein and lipid varied seasonally in relation to the reproductive activity of the animal. The seasonal variations in the biochemical composition of invertebrates are dependent upon many factors such as food availability, reproductive condition of the animal and different stress condition (Bayne, 1976; Sastry, 1979; Taylor and Venn, 1979; Pieters *et al.*, 1980; Zandee *et al.*, 1980).

Biochemical investigation on the ovary and the extra ovarian organs, which are considered to be involved in contributing to yolk precursors, has been made in several marine invertebrates (Giese, 1969). However the nature of biochemical relationship between these somatic organs as well as the maturing gonad differs greatly among different phyla. In invertebrates, such as Decapod crustaceans, which lay large number of yolky eggs, the fluctuations of organic components in the organs contributing the yolk precursors, are sharper during ovarian maturity. Furthermore, the seasonally reproducing marine invertebrates, such as those found in the temperate and polar regions also accumulate a large amount of organic substrates long before the onset of gametogenesis (Giese and Pearse, 1974).

Previous biochemical workers on molluscan reproduction have, however failed to reveal such a definite relationship with gonad maturation (Brink *et al.*, 1983). This may be due to the difficulties in the isolation of the tissues for biochemical analysis without much contamination from the adjoining organs. Nevertheless, early studies have indicated seasonal fluctuations in the nutrients of the somatic organs such as hepatopancreas, mantle and adductor muscles during gametogenic cycle. The role of mantle tissue as a storage site during the non-reproductive season and as a fertile area in the reproductive period was suggested by Bayne and Thompson (1970). The yolk particles in the eggs of bivalves have been shown to be composed of lipid material (Sastry, 1979).

In clams, as in other bivalves, a discrete biochemical storage site as the adipose tissue is lacking and the synthesized nutrients are stored in the somatic or reproductive organs (Giese, 1959). The relationship between food availability and gonad development has been studied in detail by Sastry (1968), Bayne (1975) and Gabbott (1975 and 1976). When food is in abundance, the nutrients are assimilated and stored in different organs. This may happen during the non-reproductive period. During reproductive period, these nutrient reserves are mobilized to reproductive organs.

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The role of lipids with economy of the marine invertebrates was reviewed by Giese (1966). Essentially, lipid forms the major organic component of the oocytes of molluscs (Giese, 1969; Ansell, 1974 a, b, c and d; Sastry, 1979; Shafee, 1981; Victor and Subramoniam, 1987). Conversely the lipid content of the male testis is considerably low, although the hepatopancreas and foot muscle of the male clams have considerably high level of lipids. Goddard and Martin (1966) reviewed the carbohydrate metabolism and Florkin (1966) studied the nitrogen metabolism of molluscs. The general observations have been that the amount of carbohydrate, proteins and lipids increase as gonad development proceeds and then declines following spawning (Gabbott, 1975 and 1976; Sastry, 1979; Zandee *et al.*, 1980).

Biochemical constituents related to reproductive cycle in the different body components of bivalves on a seasonal basis was studied by Galtsoff (1964), Giese *et al.* (1967), Ansell (1974 a,b,c and d), Ansell *et al.* (1964), Bayne and Thompson (1970) Widdows (1978) Thompson *et al.* (1974) Gabbot (1975), Ansell *et al.* (1973) and John (1980). Thompson (1977) described the blood chemistry, biochemical composition and the reproductive cycle in the giant scallop, *Placopecten magellanicus* from southeast Newfoundland. Jeng *et al.* (1979) studied the chemical composition of Taiwanese oysters and clams. Shafee (1981) studied the seasonal changes in the biochemical composition and calorific content of the black scallop, *Chlamys varia* from Lenveoc, Bay of Brest.

In India, biochemical composition of bivalves was studied by several workers. Venkataraman and Chari (1951) studied the biochemical variations of oysters and clams. Durve and Bal (1961) analysed the chemical composition of oyster *Crassostrea gryphoides*. Chemical composition of the clam *K. marmorata* was studied by Joshi and Bal (1965). Rahaman (1965) studied the nitrogen content of the lamellibranch *D. cuneatus*. Patel and Patel (1972) explained the biochemical aspects of blood clams. Seasonal changes in body

component indices and chemical composition in the estuarine clam *M. meretrix* was studied by Nagabhushanam and Deshmukh (1974). Krishnakumari *et al.* (1977) described some aspects of biology and biochemistry of the backwater clam *M. casta*. Changes in the biochemical composition of the bivalve molluscs, *V. cyprinoides* Var. *cochinensis* and *M. casta* in relation to season were studied by Lekshmenen and Nambisan (1979). Studies on biochemical composition of the clam, *M. casta* off Cochin Bar mouth was done by Salih (1979). The seasonal changes in biochemical composition of body components such as mantle, adductor muscle, foot, gill, gonad, siphon and digestive gland in *M. casta* were studied by Balasubrahmanyan and Natarajan (1980). Victor (1984) studied the reproductive bionomics of *D. cuneatus*. Jayabal and Kalyani (1987 b) investigated variations in protein, carbohydrate and lipid in *M. meretrix* in relation to sex, age and season to asses its nutritive value.

Biochemical changes in the estuarine clam *K. opima* collected from Vellar Estuary in relation to reproductive cycle was analysed by Jayabal (1994). The clam *M. opima* is a popular food item of people residing along the coast. The variations in the biochemical constituents may affect the nutrient quantity of the species. The organs of molluscs pose some difficulties to separate them for biochemical analysis. In *M. opima* the wet tissues mainly consists of the foot muscles, hepatopancreas, mantle and the gonadal tissues. Previous biochemical works proved that fluctuations in the organic components in the muscle and hepatopancreas are correlated to changes in the gonadal activity. Hence organ wise analysis of the organ components was studied according to different maturity stages of the animal.

5.2 MATERIALS AND METHODS

Variation in the biochemical level of different body components according to the reproductive stages was studied. Biochemical analysis was done, separately to determine carbohydrate, protein and lipid content for gonad,

hepatopancreas and foot muscle. The gonad, hepatopancreas and foot muscle were separated according to the reproductive stages of the gonad. These were weighed using a Sartorius monopan balance. Then they were kept in a hot air oven at 80°C for 24 to 30 hours for drying to the nearest of 0.01gm, until a constant weight was obtained. This was recorded as dry weight of the tissue in the same way as wet tissue weight. The dried tissues were packed in aluminium foils and kept in a desiccator. The dried materials were ground using a mortar and pestle to fine homogenised powder. For all the estimations extra pure Ranbaxy grade chemicals were used. Biochemical analysis was separately done for both the samples collected from Tuticorin and Ashtamudi.

5.2.1 QUANTITATIVE DETERMINATION OF PROTEIN

To estimate the protein in the gonad, hepatopancreas and foot muscle, Folin – Ciocalteau method (Lowry *et al.*, 1951) was followed.

Protein reacts with Folin – Ciocalteau reagent to give a coloured complex. This colour is due to the reaction of carbomyl groups in the protein and potassium ions of the reagent. The colour is intensified by the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of colour is thus related to the amount of protein in the sample. 10 mg of dried and finely powdered tissues of gonad, hepatopancreas and foot muscle from different reproductive stages like indeterminate, maturing, mature and spent were precipitated with 2 ml of deproteinising agent, 10% Tri chloro acetic acid (TCA) by keeping the tubes in ice. All the samples were centrifuged at 3000 rpm for 15 minutes. The supernatent obtained in the individual tube was used for carbohydrate estimation. The protein precipitate in each tube was dissolved in 5 ml of 1N NaOH. Three aliquots each with 0.1 ml solution were used as samples. To this, 0.4 ml of double distilled water was added and made each sample 0.5 ml. To this 0.5 ml solution, freshly prepared 5 ml alkaline mixture (48 ml of 2% Na_2CO_3 in 0.1 N NaOH + 1 ml of 0.5% copper sulphate in 1% of sodium

potassium tartarate) was added and kept at room temperature for 10 minutes. After 10 minutes, 0.5 ml of Folin reagent (diluted the 2 N stock solution with double distilled water) was added and mixed well immediately.

A standard stock solution was prepared using bovine serum albumin crystals at a concentration of 25 mg / 5 ml 1 N NaOH. Different dilutions in the range of 0.25 to 2.5 mg / ml were prepared from this stock solution and the alkaline mixture and Folin-phenol reagent were added as in the case of tissue samples. A blank was prepared with 0.5 ml double distilled water and treated the same as above.

All the test tubes were kept at room temperature for 30 minutes. After 30 minutes the samples were read for the optical density of the blue colour developed, in a spectrophotometer at 660 nm against the blank. The protein content of the tissue sample was expressed as mg protein / 10 mg dry tissue.

5.2.2 QUANTITATIVE DETERMINATION OF CARBOHYDRATE

The phenol sulphuric acid method of Dubois *et al.* (1956) was followed to estimate the total carbohydrate in the samples.

The supernatant obtained during protein estimation procedure was used for the analysis. From the above supernatant, 0.1 ml was taken and made up to 1 ml with saturated solution of benzoic acid in double distilled water and to this solution; 1 ml of sulphuric acid was added rapidly and carefully to each tube and mixed well using a cyclomixer.

A standard solution was prepared using D - glucose (Concentration – 20 mg /100 ml saturated solution of benzoic acid). Different dilutions of the working solution with the concentration of glucose ranging from 10 to 100 µg / ml were prepared and the procedure adopted for the tissue was

followed. A blank solution with 2 ml 5% phenol was prepared and the above procedure followed.

All the tubes were kept for 30 minutes at 30°C and the optical density of the orange colour developed was measured at a wavelength of 490 nm.

5.2.3 QUANTITATIVE DETERMINATION OF TOTAL LIPIDS

The total lipids were quantitatively determined by the sulphophosphovanillin method of Barnes and Blackstock (1973).

About 10 mg of foot and gonad and 5 mg of hepatopancreas samples were separately homogenized well in 2 ml of chloroform : methanol (2:1V / V) and kept overnight at 4°C for complete extraction. The mixture taken in glass stoppered centrifuge tubes was then centrifuged for 15 minutes at 300 rpm. The clear supernatant containing all lipids was transferred clean, dry glass tubes. 0.5 ml of the lipid extract of all the tissues were taken separately in clean glass tubes and dried *in vaccuo* over silica gel in a desiccator. To each dried sample, 0.5 ml concentrated sulphuric acid was added and shaken well. The tubes were then plugged with non-absorbent cotton wool and heated at 100°C in a boiling water bath exactly for 10 minutes. The tubes were rapidly cooled to room temperature under running tap water. To 0.1 ml of this acid digest, 2.5 ml of phosphovanillin reagent was added and mixed well by dissolving 80 mg of cholesterol in 100 ml of chloroform : methanol (2:1 V / V) mixture (equivalent to 100 mg of total lipid in 100 ml (2:1 V / V) chloroform : methanol mixture). Working solutions of different concentrations were prepared from the stock solution in the range 50 to 500 µg / 0.5 ml and the procedure adopted for the tissue samples were followed. 0.5 ml of 2:1 V / V chloroform: methanol mixture was treated as blank. All the tubes were kept at room temperature for

30 minutes. The intensity of the pinkish red colour developed was measured against the blank at 520 nm.

The optical density of the colour developed for total proteins, carbohydrates and lipids were measured using a UV/VS Spectrophotometer (GBC 911A) with the samples taken in silica cuvettes. Standard graphs were plotted with the concentration of each biochemical parameter in different dilutions of the working standard solution in the X-axis and optical density (O.D) in the Y-axis. The concentration of different parameters in the samples were calculated (in mg %) by comparing the optical density obtained for the sample with the values in the standard graph and also using the formula,

$$\frac{\text{(O.D of the sample – O.D of the blank)}}{\text{(O.D of the standard – O.D of the blank)}} \times \frac{\text{Concentration of standard X 100}}{\text{Weight of the sample (mg)}}$$

5.2.4 STATISTICAL ANALYSIS

Data on the differences in biochemical constituents such as carbohydrate, protein and lipid with respect to sampling station, sex, tissue and stage of gonad maturity was analysed though a Four – way Analysis of Variance with SPSS 4 computer software.

5.3 RESULTS

In the present study, changes in the biochemical composition of the three body tissues such as gonad, hepatopancreas and foot muscle was separately studied with particular reference to the sex and gonadal maturation of both the clam samples collected from Tuticorin and Ashtamudi. A comparative study of biochemical status of the same species of clam collected from two different stations has got high significance.

5.3.1. Variations in the protein composition of *M. opima*

5.3.1.1 Gonad

The value of total protein is expressed as mg/10 mg dry weight of tissue. The mean values of protein level in male gonads varied between 4.921 mg in maturing stage to 5.416 mg in matured stage at Tuticorin. For the spent male class it was 4.121 mg. In females, the protein varied between 4.517 mg in maturing stage to 5.105 mg in matured clams. For spent female clams, it was 4.063 mg (Figs. 9 and 10). In the indeterminate samples the gonad protein content was 4.461 mg.

The mean values of protein level in male gonads varied between 5.103 mg at maturing to 5.139 mg in matured clams at Ashtamudi. For the spent male clams it was 4.882 mg. In the females the protein varied between 4.846 mg for maturing clams to 5.317 mg in matured ones. For the spent females, it was 4.070 mg (Figs. 11 and 12). In the indeterminate specimens the gonad protein content was 4.928 mg.

5.3.1.2 Hepatopancreas

At Tuticorin, the mean level of protein in the hepatopancreas in the males varied between 4.728 mg for maturing clams to 5.551 mg in matured ones. In the males, the hepatopancreas in the spent stage had a protein level of 4.655 mg. In the maturing females, the protein in hepatopancreas was 4.928 mg and it was 4.825 mg in matured clams. For spent females the level was 4.024 mg (Figs. 13 and 14). In the indeterminate ones, it was 4.087 mg.

The mean level of protein in the hepatopancreas in the males varied between 5.267 mg for maturing and 5.808 mg for matured clams among the Ashtamudi population. In the males, the hepatopancreas in the spent stage had a protein level of 4.497 mg. In the females, it ranged between 4.827 mg to

5.461 mg for maturing and matured specimens. The protein level was 4.160 mg for the spent ones (Figs. 15 and 16). In the indeterminate samples, it was 5.048 mg.

5.3.1.3 Foot muscle

The protein level in the foot muscle was for Tuticorin clams was 4.787 mg, 5.423 mg and 4.042 mg respectively for maturing, matured and spent stages of male clams. In the females, it ranged between 4.462 mg for maturing to 4.983 mg in mature clams. Foot muscle of spent females had protein level of 4.550 mg (Figs. 17 and 18). In the indeterminate samples it was 4.383 mg.

The protein level in the male foot muscle was 5.032 mg, 5.752 mg and 4.296 mg for maturing, matured and spent stages respectively at Ashtamudi. In the females, it was 4.442 mg, 5.622 mg and 3.980 mg respectively for the same stages of maturity (Figs. 19 and 20). In the indeterminate samples it was 5.066 mg.

5.3.2 Variations in the carbohydrate composition of *M. opima*

5.3.2.1 Gonad

In male clams at Tuticorin, the carbohydrate level was minimum in the matured gonad at 0.521 mg and maximum in the maturing gonad (0.957 mg). Gonad of spent males had a carbohydrate level of 0.434 mg. In female, the carbohydrate level ranged between 0.891 mg in maturing ones to 0.547 mg in matured ones. In spent females, the level was 0.515 mg (Figs. 9 and 10). In the indeterminate samples it was 0.416 mg.

In males of Ashtamudi, the carbohydrate level was least in the matured gonad at 0.803 mg and maximum in the maturing gonad at 1.152 mg. Gonad of spent males had a carbohydrate level of 0.770 mg. In females, the

carbohydrate level ranged between 0.980 mg in maturing ones to 0.843 mg in matured ones. In spent females, the gonad carbohydrate level was 0.745 mg (Figs. 11 and 12) whereas; in indeterminate specimens it was 0.816mg.

5.3.2.2 Hepatopancreas

In maturing males, the carbohydrate level in the hepatopancreas was 0.761 mg at Tuticorin. In matured males it was 0.955 mg and in spent ones it was 0.772 mg. In females, the carbohydrate level was 1.107 mg in maturing to 0.955 mg in matured ones. In spent females, it was 0.774 mg (Figs. 13 and 14) and in indeterminate specimens, the carbohydrate level was 0.795 mg.

At Ashtamudi, the carbohydrate level in maturing males in the hepatopancreas was 0.765 mg. In matured males, it was 0.745 mg and in spent ones it was 0.827 mg. In females, the carbohydrate level was 0.942 mg and 0.758 mg in maturing to mature ones respectively. In spent females it was 0.676 mg (Figs. 15 and 16). In the indeterminate ones, the carbohydrate level in hepatopancreas was 0.728 mg.

5.3.2.3 Foot muscle

Carbohydrate level in the foot muscle of maturing male clams in the Tuticorin population was 0.875 mg and in matured clams, it was 0.607 mg. The level of carbohydrate was high in the females when compared to males and it ranged between 0.835 mg for maturing to 0.622 mg for matured females. In the foot muscles of the spent male clams the carbohydrate level was 0.516 mg and in spent females it was 0.480 mg (Figs. 17 and 18). In indeterminate ones, the carbohydrate in the foot muscle was 0.495 mg.

Carbohydrate level in the foot muscle of maturing male clams in the population at Ashtamudi was 0.891 mg and matured clams were 0.612 mg.

The level of carbohydrate was high in the females when compared to males and it ranged between 0.942 mg and 0.758 mg for maturing to matured females respectively. In the foot muscles of the spent male clams the carbohydrate level was 0.827 mg and in females it was 0.676 mg (Figs. 19 and 20). In indeterminate specimens, the carbohydrate in the foot muscle was 0.633 mg.

5.3.3 Variations in the lipid composition of *M. opima*

5.3.3.1 Gonad

The level of lipid in male gonads was minimum (0.785 mg) in the spent stage but was high at 1.015 mg in the maturing stage for the clams at Tuticorin. The matured males had a lipid level of 0.977 mg in the gonad. But in females, lipid level increased from 1.290 mg in the maturing phase to 1.337 mg in the matured phase. Afterwards, it decreased to 0.908 mg in the spent stage (Figs. 9 and 10). In the resting indeterminate samples the lipid level was low at 0.894 mg.

The level of lipid in male gonads was 0.513 mg in the spent stage and 0.916 mg in the maturing stage for the clams at Ashtamudi. The matured males had a lipid level of 0.731 mg in the gonad. But in females lipid level showed an increase from 1.150 mg in the maturing phase to 1.427 mg in the matured phase. But in the spent stage, it decreased to 0.984 mg (Figs. 11 and 12). In the resting indeterminate samples, the lipid level was low 0.603 mg.

5.3.3.2 Hepatopancreas

The level of lipid in hepatopancreas of Tuticorin ranged between 0.877 mg in the maturing phase to 0.800 mg in the spent phase in males. The same trend was noted in the females also. It was 1.171 mg in the maturing ones and 0.771 mg in the spent ones. The lipid level in the hepatopancreas of mature

male clams was 0.848 mg and that of mature female clams was 1.232 mg (Figs. 13 and 14). In the indeterminate samples, the lipid level was 0.827 mg.

The level of lipid in hepatopancreas ranged between 0.609 mg in the spent phase and 0.846 mg in the maturing phase in males of Ashtamudi population. The same trend was noted in the females also. It was 0.522 mg in the spent ones and 1.147 mg in the maturing ones. The lipid level in the hepatopancreas of mature male clams and that of mature female clams was 0.846 and 1.119 mg respectively (Figs. 15 and 16). In the indeterminate samples, the lipid level was 0.635 mg.

5.3.3.3 Foot muscle

In the foot muscle, the lipid level ranged between 0.795 mg in the maturing males to 0.577 mg in the spent males at Tuticorin. The matured males had a lipid level of 0.582 mg in the foot muscle, whereas in female matured ones had a lipid level of 1.133 mg. The foot muscle of the maturing and spent females showed a lipid level of 1.038 mg and 0.547 mg respectively (Figs. 17 and 18). The indeterminate clams had a lipid level of 0.570 mg in the foot muscle.

In the foot muscle, the lipid level ranged between 0.439 mg in the spent males and 0.790 mg in the maturing males of Ashtamudi. The matured males had a lipid level of 0.637 mg in the foot muscle. Whereas in females, the foot muscle of matured ones had a lipid level of 0.963 mg. The foot muscle of spent and maturing females showed a lipid level of 0.611 mg and 1.109 mg respectively (Figs. 19 and 20). The indeterminate had a lipid level of 0.774 mg in the foot muscle.

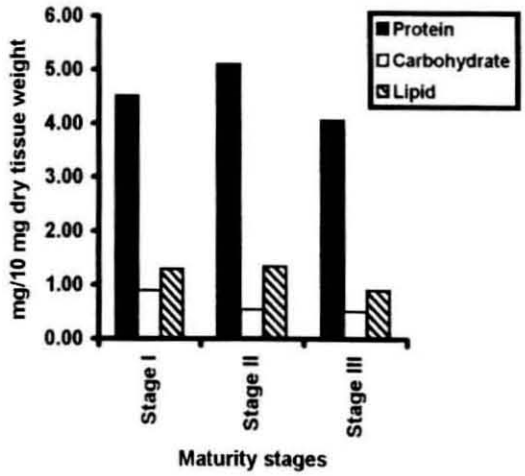

Fig. 9. Biochemical constituents of gonad at different maturity stages in female *M. opima* of Tuticorin

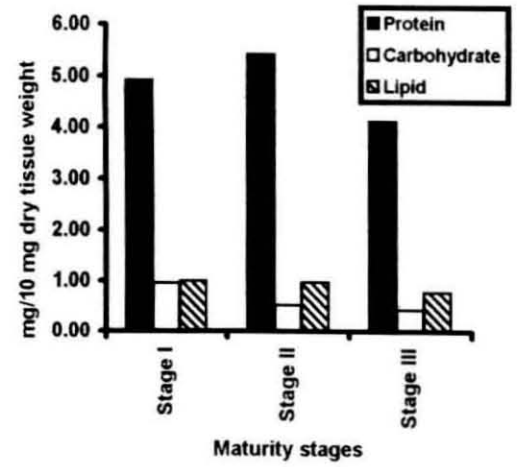

Fig. 10. Biochemical constituents of gonad at different maturity stages in male *M. opima* of Tuticorin

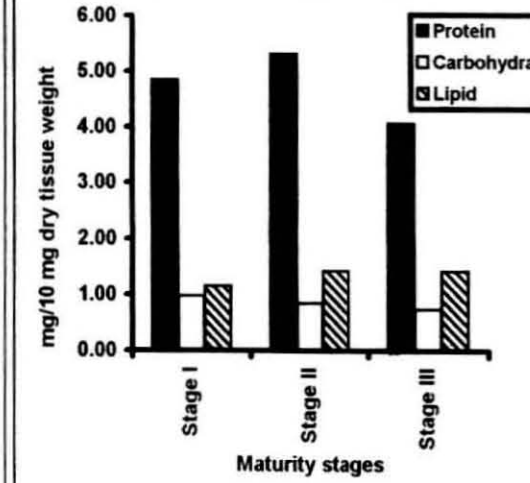

Fig. 11. Biochemical constituents of gonad at different maturity stages in female *M. opima* of Ashtamudi

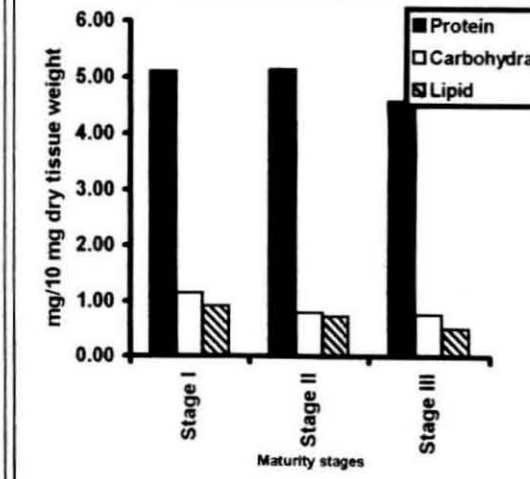

Fig. 12. Biochemical constituents of gonad at different maturity stages in male *M. opima* of Ashtamudi

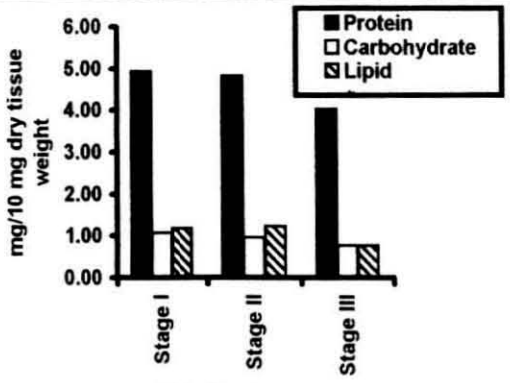

Fig. 13. Biochemical constituents of hepatopancreas at different maturity stages in female *M. opima* of Tuticorin

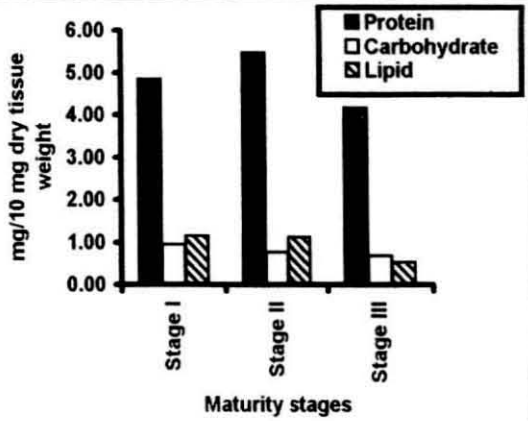

Fig. 15. Biochemical constituents of hepatopancreas at different maturity stages in female *M. opima* of Ashtamudi

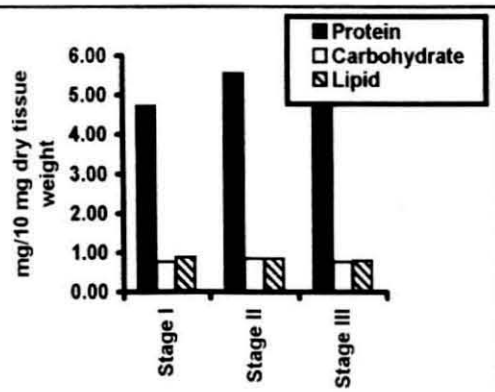

Fig. 14. Biochemical constituents of hepatopancreas at different maturity stages in male *M. opima* of Tuticorin

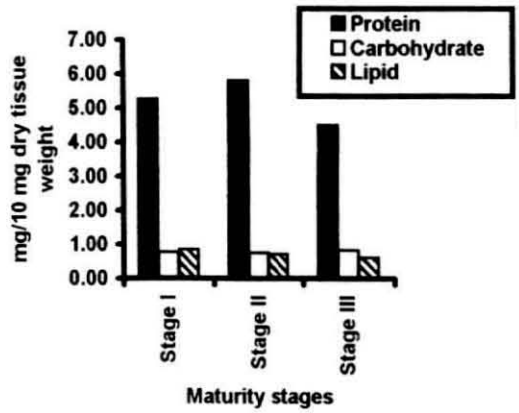

Fig. 16. Biochemical constituents of hepatopancreas at different maturity stages in male *M. opima* of Ashtamudi

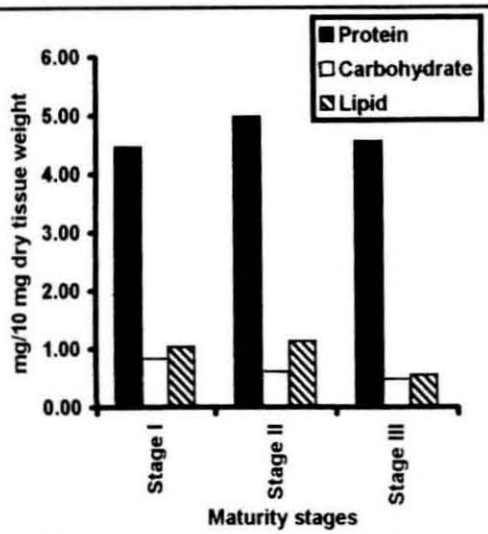

Fig. 17. Biochemical constituents of foot at different maturity stages in female *M. opima* of Tuticorin

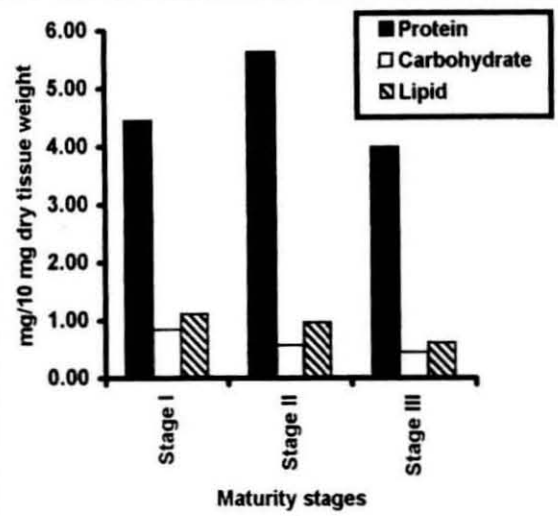

Fig. 19. Biochemical constituents of foot at different maturity stages in female *M. opima* of Ashtamudi

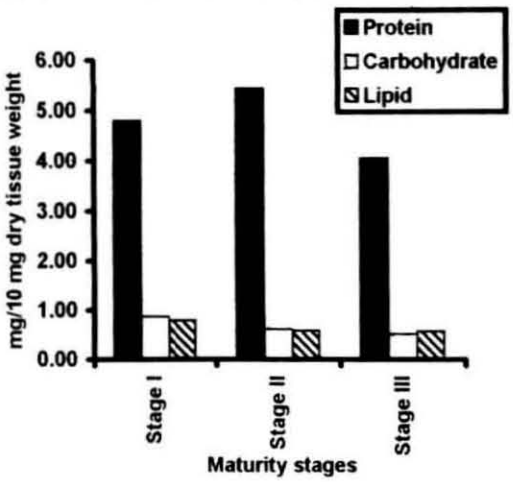

Fig. 18. Biochemical constituents of foot at different maturity stages in male *M. opima* of Tuticorin

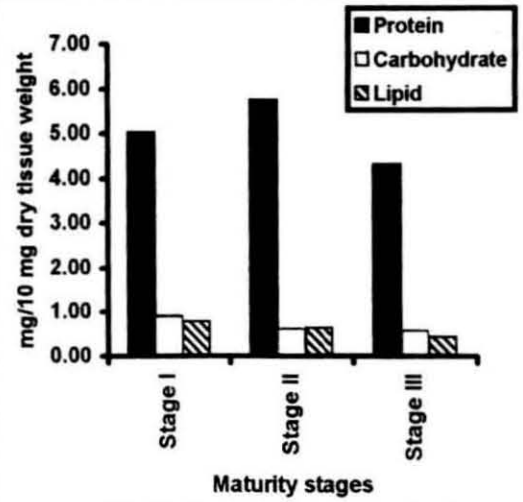

Fig. 20. Biochemical constituents of foot at different maturity stages in male *M. opima* of Ashtamudi

5.4 DISCUSSION

In the present study, comparative biochemical estimation of the gonad, hepatopancreas and foot muscle have been made in relation to the different stages of reproduction in both the sexes and also in the indeterminate clams of both the populations. These studies showed the importance of the involvement of different organ system in the reproductive processes. It was observed that the changes in the biochemical components in different tissues of male and female clams and also the indeterminate ones of both the populations, at different maturity stages showed same pattern of variation.

Single factor Analysis of Variance (ANOVA) showed that (Table. 5) there is significant difference in the protein levels between the two sampling sites, between sexes and between gonadal stages. Two – way ANOVA showed difference in the protein values between sampling location and stages. Three – way ANOVA showed difference in the protein levels between sampling location, tissues and stages. Single factor ANOVA showed that there is significant difference in the carbohydrate levels between sampling stations, sexes, tissues and stages of gonad maturity. Two – way ANOVA showed significant difference in the levels of carbohydrate between sampling stations and sex, stations and tissue, sex and tissues and tissue and gonadal stages. Three – way ANOVA showed marked significance among the carbohydrate levels of stations, tissues and gonadal stages and also among sex, tissues and stages of maturity. Lipid values showed significant differences between stations, sex, tissues and gonadal stages on single factor ANOVA. Gonadal stages and stations, sex and tissues, sex and gonadal stages, tissues and stages of maturity showed significant differences in the lipid levels when two – way ANOVA was done. Three – way ANOVA on the significant difference in the values of lipid showed favourable results among sex, tissues and gonadal stages. Only lipid showed significant difference among the levels in all the four variables such as station, sex, tissues and gonadal stages when four – way ANOVA was done. This shows the importance of lipid as a key biochemical factor in the entire physiological activities of the animal. It plays an active role in the gametogenesis of *M. opima*.

Table 5. Four way ANOVA of biochemical factors

SOURCE OF VARIATION	P VALUE		
	PROTEIN	CARBOHYDRATE	LIPID
STATION	0.001 *	0.000 *	0.000 *
SEX	0.000 *	0.508	0.000 *
TISSUE	0.663	0.000 *	0.000 *
STAGE	0.000 *	0.000 *	0.000 *
STATION * SEX	0.259	0.001 *	0.351
STATION * TISSUE	0.340	0.000 *	0.547
STATION * STAGE	0.028 *	0.123	0.000 *
SEX * TISSUE	0.958	0.000 *	0.006 *
SEX * STAGE	0.632	0.015 *	0.000 *
TISSUE * STAGE	0.098	0.000 *	0.000 *
STATION * SEX * TISSUE	0.479	0.303	0.019 *
STATION * SEX * STAGE	0.265	0.466	0.051
STATION * TISSUE * STAGE	0.036 *	0.022 *	0.309
SEX * TISSUE * STAGE	0.112	0.000 *	0.000 *
STATION * SEX * TISSUE * STAGE	0.074	0.715	0.026 *

* Significant at $P < 0.05$

In male and female clams, protein level increased to maximum in the ripe stage and declined after spawning and during maturation. In most bivalves, the protein content remains at a higher level except during the breeding season (Durve and Bal, 1961). Giese (1966) reported that in the bivalve molluscs the protein levels of the gonads are quite variable and are highest when they are gravid which presumably reflects the high protein content of the maturing gametes. John (1980) reported a decline during spawning period in lipid and protein values in *A. rhombea.* It was also observed that the protein content was more in male gonad compared to the female gonad. This may be due to the synthesis of large quantity of nucleoproteins needed during gametogenesis of the male gametes (Thompson, 1977; Shafee, 1981). In the female, protein content of hepatopancreas decreased in the ripe stage, indicating the transport of protein yolk from the digestive gland to the gonad. The protein content of the foot muscle in both the sexes increased in the matured animal and decreased after spawning.

Carbohydrate was low throughout the period of gametogenesis when compared to lipid and protein. Shafee (1981) reported that carbohydrate get converted into lipid during gametogenesis. As a result, when lipid level increases in the gonad, carbohydrate level decreases. The reduction in the carbohydrate content as oogenesis progresses may be either due to the relative increase of other substances such as lipid and protein during the intense period of vitellogenesis or carbohydrate might be consumed as energy needed in the yolk protein synthesis that occurs in the advanced stage of oogenesis. A significant negative correlation between carbohydrate and protein was reported in *V. cyprinoides* Var. *cochinensis* and *M. casta* by Lekshmenen and Nambisan (1979). In *C. virginica,* the carbohydrate level was very high in the immature animal and falls precipitously as gamete maturation advances (Galtsoff, 1964). Bayne and Thompson (1970) observed an increase in protein and lipids and a decrease in carbohydrates during winter and spring and

correlated the changes with gonad development. They also observed reduction in protein and lipid level during spawning. The reduction in the carbohydrate content was more marked in the females than in males. It may be due to the accumulation of carbohydrate in the sperms and also due to the conversion of carbohydrate into lipids in females. Carbohydrate was lower in the foot muscle and gonad but high in the hepatopancreas. Similar observation was reported in black scallop, *C. varia* (Shafee, 1981). In the female foot tissue, carbohydrate level increased in the maturing and mature clams, indicating the glycogen content in the foot tissue and began to decrease after spawning and was low in spent animals. This is in agreement with the observations made in the Taiwanese oysters and clams (Jeng *et al.*, 1979). Ansell *et al.* (1964) reported that gonad and digestive gland have relatively higher percentage of carbohydrate and fat than other organs.

Lowest lipid content was observed in the foot muscle of male and female animals. Such low lipid level was reported in *M. meretrix* by Nagabhushanam and Deshmukh (1974). The lipid level of female gonad was always more than the male gonad. The lipid level in the female gonad steadily increased from the maturing stage to the mature stage. This indicated the importance of lipid as an energy source for the gonad development. High lipid levels in the female gonad have been reported in several bivalves (Shafee, 1981; Ansell *et al.*, 1964). The greatest increase in the total lipid content was associated with the period of vitellogenesis in *D. trunculus* (Ansell *et al.*, 1973). Similar observation was made by Victor (1984) in *D. cuneatus*. The decline in the lipid level of hepatopancreas and foot muscle when compared to the gonad, in the female clams during later stages of oogenesis corresponds to its accumulation and storage as neutral lipid in the female gonad. Possibly, the stored neutral lipid in the two organs may be translated to the female gonad after undergoing hydrolysis to form the transportable form of simpler lipids units. Such lipid transport from the storage tissues to the gonad has been reported in molluscs by Giese and Pearse (1974). The observed reduction in the lipid level

in the hepatopancreas and subsequent accumulation in the gonad shows that the digestive gland functions as a storage site for lipids during the non-reproductive seasons and with the onset of gametogenesis, the lipid is transported to the gonad. A similar view has been expressed by Venkataraman and Chari (1951), Ansell *et al.* (1964), Widdows (1978), Thompson *et al.* (1974), Bayne (1975 and 1976), Thompson (1977), John (1980) and Balasubrahmanyan and Natarajan (1980).

From the present study, it is evident that there was more accumulation of biochemical components not only in gonad but also in other tissues like hepatopancreas and foot muscle during the season of gonad activity. The general declines in these substances during the spawning and post-spawning periods suggest that these body reserves are mobilized and utilized for gametogenic activities. The fluctuations and changes in the biochemical constituents of other tissues like hepatopancreas and foot muscle was associated with the changes in the gonad tissues at the time of reproduction.

CHAPTER 6

OXYGEN CONSUMPTION AND AMMONIA EXCRETION

6.1 INTRODUCTION

Gills are the main respiratory organs in molluscs. The gills of eulamellibranch are the most advanced; the adjacent filaments are united by vascular connections, leaving narrow openings or ostia between them. The two lamellae of each demibranch become attached back to back in the same way (Morton, 1979).

Oxygen consumption in an animal is always related to the body weight of an organism. Energy losses can be calculated from the oxygen consumed per unit time, from the carbon dioxide released or from the liberation of heat. An estimate of energy flow based on oxygen consumption generally assumes a mean oxy caloric equivalent of 20.08 / ml. Exogenous factors like temperature, salinity, humidity and dissolved oxygen, endogenous factors like body size, activity, gametogenic stage and sex, interaction of both exogenous and endogenous factors and other time dependent variables such as seasons affect the metabolic energy expenditure (Newell and Bayne, 1973). The metabolic rate is also affected by feeding (Hochackka, 1983).

Assimilation efficiency in various molluscs was worked out by Thompson and Bayne (1972), Forster-Smith (1975), Hibbert (1977), Bayne (1983), Newell and Bayne (1973) and Deslous *et al.* (1990).

Utilization of oxygen is a direct measure of degree of activity, food conversion and heat production in animals. Experiments on the respiration of lamellibranchs under various conditions have been carried out by some investigators. The oxygen consumption of shellfish was studied by Mitchell (1912). Berkley (1921) studied anaerobic respiration in bivalve molluscs. The normal and abnormal respirations in oysters have been described by Nozawa (1929). Galtsoff and Whipple (1930) and Ishida (1935) studied the oxygen

consumption in oysters. Van Dam (1935) had investigated the oxygen utilization in *M. arenaria* and scallops. Oxygen consumption and ammonia excretion by *Tridacna gigas* has been explained by Mingoa (1993).

Relationship between oxygen consumption and body size in snails was reported by Von Brand *et al.* (1948). Read (1962) studied the respiration of *Mytilus* and *Brachidartus* as a function of body size and temperature. By correlating oxygen consumption and reproductive cycle, Davies (1966 and 1967) opined that both temperature and body size affect the metabolic rate of the limpets *Patella vulgata* and *P. aspera*. Nagabhushanam (1966) reported the oxygen consumption of the wood boring mollusc *Martesia striata* under various conditions. The oxygen consumption of the clam *Meretrix meretrix* was studied in relation to temperature, body size, and oxygen content of water, at low salinity and low tide under starvation by Deshmukh (1979).

Jorgensen (1952) made correlative studies of respiration and pumping rate in some marine filter feeding invertebrates. The oxygen consumption of Australian freshwater mussel *Hyridella australis* in relation to osmoregulation had been reported by Hiscock (1953). Collier (1959) had measured oxygen uptake over the full range of pumping rates of oyster *C. virginica*. Moon and Pritchard (1970) observed the metabolic adaptations in vertically separated populations of *Mytilus californianus*. Hibbert (1977) studied the use of assimilation energy for respiration in clam *Mercenaria mercenaria*. The respiration rates of scallops in general, and *Pecten magellanicus* in particular, had been studied by Vahl (1978), Shafee (1982), Shafee and Lucas (1982), Barber and Blake (1985), Mac Donald and Thompson (1986 a and b), Bricelj *et al.* (1987) and Shumway *et al.* (1988). Aerial respiration rate is influenced by temperature, season and the availability of food and acclimatization conditions in sessile intertidal animals (Shumway *et al.*, 1988).

Correlating other factors of energy budget, the energy lost for metabolic activity is normally more than that of other energy expenditure (Deslous *et al.*, 1990).

Studies on the relationship between body size and metabolic rate have established that metabolism is proportional to the body weight as described by the linear equation,

$$Y = a + bX$$

Or in the form of a exponential equation,

$$Y = aX^b$$

Where 'Y' is the metabolic rate as oxygen consumption, 'X' is the body size, 'b' is the exponent and 'a' denotes the level of the metabolic rate of an organism of unit body weight. The value of 'a' varies according to a number of factors including activity and temperature. The value for the exponent 'b' is less variable (Bayne and Newell, 1973). The regression formula and 'a' and 'b' values related to oxygen consumption in various molluscs was worked out by, Zeuthen (1953), Boyden (1972 a and b), Dame (1972), Bayne *et al.* (1976), Newell (1973) and Branch and Newell (1978).

The excretory organs of molluscs are known as Keber's organs. The excretory functions are taken up by pericardium in molluscs. In bivalves, the kidneys and paired tubes, the proximal limb is glandular and opens from the pericardium while the distal limb is a thin walled bladder opening into the mantle cavity (Morton, 1979).

Excretion rates have been known to be a function of body size (Emerson, 1969), temperature (Bayne and Scullard, 1977) and season (Widdows and Shick, 1985). The major nitrogenous excretory product in bivalves is ammonia (Hammen, 1969; Campbell and Bishop, 1970; Bishop *et al.*, 1983; Heavers and Hammen, 1985). Ammonia is known to participate in the acid-base balance of the extra cellular fluids during low oxygen conditions, for instance, by restricting the rate of shell decalcification (Bayne and Newell, 1973). It has been suggested that ammonia may be involved in anaerobiosis (Bayne, 1976) possibly in maintaining the acid-base equilibrium in the body fluids of bivalves during hypoxia (Shumway *et al.*, 1988). Ammonia may come from several sources, for instance by deamination of adenosine monophosphates during low anoxia exposures or catabolism of protein substrates for anaerobiosis or by amino acid catabolism (Bishop *et al.*, 1983).

Regarding the urinary loss in the energy budget for molluscs, ammonia is the major excretory product. It comprises a significant component to total energy loss (Hochackka, 1983). Ammonia comprised between 60 - 90% of total measured nitrogen excretion in a majority of bivalves (Easterson, 1987). Ammonia excretion in molluscs was studied by Zeuthen, (1953), Carefoot (1967), Paine (1971) and Bayne (1976). Langton *et al.* (1977) explained the relationship between ammonia excretion and protein consumption in *Tapes japonica*.

The relationship between ammonia excretion rates and body size can be variable in bivalves, because smaller individuals rely on protein catabolism for energy production (Bayne and Scullard, 1977). The relationships between ammonia excretion and body size of organisms have been studied by Mace and Ansell (1982) and also by Stickle and Bayne (1982). Though the ammonia excretions by bivalves show high and varied rates, the contribution of this to total energy losses in the energy budget may normally be rather small but

nevertheless significant. In the accurate construction of energy budgets, information of these losses may be important (Newell and Bayne, 1973).

6.2 MATERIALS AND METHODS

6.2.1 ESTIMATION OF OXYGEN

Estimation of the quantity of dissolved oxygen used for respiration was carried out in a 3 L glass beaker containing 1300 ml of filtered and well-aerated seawater. For sampling seawater a glass siphon was placed in it. Then one clam per unit was kept well immersed. The clams were acclimatized for 45 minutes in the beaker. Then the aeration was stopped for five minutes. Absence of air bubbles was confirmed and 300 ml of the water was siphoned into another 300 ml BOD bottle to estimate the initial oxygen content. Immediately, sufficient liquid paraffin was poured carefully over the water in the beaker to form a thin film in order to prevent oxygen exchange. The set up was kept undisturbed. After one hour the water was siphoned out into a 300 ml BOD bottle and fixed by the addition of Winkler A and B reagents. By using Winkler method (Strickland and Parsons, 1972), the amount of dissolved oxygen was estimated. The difference in dissolved oxygen between the two samples was taken as the quantity of oxygen utilized for respiration by the clams for one hour. From this the oxygen consumed by gram meat weight of individual clam in one hour was calculated. Using the oxy calorific coefficient of 4.83 gm cal / ml of oxygen utilized, the energy utilized for respiratory metabolism by one-gram meat weight of each clam in one day was calculated by multiplying the oxy calorific coefficient with the oxygen consumed by one gram meat weight.

6.2.2 ESTIMATION OF AMMONIA

Phenol-hypochlorite method (Zolarzono, 1969) was followed to calculate the quantity of ammonia excreted by the clam. The clam was kept immersed in 100 ml filtered seawater for 1hour. Ammonia content was estimated

in the seawater samples before and after one hr. For the estimation of ammonia, 50 ml of sample was taken in an Erlenmeyer flask. To this, 2 ml of phenol solution, 2 ml of sodium nitroprusside solution and 5 ml of oxidizing solution (0.5 ml of sodium hypochlorite solution in 100 ml alkaline sodium citrate) were added. The sample was mixed thoroughly and kept at 20°C for one hour. The top of the flasks was covered with aluminium foils to prevent contamination by atmospheric ammonia. After one hour, the samples were read at 640 nm in a spectrophotometer against distilled water. The increase in ammonia content was taken as the quantity of ammonia excreted by the clam. From this the ammonia excreted by one gram meat weight of individual clam in one hour was calculated. The quantity of ammonia nitrogen excreted was multiplied by a factor of 6.25 to arrive at the quantity of protein catabolised.

6.2.3 STATISTICAL ANALYSIS

Linear Regression equation was fitted between the parameters such as consumed oxygen and meat weight, excreted ammonia and meat weight and also protein catabolised and meat weight. Correlation coefficient, 'r' for the same factors was worked out.

6.3 RESULTS

6.3.1 OXYGEN CONSUMPTION

Oxygen consumption was calculated for each clam in terms of whole meat weight of the clam and also in terms of one gram meat weight of individual clam. The former ranged between 0.0645 to 1.5532 ml hr^{-1} for the experimental clams studied. The oxygen consumption in terms of gram meat weight ranged between 0.107 to 0.41 ml hr^{-1} gm^{-1} and the mean value was 0.227 ml hr^{-1} gm^{-1}. That works out to 2.57 - 9.84 ml / day / gram meat weight with a mean of 7.49 ml / day / gram meat weight. Consumption of oxygen as a function of meat weight is given in Fig. 21.

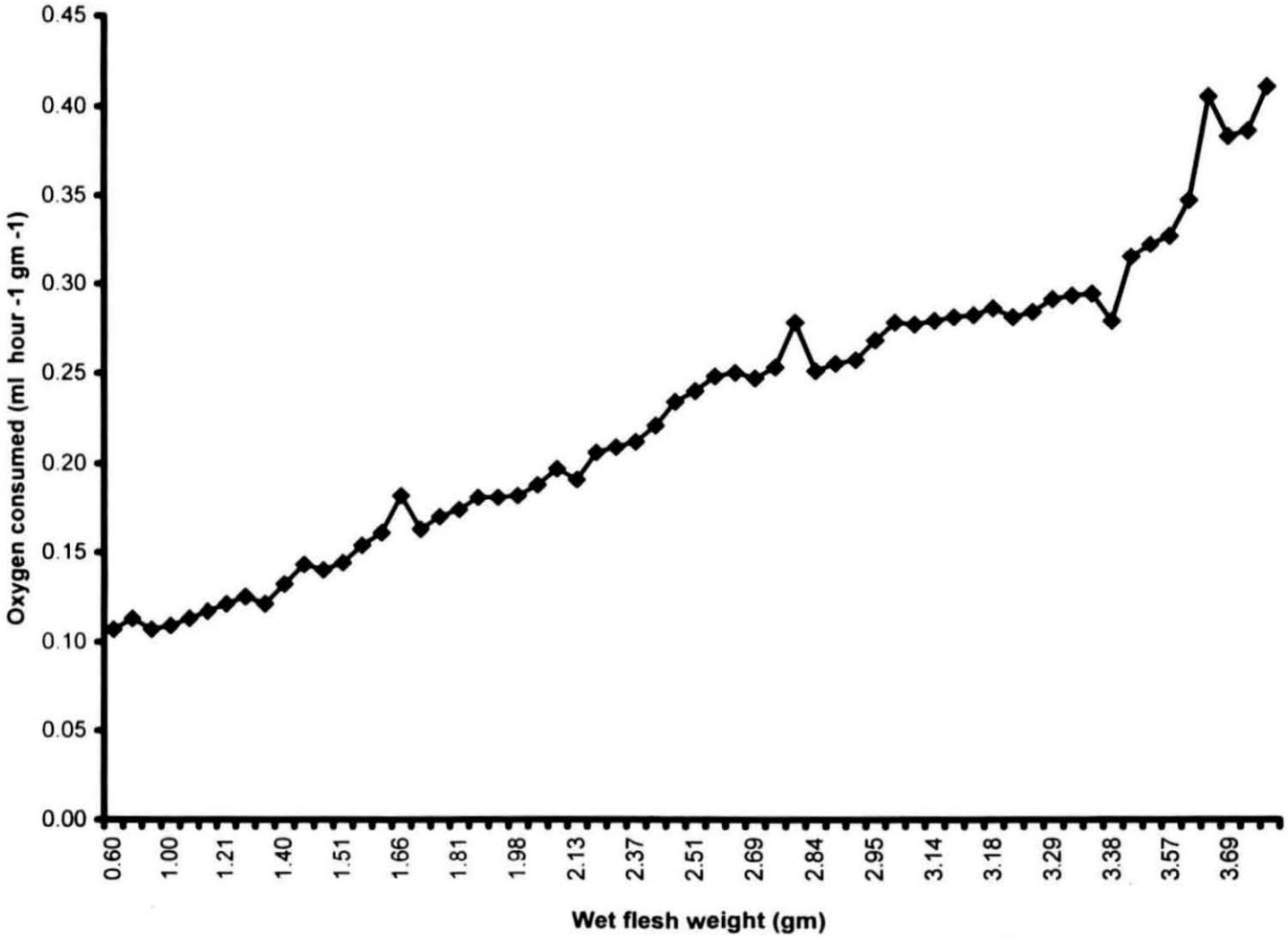

Fig. 21. Consumption of oxygen as a function of meat weight

As explained by Newell and Bayne (1973), the relationship between body size, which is expressed as wet meat weight of clam in grams and the rate of oxygen consumption, could be expressed in the form of linear regression equation,

$$Y = 0.0193 + 0.0867 X$$

Where 'Y' is the rate of oxygen consumption in ml hr^{-1} gm^{-1}, 'X' is the wet meat weight of clam in grams. The above said equation showed a positive correlation and supported that oxygen consumption is directly related to the meat weight of the clam.

The calculated correlation coefficient 0.9703 showed that the two parameters *viz.* oxygen consumption and meat weight are highly significant.

6.3.2 ENERGY EXPENDITURE

The calories spend on respiratory metabolism ranged between 12.40 to 47.53 gm cal / day / gram meat weight with a mean value of 26.36 gm cal / day / gram meat weight.

6.3.3 AMMONIA EXCRETION

Ammonia excretion is calculated for each clam in terms of whole meat weight of each clam and also in terms of gram meat weight of individual clam. The former ranged between 0.0021 mg hr^{-1} to 0.0857 mg hr^{-1} with a mean of 0.0439 mg hr^{-1}. The ammonia excretion in terms of one gram meat weight ranged between 0.0035 to 0.0116 mg hr^{-1} gm^{-1} where in the mean value was 0.008. Ammonia excretion as a function of meat weight is given in Fig. 22.

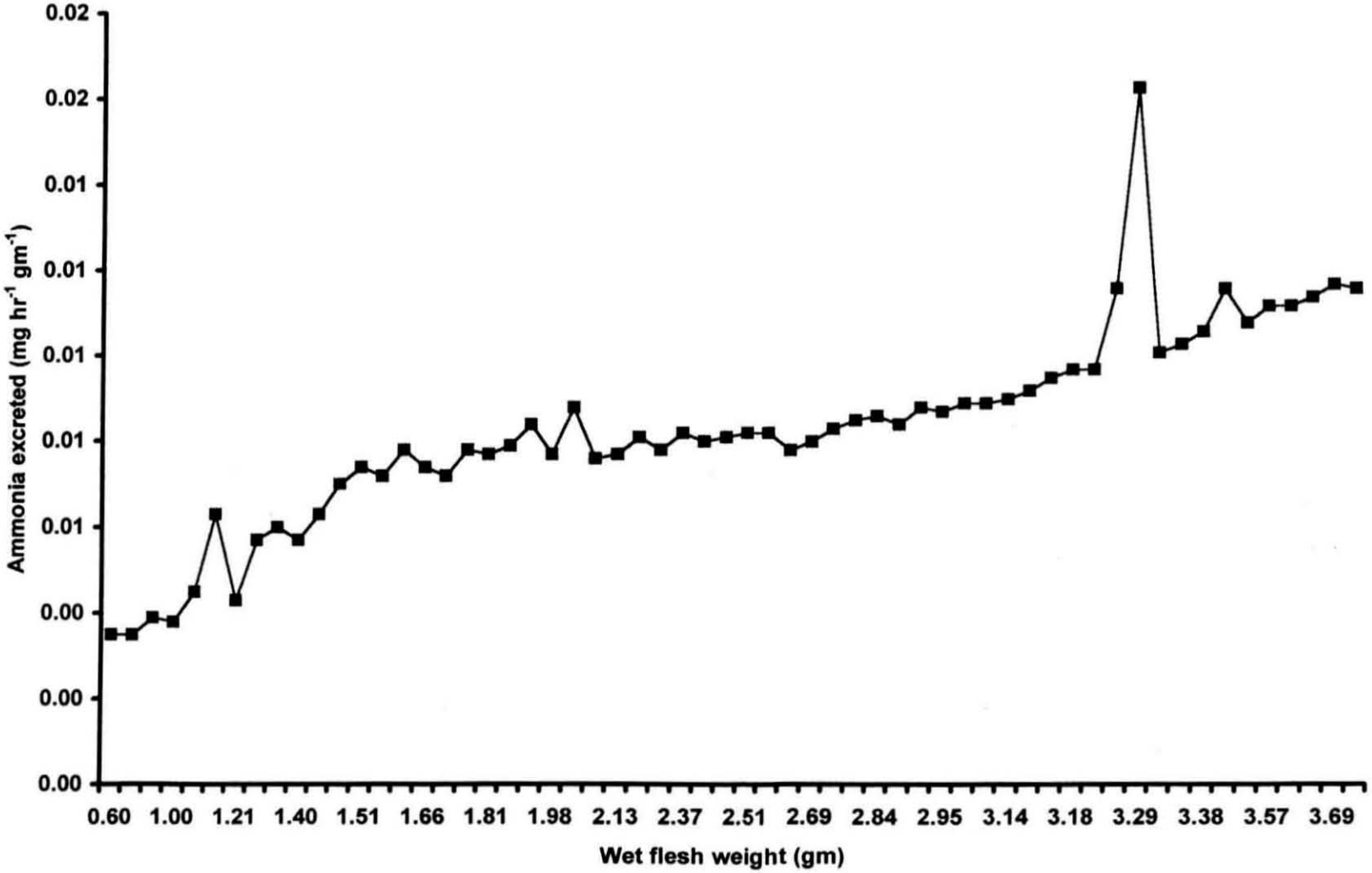

Fig. 22. Excretion of ammonia as a function of meat weight

According to Newell and Bayne (1973), the relation between the body size expressed in terms of gram meat weight of clam and the quantity of ammonia excreted by gram weight of each clam could be expressed in the form of a linear equation. The equation for the present study is expressed as,

$$Y = 0.8028 + 0.0023 X$$

Where 'Y' is the rate of ammonia excretion in mg hr^{-1} gm^{-1}, 'X' is the wet meat weight of clam in grams. The calculated correlation value 'r' was 0.8879, which showed significance. From the linear equation, it was observed that ammonia excretion and meat weight are positively related to each other.

6.3.4 QUANTITY OF PROTEIN CATABOLISED BY THE CLAMS

Ammonia is produced as an excretory product of protein breakdown; therefore, the protein catabolised was calculated from the quantity of ammonia excreted. Thus for one gram meat weight of clam the protein catabolised ranged between 0.018 to 0.226 mg with a mean value of 0.110. Protein catabolism as a function of meat weight is given in Fig. 23.

The relationship between the body size, which is expressed as wet meat weight of clam and the protein catabolised by one gram meat weight in one hour could be expressed in the form of a linear equation (Newell and Bayne, 1973).

For the present study the regression equation is,

$$Y = 0.0113 + 0.01509 X$$

Where 'Y' is the protein catabolised in mg hr^{-1} gm^{-1}, 'X' is the wet meat weight of clam in grams. The linear equation showed a positive correlation. The calculated correlation value was 0.9375, which showed high significance.

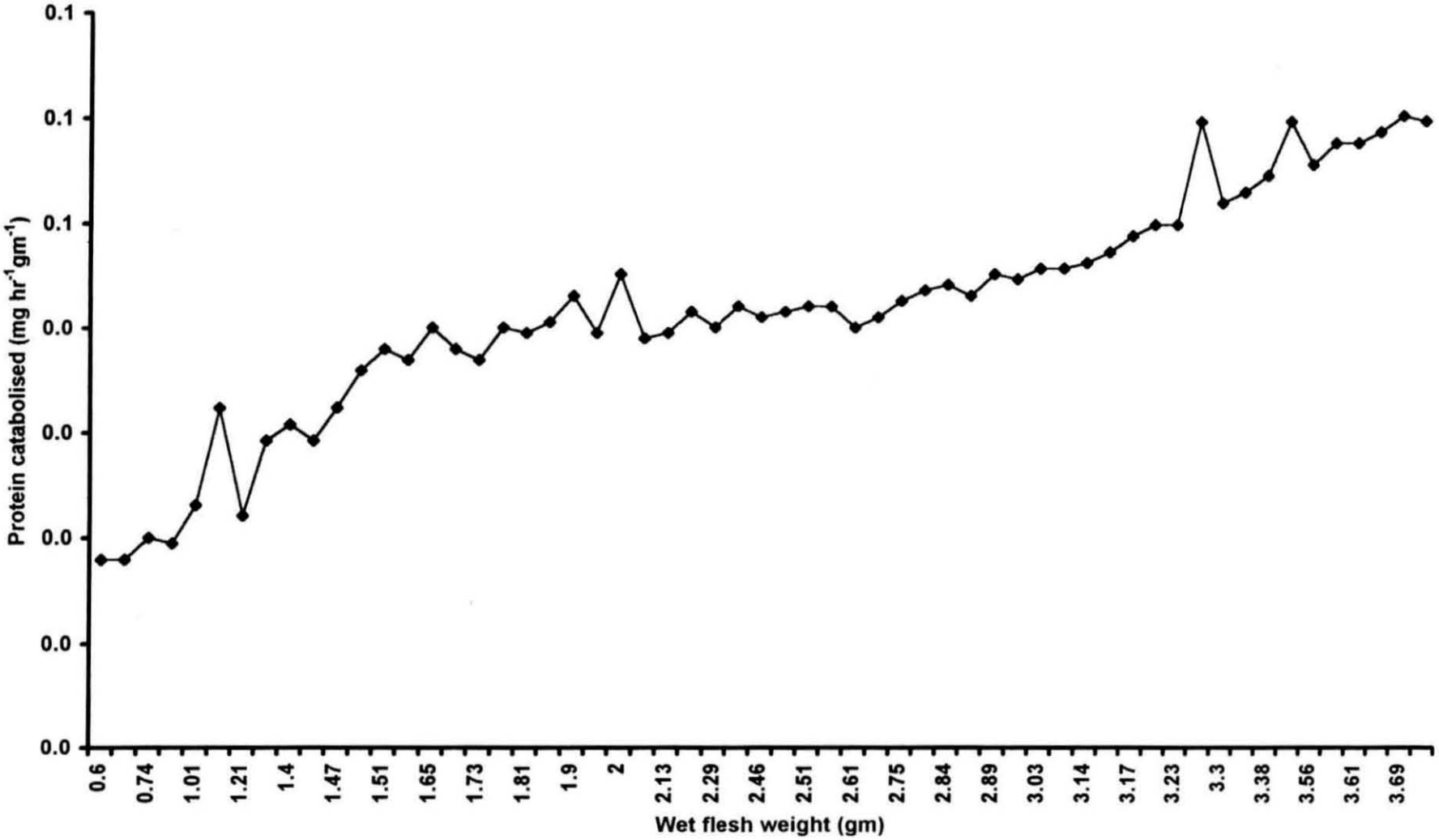

Fig. 23. Protein catabolised as a function of meat weight

6.4 DISCUSSION

Seasonal variations in oxygen consumption rate are intimately linked with the gametogenic cycle in invertebrates (Newell and Bayne, 1973). Davies (1966) reported that, in *P. vulgata the* respiration rate of high and low level can be correlated with the reproductive cycle and hence on the basis of fresh and dry tissue weight. The result of present study agrees with that of Davies (1967). From the present study it is understood that according to different gonad stages, the weight of the animal changes and as a result a variation in oxygen consumption is also observed. Vahl (1978) suggested that the rate of oxygen consumption in the Iceland scallop *Chlamys islandica* is varied according to the gonad growth. Von Brand *et al.* (1948) reported that the rate of oxygen consumption in snails was inversely proportional to the size of the animals when calculated on the basis of weight. Similar observations were made by Nagabhushanam (1966) in *Martesia striata*. Deshmukh (1979) found that the rate of oxygen consumption in *Meretrix meretrix* decreased with the increase in the body weight. These results are in general agreement with those of Mac Donald and Thompson (1986, a and b) but do not agree with that of the result of present study, where a clear indication of linear relationship between oxygen consumption and meat weight were observed.

In the present investigation, 'a' and 'b' values obtained from the regression formula, worked out for oxygen consumption were 0.0192 and 0.0867 respectively. Branch and Newell (1978) had calculated 'a' and 'b' values in *Patella granularis* as 1.160 and 0.800 respectively. Dame (1972) suggested that 'a' and 'b' value for *C. virginica* at 10°C were 0.171 and 0.734, and at 30°C, the values were 0.423 and 0.603 respectively. Newell (1973) found out 'a' and 'b' values for *O. edulis* at 5°C, 15°C and 25°C were 0.364 and 0.899, 0.962 and 0.753 and 2.655 and 1.090 respectively. For *Cerastoderma edule*, 'a' and 'b' values were 0.410 and 0.530 respectively (Newell, 1973). Boyden (1972 a and b) worked out 'a' and 'b' values for the same species as 0.200 and 0.438

respectively. Bayne *et al.* (1976) observed that for *Mytilus edulis* in winter `a' and `b' values were 0.549 and 0.744 and in summer it was 0.339 and 0.702.

The assimilation efficiencies in molluscs with respect to respiration, quoted by various authors differed very much. Thompson and Bayne (1972) quoted assimilation efficiency from 20 to 39% in *M. edulis*. Forster-Smith (1975) for the same bivalve worked it out to be 33 – 40%. Bayne (1983) reported for *O. edulis* larvae; assimilation efficiency is in the order of 30 – 46% and for *M. edulis* larvae from 18 to 44%. Hibbert (1977) calculated assimilation efficiency ranging from 26 to 31% in the clam *Mercenaria mercenaria*. Newell and Bayne (1973) made certain generalization that the suspension feeders which feed on living algal diets usually had very high assimilation efficiency of order 80%. A review of assimilation efficiencies for different tropic groups of molluscs were explained by Newell and Bayne (1973). According to them the assimilation efficiency for grazers is 27.1 to 66.4% and for suspension and deposit feeders it ranges from 21.1 to 9.0%. Carnivorous animals show an assimilation efficiency of 12.8 to 48.0% and for wood boring bivalves it is 66.7%.

Bayne and Scullard (1977) found in *Mytilus edulis*, the 'a' and 'b' values in the regression equation, showing the relation between ammonia excretion and meat weight, are 0.482 and 1.480 respectively. Mace and Ansell (1982) calculated the 'a' and 'b' values at 0.711 and 0.872 for *P. alderi*. Stickle and Bayne (1982) found a common value of 'a' and 'b' which is 0.610, for *T. lapillus*. In the present study, the 'a' and 'b' values worked out in the regression equation were, 0.8028 and 0.0023 respectively.

The quantity of ammonia excreted as quoted by Carefoot (1967) for three species *of* opisthobranch molluscs ranged between 42.25 to 94.7 µg NH–N / day / gm body weight and 15% of absorbed energy was lost in excretion. The ammonia excretion for *M. opima* in the present study, ranged

between 0.084 to 0.278 mg NH–N / day / gm body weight with a mean of 0.181 mg NH –N / day / gm body weight.

Paine (1971) reported 10% energy loss for excretion in *Tegula funebrails*. In most of the bivalves, ammonia formed between 60 to 90% of total measured nitrogen excretion (Bayne, 1976). Langton et *al.* (1977) observed a reciprocal relationship between the rate of ammonia excretion and the weight of protein – nitrogen consumed by the bivalve *T. japonica.* Deslous *et al.* (1990) worked out an assimilation efficiency of 23% in *Mytilus edulis* out of which 8.88% was consumed towards production 12.75% for respiratory metabolism and 1.36% for ammonia excretion. For the two size groups of clams in *M. mercenaria*, Hibbert (1977) worked out the energy spent towards production, respiratory metabolism and ammonia excretion, out of consumption to be 18.7, 12.3 and 5.6% respectively.

In the present study, it is also evident that the rate of oxygen consumption, ammonia excretion and protein catabolism are directly related to the wet flesh weight of *Marcia opima* and hence probably more closely related to the reproductive activity, as the wet meat weight increases when maturation process advances. It is difficult to separate the effect (s) of food, temperature and reproductive stages on metabolic rate because they all vary simultaneously. A clear knowledge of the seasonal changes in food availability and feeding strategies in this species can throw more light into the energy allocation between somatic and gametogenic growth.

CHAPTER 7

ALLOMETRIC STUDIES

7.1 INTRODUCTION

Clams are the most important bivalve resources in India. *Meretrix*, *Paphia, Marcia* and *Villorita* are the major exploited genera of clams. Among the exploited stock, small percentage of clam is utilized for local consumption and majority is mainly used for production of lime. The clam fishery is localised and at sustenance level. Ranade (1964) indicated an annual clam production of 1.1×10^3 t along Maharashtra coast. Alagarswami and Narasimham (1973) reported the landings of clams from Ratnagiri, Goa, North Kanara, Karwar, South Kanara, Kozhikode, Beypore, Vembanad Lake and Quilon, Vaigai estuary, Cooum, Adyar, Pulicat Lake and Chilka Lake. Nayar and Mahadevan (1974) reported the fishing of clams from the Adyar, Ennore and Pulicat Lake.

Relative growth and sexual maturity of crustaceans was studied by Haley (1969) and Lewis (1977). The growth studies and energy flow measurements of marine benthos was explained by Crisp (1984). Stevenson and Dickie (1954) explained the annual growth rings and rate of growth of the scallop, *Placopecten magellanicus*. Allometric relationships of bivalves have received considerable attention in view of their application, particularly in the commercial exploitation of the species in India (Abraham, 1953; Nayar, 1955; Durve and Dharma Raja, 1965 a and b; Alagarswami, 1966; Wilson, 1969; Durve, 1970; Parulekar *et al.*, 1973; Cheriyan and Cherian, 1974; Mane, 1974; Talikhedkar *et al.*, 1976; Ansari *et al.*, 1978; Shafee, 1977; Ansell and Parulekar, 1978; Ansell and Lagardere, 1980; Mohan and Damodaran, 1981; Narasimham *et al.*,1988; Jayabal and Kalyani, 1989; Stites *et al.*, 1995; Sakurai *et al.*, 1996). It is reported that in some bivalves the allometric relationship is different for the smaller and larger size groups of animals (Spear and Glude, 1957; Cheriyan and Cherian, 1974; Shafee, 1977; Mohan and Damodaran, 1981; Jayabal and Kalyani, 1989; Sakurai *et al.*, 1996) whereas in some other bivalves the fitted regression line is the same for the entire size range of animals (Durve and

Dharma Raja, 1965 a and b; Stites *et al.*, 1995). Quayle and Bourne (1972) detailed the clam fisheries of British Columbia.

For shell lime production, 3,000 t are exploited annually along the Mangalore coast and 200,000 t along the Vembanad Lake (Silas *et al.*, 1982). Nayar and Mahadevan (1974) accounted an annual production 2000 t of clams from the regular fishing carried out in Kali River, Karnataka. Usually clam fishing is done during low tide and hand picking is the most common method. Clam fishing net is used for deeper areas. At Tuticorin, *M. opima* fishery is being conducted annually. The shells are mainly used for production of lime.

Observations on parasitic infestation were made earlier by Awati and Rai (1931) in *C. cucullata*. Samuel (1976), Stephen (1977) and Thangavelu (1983) observed parasitic attack in oysters. Durve (1964), Silas and Alagarswami (1967), Harkantra (1976) and Thangavelu and Sanjeevaraj (1985) reported parasitic infestation in *M. casta*. Hesselman *et al.* (1989) reported parasitic attack in *Mercenaria sp.*

7.2 MATERIALS AND METHODS

Samples of clams were collected from the intertidal zones of the sampling sites at Tuticorin and Ashtamudi. A wooden frame of 50 sq. m. area placed in the exposed area at low tide and clams were handpicked. At Turicorin different species of clams were obtained during the study of clam fishery. These clams were identified and grouped separately according to the species to measure their respective length and to calculate their percentage composition in the total population at the sampling site.

The collected *M. opima* were measured for their length, width and thickness to the nearest of 0.01 mm using a vernier callipers. Clams were blotted dry with tissue paper and weighed on a Sartorius microbalance for their shell- on

weight to the nearest of 0.01 gm. Then the shell was opened by severing adductor muscles. The soft tissue was shucked and blotted dry. This was weighed on a pre-weighed petridish for wet flesh weight.

In the present study, an attempt has been made to determine the allometric relationship between different parameters like length, width, thickness, shell-on weight, wet flesh weight and dry flesh weight of *M. opima* from both the populations. 't' test was applied to find out whether the 'b' values obtained in the length - weight relationship of *M. opima* population from the two sampling sites were following the cube law.

7.3 RESULTS

7.3.1 ALLOMETRY OF THE CLAM *M. opima*

In order to determine the allometric relationships, the relationships between length, width, thickness, shell-on weight, wet tissue weight and dry tissue weight were studied using linear regression techniques and correlation coefficients.

Equation for the regression line was used for expressing the relationships between various morphological attributes. The equation in the form of, Y = a + b X, where 'X' is the independent variable and 'Y' is the dependent variable and `a' and `b' are constants, was fitted using linear regression and correlation coefficients (John, 1980). The derived equations for various morphological parameters are given in Table 7a and 7b for the clams collected from Tuticorin and Ashtamudi respectively.

From the tables it is evident that the values of correlation coefficient for various combinations of body as well as shell characteristics taken for the study are very close to unity and are significant.

Table 7a. Allometric relationships between various morphological characters in *M. opima* at Tuticorin

Parameters	Regression equation	Correlation coefficient (r)
Width (Y) on Length (X)	0.7801 X - 0.2643	0.9733
Thickness (Y) on Length (X)	0.5445 X - 1.7999	0.9151
Shell on weight (Y) Length (X)	2.2485 X - 2.3337	0.951
Thickness (Y) on Width (X)	0.6575 X + 3.0661	0.9234
Shell on weight (Y) on Wet tissue weight (X)	0.1253 X + 0.5096	0.9519
Dry tissue weight (Y) on Wet tissue weight (X)	0.2366 X - 0.0382	0.7465

Table 7b. Allometric relationships between various morphological characters in *M. opima* at Ashtamudi

Parameters	Regression equation	Correlation coefficient (r)
Width (Y) on Length (X)	0.6524 X + 4.012	0.9919
Thickness (Y) on Length (X)	0.6899 X - 4.1108	0.9926
Shell on weight (Y) Length (X)	3.0070 X - 3.5317	0.9561
Thickness (Y) on Width (X)	1.0458 X - 8.0106	0.9895
Shell on weight (Y) on Wet tissue weight (X)	0.105 X + 0.4821	0.9751
Dry tissue weight (Y) on Wet tissue weight (X)	0.1822 X + 0.0063	0.9788

7.3.2 LENGTH - WEIGHT RELATIONSHIP OF *M. opima*

Data on the length frequency distribution of the clams, collected for the period from December 1998 to January 2000 from Tuticorin was used. In all, a total of 1009 clams ranging in size from 13.80 mm to 45.10 mm were measured. For studying the length - weight relationship of clams collected from Ashtamudi, data on the length frequency distribution of the clams, collected for the period from May 1999 to January 2000. In all, a total of 304 clams ranging in size from 23.1 mm to 54.6 mm were measured (Fig. 24)

The observed and calculated weights against their respective lengths are plotted in Figs. 25 and 26 for *M. opima* from Tuticorin and Ashtamudi respectively. It appeared that the points clearly agreed with the calculated weights. Using the formula: $W = aL^b$ the calculated weights have been determined from the observed weights to establish this relationship. As weight is a power function of length, the equation can be expressed in the form of an exponential relation.

Following relationship was established for length-weight of clams collected from Tuticorin:

$$W = 0.005\ L^{2.248}$$

The relationship for length-weight of clams collected from Ashtamudi was:

$$W = 0.0003\ L^{3.007}$$

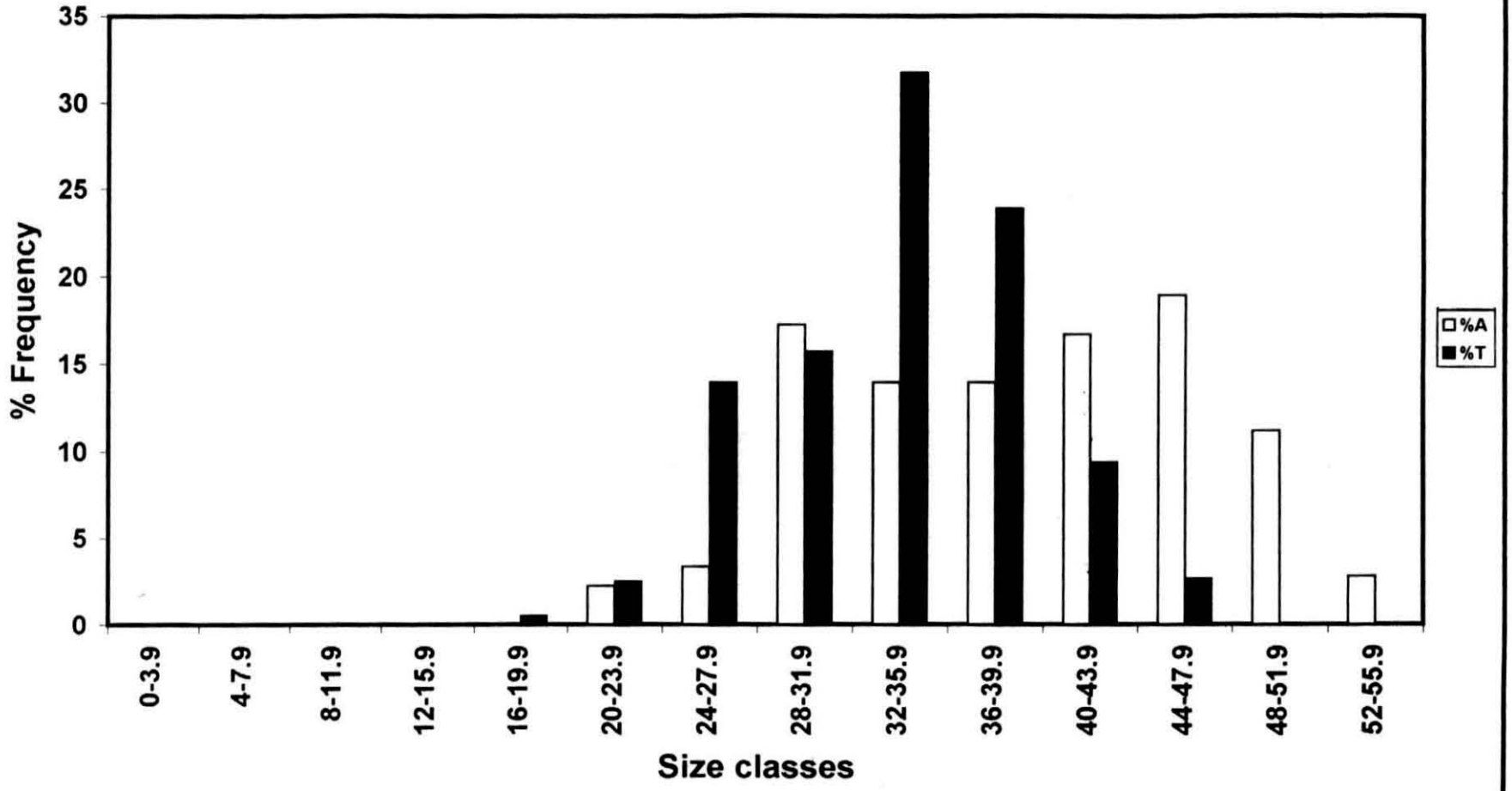

Fig. 24. Length frequency comparison of *M. opima* at Tuticorin and Ashtamudi

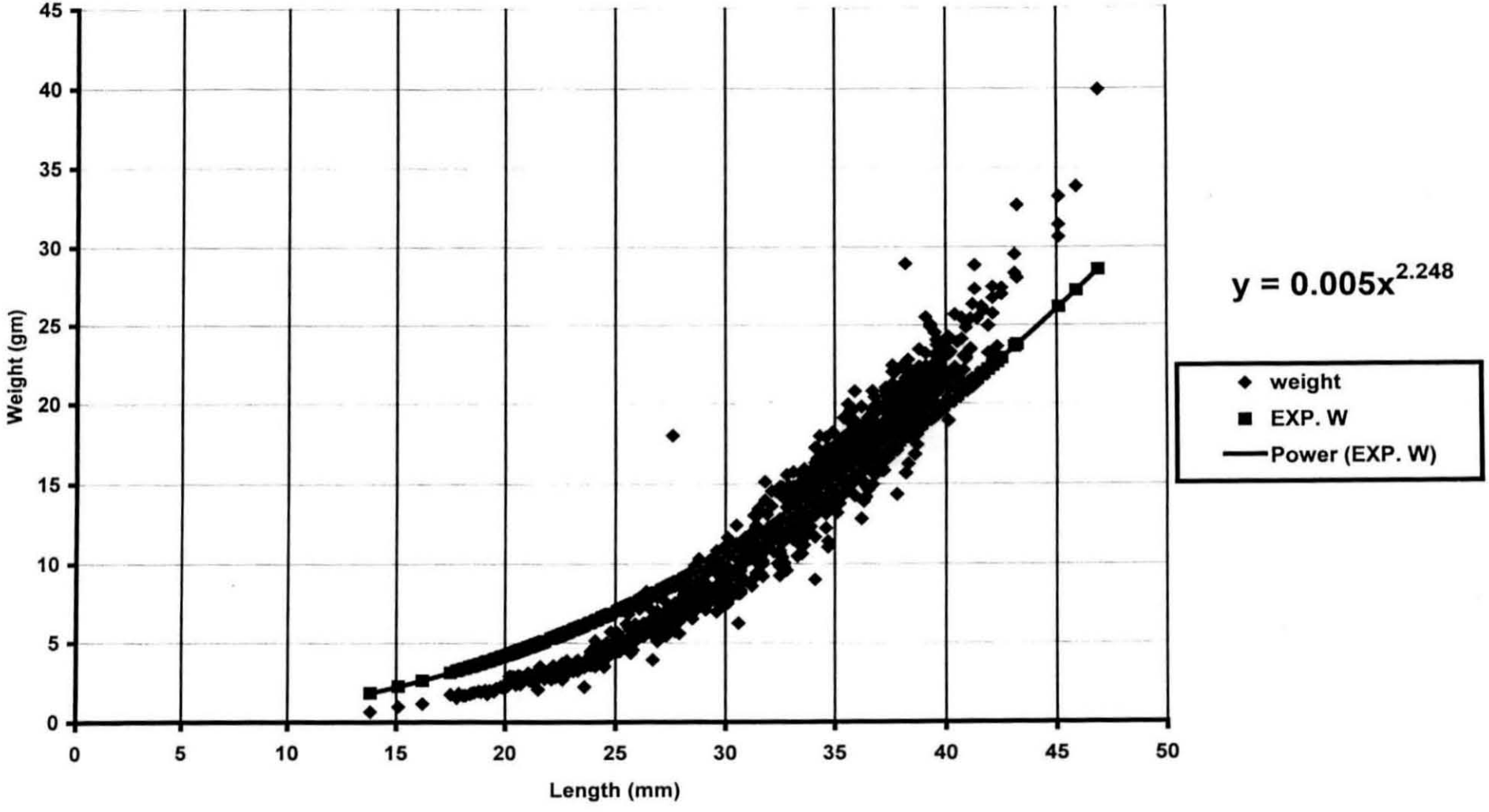

Fig. 25. Length - Weight relationship of *M. opima* at Tuticorin

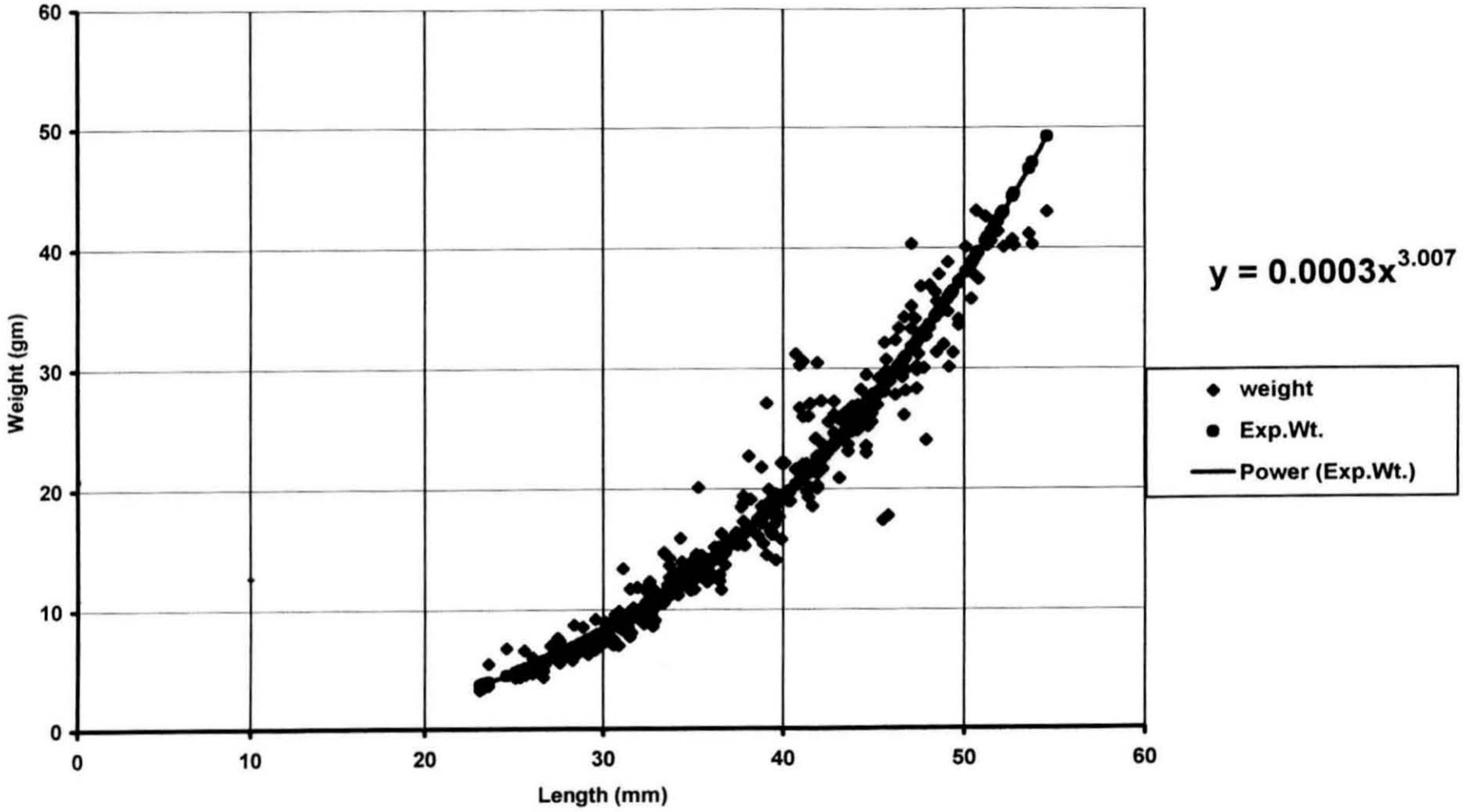

Fig. 26. Length - Weight relationship of *M. opima* at Ashtamudi

7.3.3 CLAM FISHERY IN TUTICORIN BAY

Clam fishing inTuticorin bay is done mainly by women by hand picking. The clams are usually collected in bags made of Palmyra leaves. About 23 persons are involved in clam fishing. The clam collection lasts for 7 hours from 6 am to 1 pm. The collected clams were brought ashore and kept in heaps (Plate X). A number of clams like *M. opima*, *Semele striata*, *Mesodesma glabratum, Gafrarium tumidum*, *Paphia sp.* and *Mactra cuneata* occur in Tuticorin bay, of which *M. opima* is the most important species (Plate XI). The catch per unit effort showed a range of 45 to 50 Kg.

The clams are shucked using knives or st and the meat is taken out by scrapping with nail or thumb. The meat is collected in small earthen pots and taken for sale. The meat of *M .opima* and *G. tumidum* are mainly used for edible purpose. The heaps of shells are carried by a canoe to the shell industry, for making poultry grit, for decorative purposes and for lime production.

Clam fishing begins in February and continues till October. The best clam catches were observed during March to April. The clams are sold at the rate of Rs. 1.50 to 2.00 / Kg.

7.3.4 OBSERVATIONS ON THE CLAM POPULATION AT TUTICORIN

7.3.4a SIZE GROUPS OF CLAMS

The clams were identified and grouped separately according to the species to measure their sizes and to calculate their percentage composition in the total population at the sampling site. Size class frequency on length was identified at 1.9 mm intervals and the frequency at each class was recorded for the species of clams collected. The length of *M. opima* varied between 24.7 - 44.1 mm (Plate XII). It showed maximum frequency (17.5%) in the 34 - 35.9 mm length class, followed by 36 - 37.9 mm length class (12.25%). About 94% of the sampled specimens were in the range of length 24 - 43.9 mm.

PLATE X. A view of shell heaps

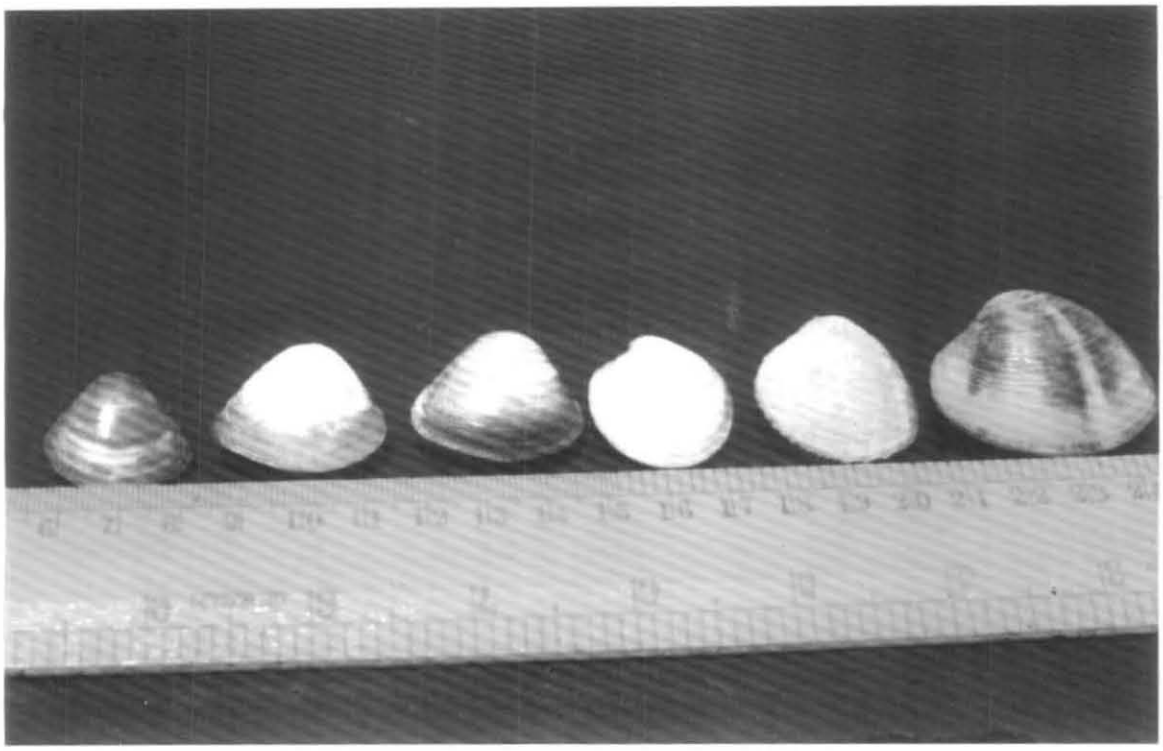

PLATE XI. SPECIES OF CLAMS FISHED AT TUTICORIN

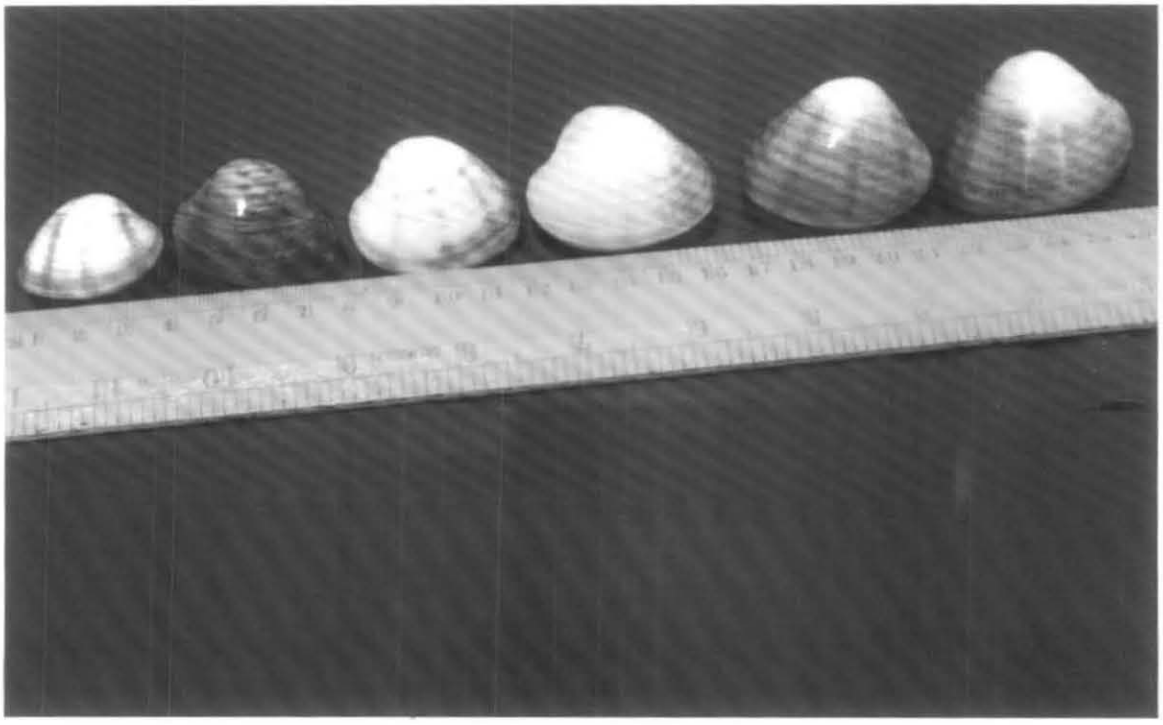

PLATE XII. DIFFERENT SIZE GROUPS OF *MARCIA OPIMA*

S. striata showed maximum frequency of 84% in the 24 - 25. 9 mm size class, with a length range of 22 - 29.9 mm. *M. glabratum* showed a range of 20— 31.9 mm although the frequency was maximum (24%) in the 26 - 27.9 mm length class. *G. tumidum* had a maximum frequency in length class of 24 - 25.9 mm (17%). The size range for this species was 20 to 41.9 mm. *Paphia sp.* showed maximum frequency in three size classes between 36 and 43.9 mm, with a total 66% of the population falling in this range. The length range for the total population was between 30 and 45.9 mm. *M. cuneata* were maximum (43%) in the 20 - 21.9 mm length class while the size ranged from 16 to 23.9 mm.

7.3.4b PERCENTAGE COMPOSITION

Major parts of the clams were constituted by *M. opima*. It formed about 51% of the total catch. *S. striata* formed 21.5%, followed by *M. glabratum* which formed 14.5%. *G. tumidum* constituted 8.75% of the total catch. *Paphia sp.* and *M. cuneata* were least in the catches forming 2.25% and 2% respectively. The percentage composition of different species of clams collected from Tuticorin bay is shown in Fig. 27.

7.3.5 PARASITIC INFESTATION

During the course of study, gonadal smear infestation by trematode *Bucephalus sp.* was noticed. In December 1998, two clams (length 34.6 mm) and in January 1999 and May 1999 one clam in each month (length 32.7 mm and 31.6 mm) were observed to be infected with larvae of trematode. The percentage of infection varied from 5 to 10% among the clams collected from Tuticorin bay.

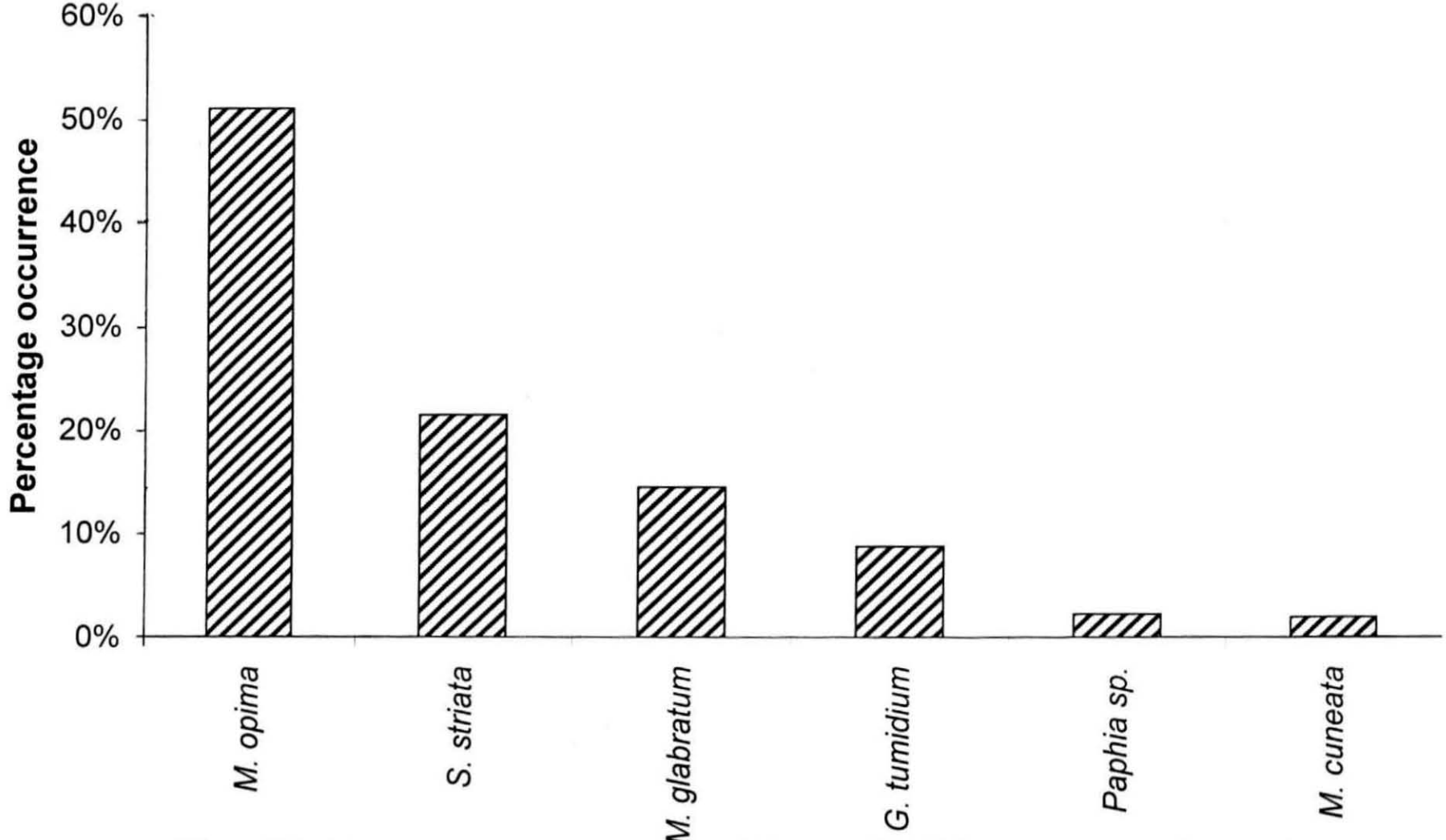

Fig. 27. Percentage composition of different species of clams collected from Tuticorin bay

There were no trematode infestations among the clams collected from Ashtamudi Lake. But there was a single incidence of fungal infection in a female clam of size 51mm in the month of March 1999. In May 1999; 10% of clams (length 34.8 mm and 40.7 mm) were found to be infected with pea crab *Pinnotheres sp.* The meat of all the infected clams was found to be watery and hence the sex could not be identified.

7.4 DISCUSSION

In the present study, different allometric relationships showed that all these factors were significantly related to each other and the correlation coefficients were near to unity as observed by earlier workers and (Nayar, 1955; Talikhekdar *et al.* 1976; John, 1980; Mohan *et al.*, 1984; Narasimham *et al.*, 1988; Jayabal and Kalyani, 1989). The linear relationships between various morphological factors are in accordance with the findings of Nayar (1955) and Talikhedkar *et al.* (1976). The proportionate increase in the width and thickness with length indicates that the general form to be more or less the same through out the life.

Changes in the constant allometry of length weight relationship are associated with increase in size and sexual maturity as reported by John (1980) and Jayabal and Kalyani (1989). Shafee (1977) suggested that in mussels the allometry of length weight relationship is associated with sexual maturity. For length-weight relationship, the slope value (b), obtained in the present study are 2.248 and 3.007 for *M. opima* collected from Tuticorin and Ashtamudi respectively. The slope value obtained in the length weight study of *D. cuneatus* was 2.8079 (Nayar, 1955) and 3.1079 (Talikhedkar *et al.*, 1976). The slope value of the present investigation followed the cube law and is very close to the slope values obtained by Nayar (1955) and Talikhedkar (1976).

Trematode infestation ranging from 5-10% was observed among *M. opima* collected from Tuticorin bay. Though trematode infestation was absent in *M. opima* from Ashtamudi, 10% had fungal infection and another 10% of clams were infected with pea crab *Pinnotheres sp.* Males were predominant in pea crab infested oysters *C. cucullata* (Awati and Rai, 1931). Durve (1964) observed infection in the gonad of *M. casta* by the Bucephalid, cercaria. Presence of commensal crab, *Pinnotheres sp.* in the clam, *M.casta* has been reported by Silas and Alagarswami (1967) and Harkantra (1976). Low percentage level (1%) of infection by trematode *Bucephalopsis haemeanus* infecting the edible oyster *C. madrasensis* was observed by Samuel (1976) and Stephen (1977). Thangavelu (1983) observed 17.37% of parasitic infection in oysters from Pulicat Lake. He also suggested that the reduction in the infection in some months may be due to the low saline condition. Thangavelu and Sanjeevaraj (1985) reported occasional occurrence of larval forms of trematode parasite *B. haemeanus* in the gonad of *M. casta* from September to November. Parasitic infestation in *Mercenaria sp.* was observed by Hesselman *et al.* (1989) in the Indian River Lagoon, Florida. The trematode has been reported to cause gonad castration. As a result, the meat of the clams is found to be thin, transparent and watery (Thangavelu and Sanjeevaraj, 1985). As these organisms tend to affect the reproduction and recruitment, future studies have to be initiated to assess the effect of parasites in the gonad development of the species studied and measures to be taken to control parasitic infestation.

SUMMARY

1. The annual reproductive cycle of *Marcia opima* at Tuticorin and at Ashtamudi was studied for a time period of 14 months from December 1998 to January 2000.

2. The gonad is divided into five stages of maturity *viz.* maturing, mature, partially spawned, spent and indeterminate.

3. Histological studies along with morphological observations could identify the maturity stages of gonad.

4. The gonad index and condition factor were directly related and showed an increasing trend as maturation advanced whereas gonad index and digestive gland index showed a reciprocal relationship.

5. Maximum value of gonad index 17.57± 2.4 was noted in March 1999, at Tuticorin. At Ashtamudi, maximum gonad index value 11.74 ± 1.3 was observed in September 1999.

6. An increase of condition factor to 90.35 ± 8.5 and 48.36 ± 7.7 was noticed in the matured clams during October 1999 at Tuticorin and in July 1999 at Ashtamudi.

7. *M. opima* is a spawns twice in a year at both the places. The spawning periods being May to June and November to December at Tuticorin. At Ashtamudi, the spawning periods are March to May and November to January.

8. Statistically significant 1:1 sex ratio was observed for *M. opima* population at Tuticorin. But at Ashtamudi male dominance was observed with male-female ratio of 1.27:1

9. The average oocyte diameter for the maturing clams was 28.24 µm and for the matured clams, the mean oocyte diameter was 48.28 µ m.

10. Dry / wet meat weight ratio of *M. opima* showed low values during May 1999 and December 1999 at Tuticorin and during November 1999 at Ashtamudi.

11. During the sampling period, the temperature ranged from 26°C to 32.5°C at Tuticorin and from 24°C to 34°C at Ashtamudi. At Tuticorin, salinity showed a range of 32-37 ‰ and at Ashtamudi 20-30 ‰.

12. The induced maturation experiment was conducted for period of 45 days. It was observed that feeding is an important factor that influences the maturity of clams. The clams attained faster growth in the fed treatments.

13. The values of gonad index, digestive gland index and condition factor were more for the fed clams kept at 28°C than that kept at 23°C.

14. Larval rearing of *M. opima* was successfully carried out. The larvae settled on day 11. They attained an average size of 272.8 µ m x 259.6 µ m on day 11.

15. The spats measured an average size of 2.8 mm x 2.37 mm on day 75.

16. The spats were reared at different salinities for 30 days. The best treatment was selected as 25 ‰ in which the spats attained a growth rate of 0.13mm / day. 100% survival was observed at salinities treatments of 10 ‰, 15 ‰, 25 ‰ and 40 ‰.

17. Biochemical analysis was done to understand the variations in the biochemical constituents of the gonad, hepatopancreas and foot muscle during different maturity stages.

18. Protein level increased to a maximum range in the ripe stage of male and female clams and decreased after spawning. Protein level was more in the male gonad than that of female gonad.

19. Carbohydrate level was low in the tissues when compared to protein and lipid throughout the period of gametogenesis.

20. Lipid content of the female gonad was more than that of the male gonad. Lowest lipid level was observed in the foot muscle of male and female clams.

21. The amount of consumed oxygen, excreted ammonia and catabolised protein showed a direct relationship with the wet flesh weight of the animal.

22. Oxygen consumption of *M. opima* was worked out to be ranging from 0.107 to 0.41 ml hr^{-1} gm^{-1} with an average value of 0.227 ml hr^{-1} gm^{-1}.

23. Ammonia excreted ranged from 0.0035 to 0.0116 mg hr^{-1} gm^{-1} with an average value of 0.008 mg hr^{-1} gm^{-1}.

24. Protein catabolised was calculated as 0.018 to 0.226 mg hr^{-1} gm^{-1} with an average value of 0.110 mg hr^{-1} gm^{-1}.

25. Clam fishery in Tuticorin bay constituted a number of species like *Marcia opima, Semele striata, Mesodesma glabratum, Gafrarium fumidum, Paphia sp.* and *Mactra cuneata* of which *Marcia opima* is the dominant species.

26. *M. Opima* at Tuticorin showed a shell length ranging from 13.8 to 45.1 mm whereas at Ashtamudi, the clam had a shell length ranging from 23.1 to 54.6 mm.

27. Values of correlation coefficients for various combinations of body as well as shell characteristics were very close to unity.

28. The slope value obtained in the length-weight relationship of *M. opima* was 2.248 and 3.007 for Tuticorin and Ashtamudi population respectively. These slope values followed the cube law.

29. The gonad of *M. opima* at Tuticorin was found to be infested with the larval trematode of *Bucephalus sp.* during the months, December, January and May. *Pinnotheres sp.* infestation was observed in the gonad of clams at Ashtamudi, during March.

REFERENCES

Abraham, K. C., 1953. Observations on the biology of *Meretrix casta* (Chemnitz), *J. Zool. Soc. India,* 5:163-190.

Abraham, K. J., 1996. Studies on gonad index of edible oyster *Crassostrea madrasensis* (Preston). M.Sc. Dissertation, CIFE, 61pp.

Alagarswami, K., 1966. Studies on some aspects of biology of the wedge clam, *Donax faba* Gmelin from Mandapam coast in the Gulf of Mannar. *J. mar. biol. Ass. India*, 8:56-75.

Alagarswami, K. and Narasimham, K. A., 1973. Clam, Cockle and Oyster resources of the Indian coasts. Proc. Symp. Living resources of the seas around India. *CMFRI Spl. Publ.*, 645-658 pp.

Alagarswami, K., Dharmaraj, S., Velayudhan T. S. and Chellam, A., 1987. Hatchery technology for pearl oyster production. *Bull. Cent. Mar. Fish. Res. Inst.*, 39:98-106.

Alagarswami, K., Dharmaraj, S., Velayudhan, T. S., Chellam, A., Victor A. C. C. and Gandhi, A. D., 1983. Larval rearing and production of spat of pearl oyster *Pinctada fucata* (Gould). *Aquaculture*, 34:287-301.

Albentosa, M., Beiras, R. and Camacho, A. P., 1994. Determination of optimal thermal conditions for growth of clam (*Venurupis pullastra*) seed. *Aquaculture*, 126:315-328.

Ansari, L. A., Ayyappan Nair, Harkantra, S. N. and Parulekar, A. H., 1978. Studies on dimensional relationship in green mussel *Mytilus viridis* from three environments. *Mahasagar,* 11:201-205.

Ansell, A. D., 1961. Reproduction, growth and mortality of *Venus striatula* (Da Costa) in Kames Bay, Millport. *J. mar. biol. Ass. U.K.*, 41:191-215.

Ansell, A. D., 1974a. Seasonal changes in biochemical composition of the bivalve *Abra alba* from the Clyde Sea Area. *Mar.Biol.*, 25:13-20.

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Ansell, A. D., 1974b. Seasonal changes in biochemical composition of the bivalve *Chlamys septemradiata* from the Clyde Sea Area. *Mar. Biol.*, 25:85-89.

Ansell, A. D., 1974c. Seasonal changes in biochemical composition of the bivalve *Nucula sulcata* from the Clyde Sea Area. *Mar. Biol.*, 25:101-108.

Ansell, A. D., 1974d. Seasonal changes in biochemical composition of the bivalve *Lina lians* from the Clyde Sea Area. *Mar. Biol.*, 27:115 - 122.

Ansell, A. D. and Lagardere, F., 1980. Observations on the biology of *Donax trunculus* and *D. vittatus* at Ile d' Ol' eron (French Atlantic Coast). *Mar.Biol.*, 57:287-300.

Ansell, A. D. and Parulekar, A. H., 1978. On the rate of growth of *Nuculana minuta* (Muller) (Bivalvia: Nuculanidae). *J. Mollusc. Stud.*, 44:71-82.

Ansell, A. D., Loosmore, F. A. and Lander, K. F., 1964. Studies on the hard shell clam *Venus mercenaria* in condition and biochemical composition. *J. Appl. Ecol.*, 1:83-85.

Ansell, A. D., Sivadas, P. and Narayanan, B., 1973. The ecology of the sandy beaches in south - west India: IV. The Biochemical composition of four common invertebrates. Spl. Publ., Mar. Biol. Ass. Ind., 333-348 pp.

Appukuttan, K. K., 1996. Recent development in molluscan aquaculture in India. *Seafood Export Journal*, 1:13-20.

Awati, P. R. and Rai, H. S., 1931. *Ostrea cucullata* (the Bombay oyster). *Indian Zool. Mem.*, 3:1-107.

Azcona, P. R., Sierra R. R. and Martin, J. B., 1996. Culture of coquina clam, *Donax trunculus*, larvae. *Aquaculture*, 139:151-155.

Balasubrahmanyan, K. and Natarajan, R., 1980. Seasonal variation in the biochemical composition of *Meretrix casta* (Chemnitz) occurring in Vellar estuary. *Bull. Cent. Mar. Fish. Res. Inst.*, 42:184-188.

Barber, B. J. and Blake, N. J. 1985., Substrate catabolism related to reproduction in the Bay scallop *Argopecten irradians concentricus,* as determined by O/N and R. Q. Physiological indices. *Mar. Biol.*, 87:13-18.

Barnes, H. and Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphophosphovanillin method for total lipids. *J. exp. Mar. Biol. Ecol.*, 12:103-118.

Bayne, B. L., 1975. Reproduction of bivalve molluscs under environmental stress. *In*: Physiological Ecology of Estuarine Organisms (ed. Vernberg, F. J.). Univ. of South Carolina Press, Columbia. pp. 259-277.

Bayne, B. L. 1976. Marine mussels: Their ecology and physiology. *In*: International Biological Programme, No: 16 (ed. Bayne, B. L.). Cambridge Univ. Press, London and New York. 506 pp.

Bayne, B. L., 1983. The physiological ecology of marine molluscan larvae. *In*: The Mollusca. Vol. III. Development (eds. Verdonk, N. H., Vander Biggelaar, J. A. H. and Tompa, A. S.). Academic Press, New York. pp. 229-343.

Bayne, B. L. and Thompson, R. J., 1970. Some physiological consequence of keeping *Mytilus edulis* in the laboratory. *Helgolender Wies. Meeresunters,* 20:526-552.

Bayne, B. L. and Newell, R. C., 1973. Physiological energetics of marine molluscs. *In*: The Mollusca. Vol. IV. Physiology (ed. Hochackka, P. W.). Academic press, New York. pp. 440-473.

Bayne, B. L. and Scullard, C., 1977. Rates of nitrogen excretion by species of *Mytilus* (Bivalvia: Mollusca). *J. mar. biol. Asso. U.K.*, 57:355-369.

Bayne, B. B., Bayne, C. J.,Carefoot T. C. and Thompson, R. J., 1976. The physiological ecology of *Mytilus californianus* Conard. 2. Adaptations to exposure to air. *Occologia*, 22:229 - 250.

Belding David L., 1912. A report upon the quahaug and oyster fisheries of Massachusetts. The commonwealth of Massachusetts, Dept. of Conservation, 1-34 pp.

Berkley, C., 1921. Anaerobic respiration in some molluscs. The relation of anaerobic respiration to glycogen. *J. Biol. Chem.*, 46(3):579-599.

Bishop, S. H., Ellis, L. L. and Burcham, J. M., 1983. Amino acid metabolism in molluscs. *In*: The Mollusca. Vol. IV. Metabolic biochemistry and molecular biomechanics (ed. Hochackka, P.W.). Academic Press, New York. pp. 243-327.

Boyden, C. R., 1972 a. The behaviour, survival and respiration of the cockles *Cerastoderma edule* and *C. glaucum* in air. *J. mar. biol. Ass. U. K.*, 52:661-680.

Boyden, C. R., 1972 b. Aerial respiration of the cockle *Cerastoderma edule* in relation to temperature. *Comp. Biochem. Physiol.*, 43:697-712.

Branch, G. M. and Newell, R. C., 1978. A comparative study of metabolic energy expenditure in the limpets *Patella cochlear*, *P. oculus* and *P. granularis*. *Mar. Biol.*, 49: 351-361.

Bricelj, V. M., Epp, J. and Malouf, R. E., 1987. Comparative physiology of young and old cohorts of the bay scallop *Argopecten irradians irradians* (Lamarck): Mortality, growth and oxygen consumption. *J. exp. Mar. Biol. Ecol.*, 112:73-91.

Brink-de Jong, M., Boer, H. M. and Joosse, J., 1983. Reproductive Biology of Invertibrates. *In*: The Mollusca. Vol. I. Oogenenis, oviposition and Oorsorption (eds. Adiyodi K. G. and Adiyodi, R. G). Academic Press, New York and London. pp. 297-355.

Broom, M. J., 1983. Gonad development and spawning in *Anadara granosa* (L) (Bivalvia : Arcidae) *Aquaculture*, 30:211-219.

Brown, C., 1983. Bacterial diseases in bivalve larval cultures and their control. *In*: Culture of marine invertebrates (ed. Berg, C. J). Hutchinson Ross, Pensylvania. pp. 230-242.

Calabrese, A., 1970. Reproductive cycle of the coot clam, *Mulinia lateralis* (Say) in Long Island Sound. *Veliger*, 12(3):265-269.

Campbell, J. W. and Bishop S. H., 1970. Nitrogen metabolism in molluscs. *In*: The Invertebrates. Vol. I. Comparative biochemistry of nitrogen metabolism (ed. Campbell, J. W.). Academic Press, London. pp. 103 - 206.

Carefoot, T. H., 1967. Growth and nutrition of three species of Opisthobranch mollusc. *Comp. Biochem. Physiol.*, 21:627-652.

Carriker, M. R., 1961. Interrelation of functional morphology, behaviour and antiecology in early stages of the bivalve *Mercenaria mercenaria*. *Sci Soc.*, 77:168 - 242.

Castagna, M. and Manzi, J. J., 1989. Clam culture in North America: Hatchery production of stock clams. *In*: Clam culture in North America (eds. Manzi, J. J. and Castagna, M.). Elsevier, Amsterdam, pp. 111-125.

Castagna, M. and Kraeutzer, J. N., 1981. Manual for growing the hard clam *Mercenaria*. Special Report in Applied Marine Science and Ocean Engineering, Virginia Institute of Marine Sciences, Gloucester, Point, V. A 110:249 pp.

Chanley, P., 1965. Larval development of the brackish water mactrid clam *Rangia cuneata*. *Cheaspeake Sci.*, 6(4):209-213.

Chen, H. C., 1984. Recent innovations in cultivation of edible molluscs in Taiwan, with special reference to the small abalone, *Haliotis diversicolor* and the hard clam *Meretrix lusoria*. *Aquaculture*, 39:11-27.

Cheriyan, P. V. and Cherian, C. J., 1974. Observation on the growth rate in *Martesia striata* (Linn.), the wood-boring pholad in the Cochin Harbour region in the south-west coast of India. Proceedings of the Indian Academy of Sciences, 79:16-28.

Cheung, S. G., 1991. Energetics of transplanted populations of the green-lipped mussel, *Perna viridis* (Linnaeus) (Bivalvia: Mytilidae) in Hong Kong. 1: growth, condition and reproduction. *Asian Mar. Biol.*, 8:117-131.

Chipperfield, P. N. J., 1953. Observations on the brrding and settlement of *Mytilus edulis* (L.) in British waters. J. *mar. biol. Ass. U. K.*, 32:449-476.

Clark, G., 1981. Staining procedures. William & Wilkins, USA, pp. 473.

Collier, A., 1959. Some observations on the respiration of the American oyster *Crassostrea virginica*. *Inst. Mar. Sci.*, 6:92-108.

Crisp, D. J., 1984. Energy flow measurements. *In*: Methods for the study of marine benthos (eds. Holone, N. A. and Mc Intyre A. D.). Blackwell and Scientific Publications, Oxford. pp. 248-372

Dame, R. F., 1972. The ecological energies of growth, respiration and assimilation in the intertidal Ameican oyster *Crassostrea virginica*. *Mar. Biol.*, 17:243-250.

Daniel, E. M., Duncan, H., Hooker, N. and Morse, A., 1977. An inexpensive chemical method for the control and synchronous induction of spawning and reproduction in molluscan species important as protein-rich food resources. *FAO Fish Bull.* 200:291-300.

Davenport, J. and Wong, T. M., 1986. Responses of the blood cockle *Anadara granosa* (Bivalvia: Arcidae) to salinity, hypoxia and aerial exposure. *Aquaculture*, 56:151-162.

Davis, H. C., 1958. Survival and growth of clam and oyster larvae at different salinities. *Biol.Bull.*, 114:296-307.

Davies, P. S., 1966. Physiological ecology of *Patella*. I. The effect of body size and temperature on respiration rate. *J. mar. biol. Ass. U.K.*, 46:647-658.

Davies, P. S., 1967. Physiological ecology of *Patella*. II. Effect of environmental acclimation on the metabolic rate. *J. mar. biol. Ass. U.K.*, 47:61-74.

Deshmukh, R. S., 1979. On the oxygen consumption of the estuarine mollusc *Meretrix meretrix* under various conditions. *J. mar. biol. Ass. India*, 21(1 & 2):1-9.

Deslous - Paoli, J. M., Boromthanarat, S., Heral, M. and Rozet, D., 1990. Energy budget of *Mytilus edulis*, L. population during its first year on bouchots in the bay of Marennes aleson. *Aquaculture*, 91:49-63.

Dix, T. G. and Ferguson, A., 1984. Cycles of reproduction and condition in Tansmanian blue mussels, *Mytilus edulis planulatus*. *Aust. J. Mar. Freshw. Res.*, 35:307-313.

Dubois, M., Giller, K. A., Hamilton, J. K., Robers, R. A. and Smith, S., 1956. Calorimetric methods for determination of sugars and related substance. *Analyt. Chem.*, 28:360-366.

Durve, V. S., 1964. Preliminary observations on the seasonal gonadal changes and spawning in the clam *Meretrix casta* (Chemnitz) from the marine fish farm. *J. mar. biol. Ass. India* 6(2):241- 248.

Durve, V. S., 1970. On the growth of the clam *Meretrix Casta* (Chemnitz) from the marine fish farm. *J. mar. biol. Ass. India*, 12(182):125-135.

Durve, V. S. and Bal, D. V., 1961. Studies as the chemical composition of the oyster *Crassostrea gryphoides* (Schlotheim). *J. Zool. Soc. India*, 13:70-77.

Durve, V. S. and Dharma Raja, S. K., 1965a. A study on the dimensional relationship in the clam *Meretrix casta* (Chemnitz). *J. mar. biol. Ass. India*, 6(2):241-248.

Durve R.S. and Dharma Raja, S. K., 1965b. A study on the dimensional relationship in the clam *Meretrix casta* (Chemnitz) collected from two localities. *J. mar. biol. Ass. Ind.* 7:68-79.

Easterson, D. C. V., 1987. Nutritional bioenergetics in fishes and shell fishes, Techinical Paper II, Summer Institute in Recent Advances in Fin fish and Shell fish Nutrition.11-30 pp.

Emerson, D. N., 1969. Influence of salinity on ammonia excretion rates and tissue constituents of euryhaline invertebrates. *Comp. Biochem. Physiol.*, 29:1115-1133.

FAO, 2000. The status of World Fisheries and Aquaculture. FAO Fisheries Circular No: 815, Rev.11. Rome. 225 pp.

Florkin, M., 1966. Nitrogen Metabolism. *In*: Physiology of Mollusca (eds. Wilber, K. M. and Yonge, C. M.). Academic Press, New York and London. pp. 309-381.

Forster-Smith, R. C., 1975. The effect of concentration of suspension on the filtration rates and pseudo faecal production for *Mytilus edulis* L., *Cerastoderma edule* L. and *Venurupis pullastra* (Montagu). *J. exp. Mar. Biol. Ecol.*, 17:1-22.

Fretter, V. and Graham, A., 1964. Reproduction. *In*: Physiology of Mollusca (eds. Wilbur, K. M. and Yonge, C. M.). Academic Presss, New York. pp. 127-164.

Gabbot, P. A., 1975. Storage cycles in marine bivalve molluscs. A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. Proc. Eur. Mar. Biol. Symp., Oben, Scotland, 191-211 pp.

Gabbot, P. A., 1976. Energy metabolism. *In*: Marine Mussels (ed. Bayne, B. L.). Cambridge Univ. Press, pp. 295-355.

Galtsoff, P. S., 1964. The American oyster, *Crassostrea virginica* Gmelin. *U.S. Fish wildl. ser. Fish Bull.*, 64:1- 480.

Galtsoff, P. S. and Whipple, D. V., 1930. Oxygen consumption of normal and green oysters. *Bull. U.S. Bur. Fish.*, 46:489-508.

Gaspar, M. B. and Monteiro, C. C., 1998. Reproductive cycles of the razor clam *Ensis siliqua* and the clam *Venus striatula* off Vilamoura, Southern Portugal. *J. mar. biol. Ass. U.K.*, 78:1247-1258.

Gibbons, M. C. and Castagna, M., 1985. Responses of the hard clam *Mercenaria mercenaria* (Linnaeus) to induction of spawning by serotonin. *J. Shellfish Res.*, 5(2):65-67.

Giese, A. C., 1959. Comparative physiology: Annual reproductive cycles of marine invertebrates. *Ann. Rev. Physiol.*, 21:547 - 576.

Giese, A. C., 1966. Lipids in the economy of marine invertebrates. *Ann. Rev. Physiol.*, 46:244-298.

Giese, A. C., 1969. A new approach to the biochemical composition of the mollusc body. *Oceanogr. Mar. Biol. Ann. Rev.*, 7:115-129.

Giese, A. C. and Pearse, J. S., 1974. Introduction: General Principles. *In*: Reproduction of Marine Invertebrates. Vol. I. (eds. Giese A. C. and Pearse, J. S.). Academic Press, New York. pp. 1-49.

Giese, A. C., Hart, M. A., Smith, A. M. and Cheung, M. A., 1967. Seasonal changes in body component indices and chemical composition in the Pismo clam, *Tivela stultorum. Comp. Biochem. Physiol.*, 22:549-563.

Goddard, C. N. and Martin, A. W., 1966. Carbohydrate metabolism. *In*: Physiology of Mollusca. Vol.II. (eds. Wilber, K. M. and Yonge, C. M.). Academic Press, New York. pp. 275-302.

Gopinathan, C. P., 1982. Methods of culturing phytoplankton *In*: Manual of Research methods for fish and shellfish nutrition: *CMFRI. Spl. Publ.* 8:113-118.

Hadfield, N. J. and Anderson, D. T., 1988. Reproductive cycles of the bivalve molluscs *Anadara trapezia* (Deshayes), *Venurupis crenata* (Lamarck) and *Anemia descripta* (Iredale) in the Sydney region. *Aust. J. Mar. Freshw. Res.*, 39:649-660.

Haley, S. R., 1969. Relative growth and sexual maturity of the Texas ghost crab, *Ocypoda quadrata* (Fabr.) (Brachyura, Ocypodidae). *Crustaceana*, 17:285-297.

Hammen, C. S., 1969. Metabolism of the oyster, *Crassostrea Virginica. Am. Zool.*, 9:309-318.

Hancock, D. A. and Franklin, A., 1972. Seasonal changes in the condition of the edible cockle (*Cardium edule* L.). *J. Appl. Ecol.*, 9:567-579.

Harkantra, S. N., 1976. Benthos of the Kali estuary, Karwar. *Mahasagar*, 8(1):53-58.

Heavers, B. W. and Hammen, C. S., 1985. Fate of endogenous free aminoacids in osmotic adjustment of *Crassostrea virginica* (Gmelin). *Comp. Biochem. Physiol.*, 82 A:571-576.

Hesselman, D. M., Barber, B. J. and Blake, N. J., 1989. The reproductive cycle of adult hard clams, *Mercenaria sp.* in the Indian River lagoons Florida. *J. Shellfish Res.*, 8:43-49.

Hibbert, C.J., 1977. Energy relations of the bivalve *Mercenaria mercenaria* on an intertidal mudflat. *Mar. Biol.*, 44:77-84.

Hiscock, I. D., 1953. Osmoregulation in Australian freshwater mussels. II. Respiration and its relation to osmoregulation in *Hyridella australis. Aust. J. Mar. Freshw. Res.*, 4(2):330-342.

Hochackka, P. W., 1983. *In*: The Mollusca, Metabolic biochemistry and Molecular biomechanics. Vol. IV. (ed. Hochackka, P. W.). Academic Press, New York, pp. 243-306.

Honkoop, P. J. C., Luttikhuizen, P. C. and Piersma, T., 1999. Experimentally extending the spawning season of a marine bivalve using temperature change and fluoxetine as synergestic triggers. *Mar. Ecol. Prog. Ser.*, 180:297-300.

Hornell, J., 1916. The utilisation of coral and shells for lime - burning in the Madras Presidency. *Bull. Madras Fish. Dept.*, 8:105-126.

Ino, T., 1972. Controlled breeding of molluscs. *In*: Coastal Aquaculture in the Indo- Pacific region (ed. Pillay, T. V. R.). Fishing News (Books) Ltd., London. pp. 260-272.

Ishida, S., 1935. On the oxygen consumption of the oyster *Ostrea gigas* under various conditions. *Sci. Rep. Tohoku. Univ. Ser.*, 4(10):619-638.

Jayabal, R., 1994. Biochemical studies in the estuarine clam *Katelysia opima* from Vellar estuary. *J. mar. biol. Ass. India*, 36(1&2):303-305.

Jayabal, R. and Kalyani M., 1986. Reproductive cycles of some bivalves from Vellar estuary, East coast of India. *Indian J. Mar. Sci.*, 15:59-60.

Jayabal, R. and Kalyani, M., 1987a. Reproductive cycle of the estuarine bivalve *Meretrix meretrix*. (Linn.) of the Vellar estuary. *Indian J. Fish.*, 34(2):229-232.

Jayabal, R. and Kalyani, M., 1987b. Seasonal variations in biochemical constituents of different body components of *Meretrix meretrix* (L). *Mahasagar.* 20(1):65-69.

Jayabal, R. and Kalyani, M., 1989. Allometric relationship in the hard clam *Meretrix meretrix* (Linnaeus). *J. mar. biol. Ass. India*, 36(1&2):319-323.

Jeng, S, S., Hsu, S. Y. and Wong, G. S., 1979. Chemical composition of Taiwanese oysters and clams. *Bull. Inst. Zool. Academia Sinica.*, 18(1):1-10.

John, G., 1980. Studies on *Anadara rhombea* (Born) (Mollusca: Bivalvia: Arcidae) from Port Novo coastal waters. Ph. D. Thesis, Annamalai University. 190 pp.

John, R. S. and Strehlow, D. S., 1983. Algal – induced spawning in the marine mussel *Mytilus califonianus. Int. J. Inv. Rep.*, 6:129-133.

Jones, S., 1968. The molluscan fishery resources of India. Proc. Symp. Mollusca, Mar. Biol. Ass. Ind., 111:906 - 918.

Jorgensen, C. B., 1946. Reproduction and larval development of Danish marine bottom invertebrates. 9. Lamellibanchia. Meddeleser Fra Kommissionen for Danmarks Fiskeri-Og Hasundersogelser, *Plankton*, 4:277-311.

Jorgensen, C. B., 1952. On the relation between water transport and food requirements in some marine filter - feeding invertebrates. *Act. Physiol. Scand.*, 5:297-304.

Joshi, M. C. and Bal, D. V., 1965. Observations on the chemical composition of the clam *Katelysia marmorata* Lamarck. *J. Zool. Soc. India*, 17(1):108 -113.

Kalyanasundaram, M. and Ramamoorthi, K., 1987. Larval development of the clam *Meretrix meretrix* (Linnaeus). *Mahasagar*, 20(20):119-120.

Kanti, A., Heffeman Pans, P. B. and Walker, R. L., 1993. Gametogenetic cycle of the southern surf clam, *Spisula solidissima similis* (Say, 1822), from St. Catherine's sound, Georgia. *J. Shellfish Res.*, 12:255-261.

Keck, R. T., Maurer, D. and Lind, H., 1975. A comparative study of the hard clam gonad developmental cycle. *Biol. Bull.*, 148:243-258.

Kinne, O., 1967. Ecology. *In*: Estuaries (ed. Lanoff, G. H.). Amer. Assoc. Advance Sci., 83. Washington, D.C. pp. 525-540.

Kinne, O., 1971. Temperature. *In*: Marine Ecology. Vol. I. (ed. Kinne, O.). Environmental Factors. Wiley-Interscience, London, pp. 407-514.

Krishna Kumari, L., Rajagopal, M. D. and Sumitra Vijayaraghavan. 1977. Some aspects on biology and biochemistry of the backwater clam, *Meretrix casta* (Chemnitz). *Mahasagar*, 10 (3&4):157-163.

Laing, I. and Millican, P. F., 1986. Relative growth and growth efficiency of *Ostrea edulis* L. spat fed varied algal diets. *Aquaculture*, 54(4):245-262.

Laing, I., Utting, S. D. and Kilada, R. W. S., 1987. Interactive effect of diet and temperature on the growth of juvenile clams. *J. exp. Mar. Biol. Ecol.*, 113:23-38.

Lango, F. R., Chavez, V. J., Claude, C. J. and Le, P. M., 2000. Oocyte size, a means to evaluate the gametogenic development of the Pacific oyster, *Crassostrea gigas* (Thunberg). *Aquaculture*, 190:183-199.

Langton, R. W., Winter, J. E. and Roels, O. A., 1977. The effect of ration size on the growth and growth efficiency of the bivalve mollusc, *Tapes japonica*. *Aquaculture*, 12:283-292.

Laruelle, F., Guillon, J. and Paulet, M., 1994. Reproductive pattern of the clams, *Ruditapes decussatus* and *R.Philippinarum* on intertidal flats in Brittany. *J. mar. biol. Ass. U.K.*, 74:351-366.

Lekshmenen, P. T. and Krishnan Nambisan, P. N., 1979. Biochemical composition of the bivalve molluscs, *Villorita cyprinoides* Var. *cochinensis* (Hanley) and *Meretrix casta (Chemnitz)*. *Indian J. Mar. Sci.*, 9:65-67.

Lewis, E. G., 1977. Relative growth and sexual maturity of *Bathyneetes superbus* (Costa) (Decapoda, Portunidae). *J. nat. Hist.*, 11:629-643.

Loosanoff, V. L., 1937. Development of the primary gonad and sexual phases in *Venus mercenaria* Linnaeus. *Biol. Bull.*, 72:389-405.

Loosanoff, V. L., 1953. Reproductive cycle in *Cyprina islandica*. *Biol. Bull.*, 104:146-155.

Loosanoff, V. L. and Davis, H. C., 1950. Conditioning of *V. mercenaria* for spawning in winter and breeding its larvae in the laboratory. *Biol. Bull.*, 93:60-65.

Loosanoff, V. L. and Davis, H. C., 1952. Temperature requirements for maturation of gonads of northern oysters. *Biol. Bull.*, 103:80-96.

Loosanoff, V. L. and Davis, H. C., 1963. Rearing of bivalve molluscs. *In*: Advances in Marine Biology. Vol. I. (ed. Russel, F. S.). Academic Press, New York. pp. 1-136.

Loosanoff, V. L. and Davis, H. C. and Chanley, P. E., 1966. Dimensions and shapes of larvae of some marine bivalve molluscs. *Malacologia*, 4(2):351-435.

Lowry,O. H., Rosebrough, N. J., Farr, A. L. and Randall, E. J., 1951. Protein measurement with the Folin - phenol reagent. *J. Biol. Chem.*, 193:265-275.

Lubet, P. and Allarakh, C., 1994. Strategie de reproduction de l' huitre *Saccostrea cucullata* (Born) a l'ile. Maurice. Comparaison avec d'autres secteurs de l'aire de repartition. *Haliotis*, 23:61-69.

Mac Donald, B. A. and Thompson, R. J., 1986a. Influence of temperature and food availability on the energetics of the giant scallop *Placopecten magellanicus*. III. Physiological ecology, the gametogenic cycle and scope of growth. *Mar. Biol.*, 93:37-48.

Mac Donald, B. A. and Thompson, R. J., 1986b. Production, dynamics and energy partitioning in two populations of the giant scallop *Placopecten magellanicus* (Gmelin). *J. exp. Mar. Biol.*, 101:285-299.

Mac Donald, B. A. and Thompson, R. J., 1988. Intraspecific variation in growth and reproduction in latitudinally differentiated populations of the giant scallop *Placopecten magellanicus* (Gmelin). *Biol. Bull.*, 175:361-371.

Mace, A. M. and Ansell, A. D., 1982. Respiration and nitrogen excretion of *Polineces elderi* (forbes) and *Polineces catena* (da Costa) (Gastropoda : Naticidae). *J. exp. Mar. Biol. Ecol.*, 60:275-292.

Mane, U. H., 1974. Growth and breeding habits of the clam, *Katelysia opima* in the Kalbadevi estuary at Ratnagiri. *Indian J. Fish.*, 21(2):386-398.

Marsden, D. I., 1999. Reproductive cycles of the surf beach clam *Paphies donacina* Spengler, 1793) from New Zealand. *J. Shellfish Res.*, 18(2):539-546.

Masumoto, B., Masumoto, M. and Hibino, M., 1934. Biochemical studies of Magaki (*Ostrea gigas*). II. The seasonal variation in the chemical composition of *Ostrea gigas*. *J. Sci. Hiroshima Univ.*, 4:47-56.

Mingoa - Licuanan, S. S., 1993. Oxygen consumption and ammonia excretion in juvenile *Tridacna gigas* (Linne, 1758): Effects of emersion. *J. exp. Mar. Biol. Ecol.*, 171:119-137.

Mitchell, P. H., 1912. The oxygen requirements of shellfish. *Bull. U.S. Bull. Fish.*, 32: 207-222.

Mohan, M. V. and Damodaran, R., 1981. Allometric relationship in the clam *Suratta scripta* L. *Ind. J. Mar. Sci.*, 10:198-200.

Mohan, M. V., Damodaran, R. and Salih, K. Y. M., 1984. Allometric studies in the clam *Meretrix casta* Chemnitz. *Mahasagar*, 17(2):119-123.

Moon, T. W. and Pritchard, A. W., 1970. Metabolic adaptations in vertically separated populations of *Mytilus californianus*. *J. exp. Mar. Biol. Ecol.*, 5:35-46.

Morton, J. E., 1979. Reproduction. *In*: Molluscs. Vol. V. (ed. Hochackka, P. W.). Hutchinson and Co publishing Ltd., London. pp. 90 - 126.

Muthiah, P., Narasimham K. A., Gopinathan C. P. and Sundararajan, D., 1992. Larval rearing spat production and juvenile growth of the blood clam *Anadara granosa*. *J. mar. biol. Ass. India*, 34(1&2):138-143.

Nagabhushanam, R., 1966. On the oxygen consumption of the wood - boring mollusc *Martesia striata*, under various conditions. Proc. 2nd All India Congr. Zool., (1962), 2:154-159.

Nagabhushanam, R. and Deshmukh, R. S., 1974. Seasonal changes in body component indices and chemical composition in the estuarine clam, *Meretrix meretrix*. *Indian J. Fish.*, 21(2):531-542.

Nagabhushanam, R. and Mane, V. H., 1975. Reproduction and breeding of the clam, *Katelysia opima*, in the Kalbadevi estuary at Ratnagiri, West coast of India. *Indian J. Fish* ., 4:86-92.

Namaguchi, K. and Tanaka, Y., 1987. Effects of temperature and salinity on growth of early young hard clam *Meretrix lusoria*. *Bull. Nat. Res. Inst. Aquaculture*, 11:35- 40.

Narasimham. K. A., 1980. Culture of blood clam at Kakinada. *Mar. Fish. Inform. Ser., T & E Ser.*, 23:7-9.

Narasimham, K. A., 1988. Biology of the Wedge clam, *Anadara rhombea* (Born) in Kakinada Bay. *Bull. Cent. Mar. Fish. Res. Inst.*, 42(1):135-144.

Narasimham, K. A., 1991. Present status of clam fisheries of India. *J. mar. biol. Ass. India,* 53(1&2):76-88.

Narasimham, K. A., 1993. Ranching of clams in the Ashtamudi Lake. *Mar. Fish. Inform. Ser., T & E Ser.,* 12 A:14-75.

Narasimham, K. A., 1997. Clam culture, paper presented on National Aquaculture week, Mandapam Camp, CMFRI.

Narasimham, K. A., Selvaraj, G. S. D. and Lalitha Devi, S., 1984. The molluscan resources and ecology of Kakinada Bay. *Mar.Fish. Inform. Ser. T & E Ser.* No. 59:1-16.

Narasimham, K. A., Syda Rao, G. and Achary, G. P. K., 1986. The clam resources. R & D series for Marine Fishery Resources Management, CMFRI, Cochin.

Narasimham, K. A., Muthiah, P., Gopinathan, C. P. and Gandhi, A. D., 1988. Larval rearing and spat production of the great clam, *Meretrix meretrix* (Linnaeus). *Indian J. Fish.*, 35(2):107-112.

Narasimham. K. A., Muthiah, P., Sundararajan. D. and Vaithianathan, N., 1988. Biology of the great clam, *Meretrix meretrix* in the Korampallam Creek, Tuticorin. *Indian J. Fish.*, 35(4):288-293.

Natarajan, R. and John, C., 1983. Reproduction in the edible ribbed clam, *Anadara rhombea* (Born) from the back waters of Port Novo. *Indian J. mar. Sci.*, 12 (2):90-95.

Nayar, K. Nagappan., 1955. Studies on the growth of the wedge clam *Donax* (Latona) *cuneatus* Linnaeus, *Indian J. Fish.*, 2(2):325-348.

Nayar, K. Nagappan. and Mahadevan S., 1974. Edible Bivalves: Clams and others. The commercial molluscs of India. *Bull. Cent. Mar. Fish. Res. Inst.*, 24:40-53.

Nayar, K. Nagappan., Rajapandian, M. E., Gandhi A. D. and Gopinathan, C. P., 1984. Larval rearing and production of spat of the oyster *Crassostrea madrasensis* (Preston) in an experimental hatchery. *Indian J. Fish.*, 31(2):233-243.

Nayar, K. Nagappan., Satyanarayana Rao, K., Rajapandian, M. E., Gopinathan, C. P. and Gandhi, A.D., 1987. Production of oyster seed in a hatchery system. *In*: (eds. Nayar, M. N. and Mahadevan, S.). Oyster Culture - status and Prospects. *Bull. Cent. Mar. Fish. Res. Inst.*, 38:52- 58.

Nell, J. A., O'Connor, W. A., Heasman, M. P. and Guard, L. J., 1994. Hatchery production for the venerid clam *Katelysia rhytiphora* (Lamy) and the Sydney cockle *Anadara trapezia* (Deshyes). *Aquaculture*, 119:149-156.

Newell, R. C., 1973. Factors affecting the respiration of intertidal invertebrates. *Am. Zool.*, 13:513-528.

Newell, R. C., 1980. The maintenance of energy balance in marine invertebrates exposed to changes in environmental temperature. *In*: Animals and

environmental fitness. Vol. I. (ed. Gilles, R.). Pergamon Press, Oxford, pp. 561-582.

Newell, R. C. and Bayne, B. L., 1973. A review on temperature and metabolic acclimation in intertidal marine invertebrates. *Neth. J. Sea Res.*, 7:421-433.

Newell, R. C. and Branch, G. M., 1980. The influence of temperature on the maintenance of metabolic energy balance in marine invertebrates. *Adv. Mar. Biol.*, 17:329-396.

Nozawa, A., 1929. The normal and abnormal respiration in oyster *Ostrea circumpicta. Sci. Rep.Tohoku. Imp. Univ., ser.*, 4:259.

Paine, R. T., 1971. Energy flow in a natural population of the herbivorous gastropod *Tegula funebralis. Limnol. Oceanogr.*, 16:86-98.

Parulekar, A. H., Dwivedi, S. N. and Dhargalka, V. K., 1973. Ecology of clam beds in Mandori, Cambarjua and Zuari estuarine system of Goa. *Ind. J. Mar. Sci.*, 2:122-126.

Patel, B. and Patel, S., 1972. Blood clams - Material for physiological and biochemical studies. *J. mar. biol. Ass. India*, 14(2):555-563.

Peddichord, R. K. 1977. Salinity and substratum effects on condition index of the bivalve *Rangia cuneata. Mar. Biol.*, 39:351-360.

Pfitzenmeyer, H. T., 1962. Periods of spawning and setting of the soft-shelled clam, *Mya arenaria* at Solomons, Maryland. *Chesapeake Sci.*, 3:114-120.

Pfitzenmeyer, H. T., 1965. Annual cycle of gametogenesis of the soft-shelled clam, *Mya arenaria* at Solomons, Maryland. *Chesapeake Sci.*, 6:52-59.

Pieters, H., Kluytmans, J. H., Zandee, D. I. and Cadne, G.C., 1980. Tissue composition and reproduction of *Mytilus edulis* dependent on food availability. *Neth. J. of Sea Res.*, 14:349-361.

Porter, H. J., 1964. Seasonal gonadal changes of adult clam, *Mercenaria mercenaria* (L.) in North Carolina. Proc. Natl. Shellfish Ass., 55:35-52.

Prosser, C. L., 1955. Physiological variation in animals. *Biol. Rev.*, 30:229-262.

Quayle, D. B. and Bourne, N., 1972. The clam fisheries of British Columbia. *Bull. Fish. Res. Canada.* 179:1-70.

Rahaman, A. A., 1965. Nitrogen content of the lamellibranch *Donax cuneatus* (L). Proc. Indian Acad. Sci., 62:188-194.

Rajapandian, M. E. and Rajan C. T., 1987. Biological aspects of oysters. *In*: Oyster culture: Status and prospects (ed. Nagappan Nayar, K. and Mahadevan, S.). *Bull. Cent. Mar. Fish. Inst.*, 38: 30-39.

Ramadoss,K., Velayudhan, T. S., Kripa, V., Laxmilatha, P., Ramachandran, N. Sreenivasan, P. V., Sarvesan, R., Natarajan, P., Asokan P. K., Sujitha Thomas, Geeta Sasikumar and Boby Ignatius., 2001. Investigations on the resource characteristics of bivalves and gastropods. CMFRI Annual report 2000-2001. 76-77 pp.

Ram Mohan, M. K., and Velayudhan, T. S., 1998. Studies on the salinity tolerance of the venerid clam, *Paphia malabarica* (Chemnitz). *J.mar. biol. Ass. India*, 40(1&2):185 - 188.

Ranade, M. R., 1964. Studies on the biology, ecology and physiology of the marine clams. Ph. D. thesis, University of Bombay, 286 pp.

Ranade, M. R. and Kulkarni, C. V., 1972. Observations on the behaviour of clams in warters of low salinity. *J. Bombay Nat. Hist. Soc.*, 69(3):616-634.

Rao, K. S., 1941. Indian shell fish and their fisheries. *Sci. and cul.*, 7:69-78.

Rao, K. S., 1967. Annual Reproductive cycle of the wedge clam, *Donax cuneatus* Linnaeus. *J. mar. biol. Ass. India*, 9(1):141-146.

Rao, K. S. and Rao, G. S., 1989. Experimental clam culture at Mulki, Dakshina Kannada. Proc. Symp. Coastal Aquaculture, Mar. Biol. Ass. Ind., 2:557-560.

Rao, K. V., 1951a. Studies on the growth of *Katelysia opima* (Gmelin). Proc. Indo-Pacific Fish. Council, Sec.11.

Rao, K. V., 1951b. Observations on the probable effects of salinity on the spawning, development and setting of the Indian backwater oyster *Ostrea madrasensis* (Preston). Proc. Indian Acad. Sci., Sec.B., 33:231-256.

Rao, K. V., 1963. Edible molluscan shell fish of India. *Indian Fish. Bull.*, 103:23-27.

Rasalam, E. J. and Sebastian, M. J., 1976. The lime shell fisheries of Vembanad Lake, Kerala. *J.mar. biol. Ass. India.* 18(a):323 - 355.

Read, K. R. H., 1962. Respiration of the bivalve molluscs *Mytilus edulis* and *Brachidontus plicatulus* as a function of size and temperature. *Comp. Biochem. Physiol.*, 7:89-101.

Robert, R., Toyt, G. and Laborele, J. L., 1993. Growth, reproduction and gross biochemical composition of the Manila clam, *Ruditapes philippinarum* in the Bay of Arcachon, France. *Mar. Biol.*, 116:291-299.

Rogers, W. E., 1959. Gonad development and spawning of soft clam. *Maryland Tide Water News*, 15:9-10.

Ropes, J. W., 1968. Reproductive cycle of the surf clam *Spisula solidissima* in offshore New Jersey. *Biol. Bull.*, 135(2):349-365.

Ropes, J. W. and Stickney, A. P., 1965. Reproductive cycle of *Mya arenaria* in New England. *Biol.Bull.*, 128:315-327.

Runnstorm, S., 1927. Ober die Thermopathie der fortpflanzung und endwicklung mariner tiere in Beziehung Zu ihrer geographischan Verbreitung. *Bergens. Mus. Arbog.*, 2:1-67.

Sakurai, I., Kurata, M. and Abe, E., 1996. Age structure and mortality of the Sydney Surf Clam, *Mactra chinensis* off Tomakomai, Southwest Hokkaido. *Fish. Sci.*, 62(2):168-172.

Salih, K. Y. M., 1979. Studies in biochemical composition of the clam, *Meretrix casta* (Chemnitz) off Cochin Bar mouth. *Cochin Univ. Bull. Mar. Sci.*, 47-73.

Samuel, D., 1976. A digenetic trematode infection in the edible oyster, *Crassostrea madrasensis* (Preston). *Indian J. Fish.*, 23(1&2):153-159.

Sastry, A. N., 1966. Temperature effects in reproduction of the Bay Scallop, *Acquipecten irradians* Lamarck. *Biol. Bull.*, 130:118 - 134.

Sastry, A. N., 1968. Relationship among food, temperature and gonad development of the bay scallop, *Acquipecten irradians* Lamarck. *Physiol. Ecol.*, 41:44-53.

Sastry, A. N., 1970. Reproductive Physiological variation in lattitudinally separated populations of the Bay Scallop, *Acquipecten irradians* Lamarck. *Biol. Bull.*, 138:56-65.

Sastry, A. N., 1979. Reproduction of marine invertebrates Pelecypode (excluding Ostreidae). *In*: Reproduction (eds. Giese, A. C. and Pearse, J. S.). Academic Press, New York. pp. 113-293.

Sastry, A. N. and Blake, N. J., 1971. Regulation of gonad development in the Bay scallop, *Acquipecten irradians* Lamarck. *Biol. Bull.*, 140:274-283.

Sbrenna, G. and Campioni, D., 1994. Gametogenic and spawning patterns of Manila clams *Tapes Philippinarum* (Bivalvia: Veneroida) in two lagoons of the River Po Delta, Italy. *J. Shellfish Res.*, 13:37-46.

Seed, R., 1976. Ecology. *In*: Marine Mussels; their ecology and Physiology (ed. Bayne, B. L.). Cambridge University Press, London. pp. 13-65.

Shafee, M. S., 1977. Studies on the various allometric relationships in the intertidal green mussel, *Perna viridis* Linnaeus of Ennore estuary, Madras. *Ind. J. Fish.*, 23:1-9.

Shafee, M. S., 1981. Seasonal changes in the biochemical composition and calorific content of the black scallop, *Chlamys varia* (L) from Lenveoc, Bay of Brest. *Oceanol. Acta*, 4:331-341.

Shafee, M. S., 1982. Variations saisonniers dela consommation d'oxygene chez petonde noir *Chlamys varia* (L) de Lenveoc (rade de Brest), *Oceanol. Acta.*, 5:189-197.

Shafee, M. S. and Daouadi, M., 1991. Gametogenesis and spawning in the carpet shell clam, *Ruditapes decussatus* (L.) (Mollusca: Bivalvia), from Atlantic coast of Morocco. *Aquaculture and Fisheries Management*, 22:203-216.

Shafee, M. S. and Lucas, A., 1982. Variations saisonniers dubilan energetique chez les individus d'une population de *Chlamys varia* (L): Bivalvia, Pectinidae, *Oceanol. Acta.*, 5:331-338.

Shafee, M. S., Berraho A. and Rafik, M., 1998. Culture of carpet - shell clam, *Ruditapes decussatus* (L) on the Atlantic coast of Moroco. *J. Aqua. Trop.*, 13(1):17-36.

Shaw, W. N., 1962. Seasonal changes in female soft-shell clam, *Mya arenaria* in the Tred Avon River, Maryland. Proc. Natl. Shellf. Ass., 53:121-132.

Shaw, W. N., 1965. Seasonal gonadal cycle of the male soft-shell clam, *Mya arenaria* in Maryland. U. S. Fish and Wildl. Serv., Spec. Sci. Rept., Fish. No.508, 5 pp.

Shumway, E. S., Baster J. and Stahlneeker J., 1988. Seasonal changes in oxygen consumption of the giant scallop, *Placopecten magellanicus* (comclin). *J. Shellfish Res.*, 7:77-82.

Silas, E. G. and Alagarswami, K., 1967. On an instance of parasitization by the pea-crab (*Pinnotheres sp.*) on the backwater clam *Meretrix casta* (Chemnitz) from India with a review on the work on the systematics, ecology, biology and ethology of pea-crabs of *Pinnotheres latereilla*. Proc. Symp. Crustacea, Mar. Biol. Ass. Ind., 3:1161-1127.

Silas, E. G., Alagarswami, K., Dharmaraj, S., Narasimham, K. A., Appukkuttan K. K. and Muthiah, P., 1982. Country Report - India. *In*: Bivalve culture in Asia and Pacific (ed. Davy, F. B. and Graham, M.). Proc. Workshop, Singapore, Ottawa, Canada. 34-43 pp.

Spear, H. S. and Glude, J. B., 1957. Effects of environment and heredity on growth of the soft clam (*Mya arenaria*). *Fish. Bull. U.S.*, 57(114):279-292.

Sreenivasan, P. V., 1983. Distribution of clams of *Meretrix casta* (Chemnitz) in Vellar estuary. Proc. Symp. Coastal Aquaculture, 2:561-563.

Stephen, D., 1977. Parasitic castration of the Indian backwater oyster, *Crassostrea madrasensis* (Preston) by the larval trematode *Bucephalus Sp. Sci. Cult.*, 43:387-388.

Stevenson, J. A. and Dickie, L. M., 1954. Animal growth rings and rate of growth of the scallop. *Placopecten magellanicus* (Gmelin) in the Digby area of the bay of Fundy, *J. Fish. Res. Ed. Can.*, 11:660-671.

Stickle, W. B. and Bayne, B. L., 1982. Effects of temperature and salinity on oxygen consumption and nitrogen excretion in *Thais* (nucella) *lapillus*. *J. exp. Mar. Biol. Ecol.*, 58:1-17.

Stites, D. L., Benke, A. C. and Gillespic, D. M., 1995. Population dynamics, growth and production of the Asiatic clam *Corbicula fluminea*, in a blackwater river. *Can. J. Fish Aquat. Sci.*, 52:425-437.

Strickland. J. D. H. and Parsons, T. R., 1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Canada*. 311 pp.

Sundaram, K. S. and Shafee, M. S., 1994. Salinity tolerance of some bivalves of Ennore estuary; *J. mar. biol. Ass. India*, 31(1 & 2):299-302.

Talikhedkar, P. M., Mane, V. H. and Nagabhushanam, R., 1976. Growth rate of the wedge clam *Donax cuneatus* at Miriye Bay, Ratnagiri. *Indian J. Fish.*, 23:183-193.

Taylor, A. C. and Venn, T. J., 1979. Seasonal variation in weight and biochemical composition of the tissues of the Queen scallop, *Chlamya opercularis*, from the Clyde sea area. *J. mar. biol. Ass. U. K.*, 59:605-621.

Thangavelu, R., 1983. Ecophysiology of the edible oyster *Crassostrea madrasensis* (Preston) from the Pulicat Lake, South India. Ph. D. Thesis, University of Madras. 228 pp.

Thangavelu, R. and Sanjeevaraj, P. J., 1985. Fishery and biologyof the clam *Meretrix casta* (Chemnitz) in the Pulicat Lake. *J. mar. biol. Ass. India*, 27(1 & 2):75-83.

Thangavelu, R. and Poovannan, P., 1994. On some aspects of ecology of the clam *Meretrix casta* (Chemnitz) in Muttukadu backwater. *J. mar. biol. Ass. India*, 36(1 & 2):77-80.

Thompson, R. J., 1977. Blood Chemistry, biochemical composition and the annual reproductive cycle in the giant scallop *Plaeopectes magellanicus*, from southeast Newfoundland. *J. Fish. Res. Board Can.*, 34:2104-2116.

Thompson, R. J. and Bayne, B. L., 1972. Active metabolism associated with feeding in the mussel *Mytilus edulis* L. *J. exp. Mar. Biol. Ecol.*, 8:191-212.

Thompson, R. J., Ratchcliffe, N. A. and Bayne, B. L., 1974. Effects of starvation on structure and function of the digestive gland of the mussel (*Mytilus edulis* L.). *J. mar. biol. Ass. U. K.*, 54:699-712.

Tranter, D. J., 1958. Reproduction in Australian pearl oyster (Lamellibranch) *Pinctada albina* (Lamarck): Primary gonad development. *Aus. J. Mar. Freshw. Res.*, 9:135-143.

Tumer, H. J. and George, C. J, 1955. Some aspects of the behaviour of the quahaug, *Venus mercenaria*, during the early stages. Eight Rept. on Invests. of Shellfish of Mass., Dept. of Nat. Res., 5-14 pp.

Utting, S. D. and Spencer, B. E., 1991. The hatchery culture of bivalve molluscs larvae and juveniles. Leafl. No. 68. MAFF Fish. Res., Lowesfoft, U.K. 31 pp.

Vahl, O., 1978. Seasonal changes in oxygen consumption of the Icelandic scallop Chlamys islandica (Muller, O. F.) from 70°N. Ophelia, 17 (1):143-154.

Van Dam, L., 1935. On the utilisation of oxygen by *Mya*. *J. Exp. Biol.*, 12:86.

Velez, A. and Epifanio, C. E., 1981. Effects of temperature and ration on gametogenesis and growth in the tropical mussel *Perna perna* (L.). *Aquaculture*, 22:21-26.

Venkataraman, R. and Chari, S. T., 1951. Studies on oysters and clams - Biochemical variations. *Indian J. Med. Res.*, 39:533-541.

Victor, A. C. C., 1984. Studies on the reproductive bionomics of selected marine bivalve molluscs (*Donax cuneatus* Linnaeus) Ph. D. Thesis. University of Madras.

Victor, A. C. C. and Subramoniam T., 1987. Reproductive biology of the wedge clam, *Donax cuneatus* Linnaeus. *Bull. Cent. Mar. Fish. Res. Inst.*, 24:177-188.

Von Brand, T. M., Nolen, O. and Mann, E. R., 1948. Observations on the respiration of *Australorbis gratratus* and some other aquatic snails. *Biol. Bull.*, 95:199-215.

Walne, P. R., 1974. The culture of marine bivalve larvae. *In*: Physiology of Mollusca. Vol. I. (ed. Hochackka, P. W.). Academic Press, New York and London. pp. 197-207.

Wells, W. F., 1927. Report of experimental shellfish station. Report. Rep. Cons. Comm. St. of New York. Conservation Commission, State of New York, 1926:1-26.

Widdows, J., 1978. Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*. *J. mar. biol. Ass. U. K.*, 58:109-124.

Widdows, J. and Shick, J. M., 1985. Physiological response of *Mytilus edulis* and *Cardium edule* to aerial exposure. *Mar. Biol.*, 85:217-232.

Wilson, B. R., 1969. Survival and reproduction of the mussel *Xenostrobus securis* (Lamarck) (Mollusca, Bivalvia, Mytilidae) in a Western Australian estuary. *J.Nat. Hist.*, 3:93-120.

Wilson, G. J., 1969. Population dynamics and energy budget for a population of *Donax variabilis* (Say) on an exposed South Carolina beach. *J. exp. Mar. Biol. Ecol.*, 239:61-83.

Wilson, J. H. and Hodgkin, W. P., 1967. Z comparative account of the reproductive cycle of five species of marine mussels (Bivalvia: Mytilidae) in the vicinity of Freneatle, Western Australia. *Aust. J. Mar. Freshw. Res.*, 18:175-203.

Wong, T. H., Lin, T. G. and Rai, H. S., 1986. Induced spawning, larval development and juvenile growth of *Anadara granosa* (L.) in the laboratory. Workshop on the biology of *Anadara granosa* in Malaysia, Jan. 22-23, Penang, Malaysia, 10 pp.

Zandee, P. I., Kluytmans, J. H., Zurburg, N. and Pieters, N. 1980. Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. *Neth. J. of Sea Res.*, 14:1-29.

Zeuthen, E., 1953. Oxygen uptake as related to body size in organisms. *Q. Rev. Biol.*, 28:1-12.

Zolorzano, L., 1969. Estimation of ammonia, *Limnol. Oceanogr.*, 14:799-801.