Studies on the Biology of the clam *Marcia opima* Gmelin from Kayamkulam Lake

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CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried out by Mr. T.K. Maqbool, under my scientific supervision and guidance in the Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science & Technology, in partial fulfillment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science & Technology under the Faculty of Marine Sciences, and no part thereof has been presented for the award of any other degree, diploma or associateship in any University.

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DECLARATION

I, T.K. Maqbool, do hereby declare that this Thesis entitled "STUDIES ON THE BIOLOGY OF THE CLAM MARCIA OPIMA GMELIN FROM KAYAMKULAM LAKE" is a genuine record of the research work done by me under the scientific supervision of Dr. K.Y. MOHAMMED SALIH, Reader, School of Marine Sciences, Cochin University of Science & Technology, and has not previously formed the basis for the award of any degree, diploma, or associateship in any University.

(T.K. MAQBOOL)

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CONTENTS

PREFACE

In the early dawn of classification of living forms, the word 'Mollusca' actually fortified a group of highly adaptive and diversified organisms. Of the major classes of the phylum mollusca, the lamellibranchs comprises the majority of the bivalves and constitutes the most successful organisms among the molluscan genus in the sea. The venerid bivalve, *Marcia opima*, constitutes the newly recognised genus of mollusc, hitherto not reported in the coastal waters of Kerala State, India. Incidentally this species is acclaimed for its abundance and high fishery potential.

The untapped molluscan resource turnover in terms of calorific value, clubbed with the urgent human requirements, has resulted in the promotion of study leading to better understanding of its fishery. To a large extent, the recent awakening in the tempo of human desire towards understanding the molluscan biology associated with the firm belief that the knowledge would help to improve its fishery and cope up with the demands of animal and human nutrition, shall alleviate the problems of malnutrition.

The backwaters of Kerala are highly prone to the toxic effects of various kinds of pollutants. A unique feature encountered in these waters are the coconut husk retting yards due to the plentiful availability of the raw material used in the manufacture of coir. The remarkable feature associated with retting is the depletion of oxygen leading to anoxic condition juxtapositioned with increase in hydrogen sulfide in the surface waters. This situation often depletes the fauna of the retting zone when compared to non-retting zones.

This investigation has been undertaken in the light of the above mentioned factors. The study addresses some specific biological and biochemical aspects and trends in bioconcentration of some selected metals on the aforesaid organism. The influence of pollutants from the coconut husk retting grounds on the clam is also highlighted.

Chapter I

INTRODUCTION

The world seafood industries are rapidly blooming, on being adequately supported by scientific know how. India, ranking among the world's leading nations, has to attempt at oceans of profit from the hitherto unexploited hidden treasures of the sea, amounting to over 4.5 million tonnes. This vast untapped wealth presents lucrative opportunities especially for this developing country, both to satisfy domestic requirements and earn foreign exchange. Interestingly, molluscs contribute a substantial part of the total fishery resource.

The phylum mollusca forms one of the largest divisions of the animal kingdom, for, probably hundred thousand different kinds of molluscs are now known. The name mollusca given to this group of animals date from the use of the word by Aristotle although he employed it only for the cuttle fish. The word actually means "soft-bodied" and except for the shells, they are quite soft bodied and have no other skeleton.

The phylum mollusca is represented by the highly active squids to the slow and sluggish snails; from the huge tridacna clams (upto 180kg) to minute wood-land snails. Adult molluscs vary in body length from 1 or 2 mm to over 22 meters as in some deep sea squids. They have a life span as short as a couple of months to more than 100 years (Sloan and Robinson, 1984) and occupy almost every type of ecological niche. They

are found in the deepest parts of the ocean; all bodies of fresh water including arctic pools and thermal springs, from tropical swamps to the vegetation on the highest mountains. Its world wide distribution and extreme abundance both in species and numbers of individuals could be the potential answer to problems related to malnutrition in developing and underdeveloped countries.

Clams remain a less exploited food resource, of great market potential which are adapted to various habitats, with fast growth rates and have high efficiency in converting primary productivity into body tissue. Though India does have a rich molluscan resources, it has not been properly utilized especially in the case of bivalve fishery. Thousands of square kilometers of our coastal seas, backwaters and estuaries form an ideal habitat for the growth of these bivalves. The clam meat has a high nutritive status and contains high percentage of glycogen, protein and minerals in an easily assimilable form. It is a fact that clam meat is not having as much demand in the domestic market as in the case of other fishes and a lions share of it is being now wasted unexploited. In a country like India, where nearly 30 % of the population is suffering from the consequences of malnutrition, it is of urgent necessity for educating the public, about the nutritive value of the clam meat towards maximum exploitation of the fishery resource.

Characteristic feature of the molluscs is a fleshy fold known as the mantle which surrounds most of the body,

forming a protective cavity for the gills or ctenedia, excretory and genital ducts; performs various functions such as formation of siphons, secretions of the shell and skeleton etc. In all the classes, except bivalvia, the foregut bears a toothed chitinous ribbon, the radula. This typical molluscan organ is used to rasp and tear food. There is no well developed brain, but there are several highly organised ganglia. By and large bivalvia form the second largest class of the mollusca. Clams, oysters, mussels, scallops and shipworms constitute this class.

Bivalvia are laterally compressed molluscs lacking head, radula and buccal mass. The sensory functions being delegated to the mantle margins. The single enveloping mantle secretes the two shell valves, connected by a mantle isthmus secreting proportionately less crystalline calcium carbonate and more elastic tanned protein to form the ligament. Associated with it is a thickening of dorsal margin of the shell valves to form hinge plate bearing hinge teeth and sockets. The number and arrangement of hinge teeth are important in taxonomic features of bivalvia. Variously developed pallial muscles include anterior and posterior adductor attached to each valve. Closure of the shell results from contraction of the adductor muscle, opposing the elastic property of the hinge ligament.

Bivalves are dioecious having the sexes in separate individuals. Eggs develop predominantly by spiral cleavage into first trochophore larva with a ring of hair like cilia

around the oral cavity and second, in many marine forms, into a free swimming veliger stage. Although widely distributed in aquatic habitats, burrowing mode of locomotion, their filter feeding properties and pelagic development prevented them from becoming established on land.

Presently on an average, only about 10 gram of fishery product per day per head is consumed in India, which is very low compared to the 275 gm per day per head available in Japan. The demand for fishery products by the turn of this century is estimated at 15 million tonnes. Even at 50% level of contribution from marine fisheries, it should produce 7.5 million tonnes which would be about 4 times the present level of input. In order to meet the required level of demand, we have to find unconventional sources apart from the conventional ones. In this regard, molluscs have great prospects to constitute the unconventional sources. A 65 fold increase was achieved in the Indian export of frozen clam meat from 16 tonnes in 1981-82 to 1033 tonnes in 1984-85, which indicates the potentiality of this resource. Increase in the bivalve fishery production attained world wide indicate that the requisite knowledge is being attained in scientific exploitation and fishery management to reach new heights. According to FAO estimates (Anon, 1991) the Indian marine mollusca production was 3000 tonnes in 1989 while that of N.Korea during the same period was 200,000 tonnes.

The world sea food leans heavily on scientific information for fishery enhancement and optimum exploitation.

Of all the fish consumed by man, edible bivalves are especially suited for cultivation because they are sessile and can be cultivated without caging or other barriers. They have a high degree of productivity owing to the faster growth rate and constitutes low level in the food chain. Unfortunately, bivalve fishery has not been properly managed and well exploited. It has an immense potential of development if challenged in a scientific manner.

Marcia opima forms the dominant species of the benthic community of the barmouth region of Kayamkulam lake. The distribution of species is not continuous along the Kerala coast but limited to some isolated patches; Ashtamudi and Kayamkulam barmouth regions. Still the clam form an integral part of the clam fishery and a record of a substantial quantity of clam landing during 1982-83 is available (Appukuttan et al., 1985) from Ashtamudi backwaters. However no study has been conducted on the species in order to bring out the biological and biochemical aspects as well as on the factors adversely affecting its survival, which is fundamental for proper exploitation of the species.

This study on the species deals with identification, distribution and population density of the clam; studies on the physical and chemical characteristics of the environment which are likely to have influence on the survival and reproduction. This would help in identifying the conditions favourable for the growth of the organism, as well as those adversely affecting the growth. Biological aspects like size at sexual

maturity and reproductive cycle were studied using histological section of gonads. A proper understanding of the reproductive biology was also focussed especially for the scientific management of the natural fishery and also for artificial culture. This study also covers the quantitative analyses of biochemical components, which provides information of nutritive status of the organism and its seasonal variation in relation to physiological and ecological factors. The relative importance of various substrates, their sites of storage and timing of utilisation in relation to seasons vary between species as well as between population of same species (Barber and Blake, 1981; Bayne, 1976). Energy storage usually occurs during favourable seasons with nutrient abundance, which is utilized during periods of energy demand like unfavourable periods and spawning (Ansell and Trevellion, 1976; Bayne, 1976). These aspects were also probed into during the course of this study. The estimation of bioconcentration of some metals and the toxicology of pollutants from coconut husk retting yards was also under taken. These information, it is hoped would provide support for the scientific exploitation that would help to cope up with the growing demands of molluscan fishery and to disencumber the problems that adversely affect the fishery potential of this clam.

The clam with its high potential and nutritive value is suitable for culture in a large scale and can also be transplanted to similar areas of the southwest coast of India. This study conducted on different aspects of the clam would

definitely help to understand the problems facing the fishery as well as those involved in the culture of such species. The thesis has been divided into six chapters. The first chapter sets the stage to introduce the topic. A brief description of the morphological, ecological and fishery aspects of the clam is given in the second chapter. The third chapter describes the reproductive biology in the light of the histological studies conducted. Seasonal change in the biochemical constituents has been assessed in the fourth chapter, while the seasonality of mineral accumulation has been given in the fifth chapter. Some aspects dealing with the impact of pollution due to the coconut husk retting on the species selected for the study has been described in the sixth chapter. Salient features of the investigation has been highlighted in the seventh chapter followed by a list of references.

Chapter II

MARCIA OPIMA
MORPHOLOGY, ECOLOGY AND FISHERY

The vast expand of clam beds located near the barmouth of the Kayamkulam lake include different species of venerid bivalves like *Villorita cyprinoides*, *V.cyprinoides* var *cochinensis*, *Meretrix meretrix* and *Tapes* sp. In the present study, an attempt was made to identify a species which was initially identified as *Katelysia opima* Gmelin, 1791 using the available taxonomic literature. However, it was later reidentified* as *Marcia opima* Gmelin 1791 by Dr.Akimichi Hosomi and Prof. Habe of Japan and the identity was confirmed by Dr. Graham Oliver of National Museum of Wales.

M. opima, has acclaimed considerable importance, both as a source of protein food and in the use of its shell, as a raw material for the manufacture of lime, cement and paints. These clams inhabiting the Kayamkulam lake close to the barmouth are being reported from this area for the first time. A scientific study of the species, covering the biological and biochemical aspects, along with the environmental factors supporting the survival of the species is of vital importance and has been attempted here as a pioneer work regarding the species.

Large quantities of *M. opima* are collected daily by hand picking and by using hand operated dredge by the local fishermen, along with small proportion of *Meretrix casta* and

* - paper communicated

Tapes spp. John (1958) recorded other bivalves from Kayamkulam lake.

The genus has been reported from Maunmagan, Lower Burma by Ray (1977). *M. opima* has been reported from Arabian Gulf by Kathleen (1970) and studies on *M. japonica* have been reported from Hong Kong (Kueh, 1987). Literature on the scientific study of the species is lacking from any part of India. The genus is not mentioned either in the taxonomical records like Genera of recent mollusca, Fauna of British India and Bulletin, Madras Government Museum.

However, the fishery of the species has been reported from Ashtamudi backwaters, harbour south of Kayamkulam barmouth by Appukuttan *et al.*(1985), mentioning the species as *Katelysia opima*.

The systematic position of the species is

Phylum	:Mollusca
Class	:Bivalvia
Super Family	:Veneracea
Family	:Veneridae
Genus	:*Marcia*
Species	:*opima*

MORPHOLOGY

Marcia opima has an inflated shell and occurs in different shades of brownish cream and dark grey colours. The shell surface is variously patterned with dark markings radiating from the umbo towards the shell margin (Fig. II.1). The shell surface is more or less smooth with the shell rings not formed under normal conditions. It has a well defined, heart-shaped lunule and an elongated hinge ligament (Fig. II.2).

FIG. II. 1 *Marcia opima*

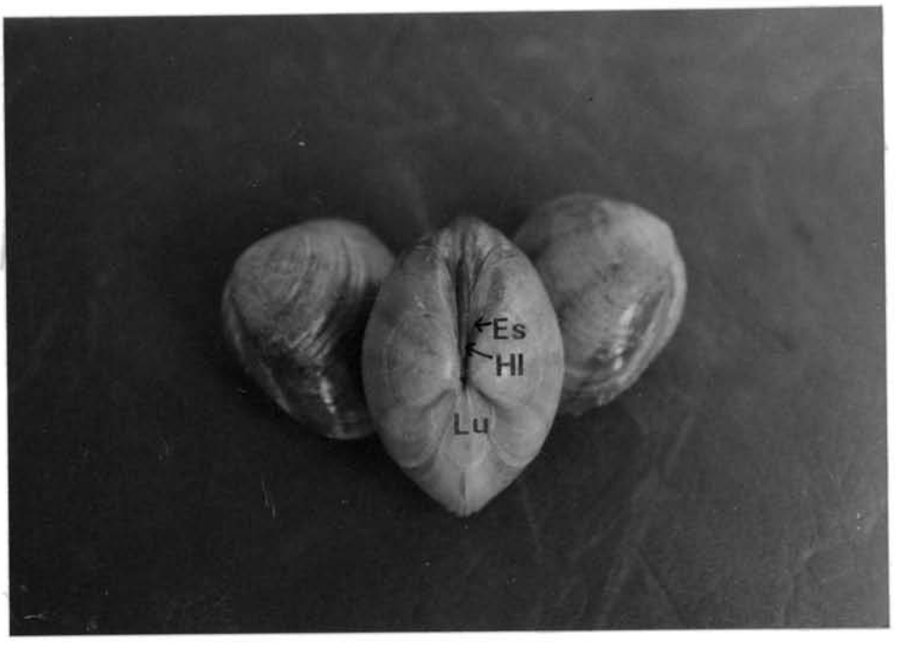

FIG. II. 2

Lu - Lunule, Hl - Hinge ligament, Es - Escutcheon

Escutcheon is weakly defined with a light ash colour, which merges with the colour of the shell surface. The pallial line is deeply sinuate (Fig. II.3). Shell is thin and the maximum shell length recorded was 58mm.

M. opima has long siphons and these clams are seen at a depth of 2-3 inches in the muddy sand substratum. The clams are found to prefer high salinity and they occupy the regions close to the barmouth. Often minor changes in the position of the clam-bed were noticed, which could either be due to exploitation or changes in the nature of the substratum by the wave action. A maximum density of 548 clams per meter square was recorded during the study period. None of the clams used for the present study was found to be infested by pea-crab, which is noticeably high in *Meretrix casta* in the same locality.

ECOLOGY

The clam-bed is located close to the barmouth of Kayamkulam lake. The Kayamkulam Lake lies between 9°2' and 9°16' N ; 76° 26' and 76°32' E, almost parallel to the south-west coast of India (Fig. II.4). The backwater system, which is narrow and elongated, except near the barmouth, is separated from the sea by a narrow strip of sandy beach. The average tidal range is about 1 Metre near the barmouth, which diminishes to the interior and thus the backwater provides marine, estuarine and fresh water environments.

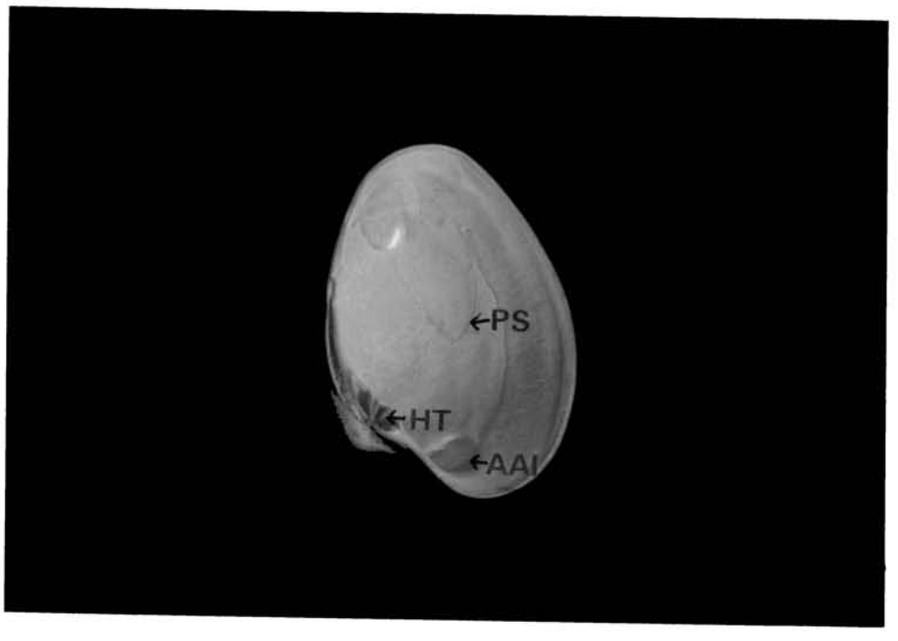

FIG. II.3

PS - Pallial Sinus, AAI - Anterior Adductor Impression

HT - Hinge teeth

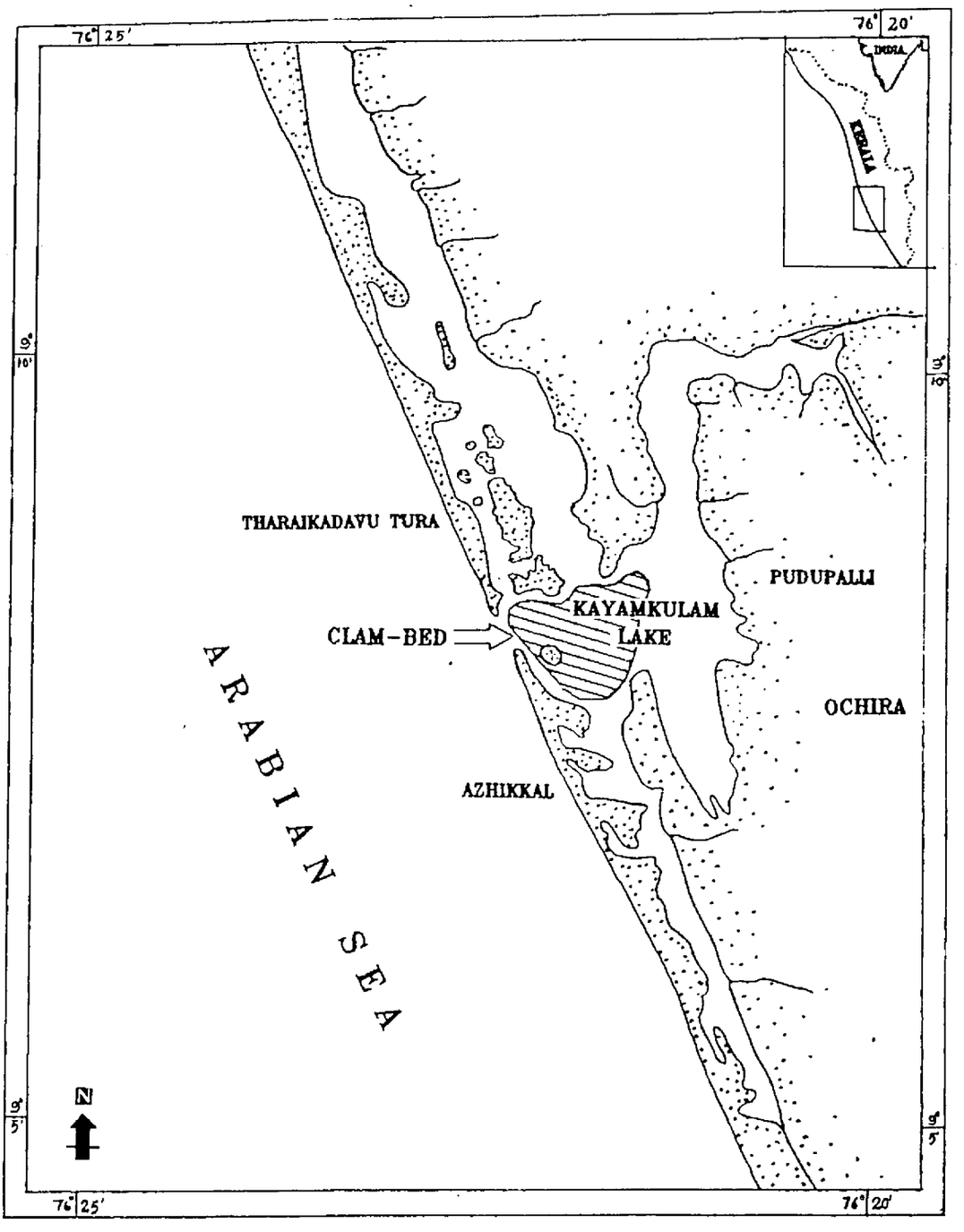

FIG. II.4

MAP OF KAYAMKULAM LAKE SHOWING THE CLAM-BED

Studies on the ecological factors and regional distribution and range of fauna of Kayamkulam lake was made by John (1958). Other important studies on the ecological factors of Kayamkulam lake include those of Nair (1971) on the hydrology of Kayamkulam estuary and Antony (1975) who conducted studies on the distribution of foraminifera from Kayamkulam lake, correlating between its faunistic composition and salinity. Gopakumar (1992) studied the effects of sulphates on the biology of the prawn *Penaeus indicus* from Kayamkulam lake.

The Kayamkulam estuary differs from a typical estuary since the barmouth, which opens to the Arabian Sea, remains closed by a sand bar for a period of almost three months in the summer. This phenomenon is largely responsible for the marked variation in the hydrographic features of the estuary. The rivers Pampa and Achankoil which flow into the lake, empty large quantity of fresh water during the monsoon season (i.e. June - September). Kayamkulam lake is very shallow (4 - 6 feet) except the deeper inland navigation channel which connects Alappuzha and Kollam. The sediment is sand/silt/clay type and the proportion of sand decreases considerably from the barmouth towards the eastern parts.

The large quantity of fresh water discharged into the lake during monsoon creates a marked difference in the salinity of the lake, (Fig. II.5) especially when the barmouth remains closed. This is an important factor adversely affecting the fishery of the area. The salinity recorded show maximum value

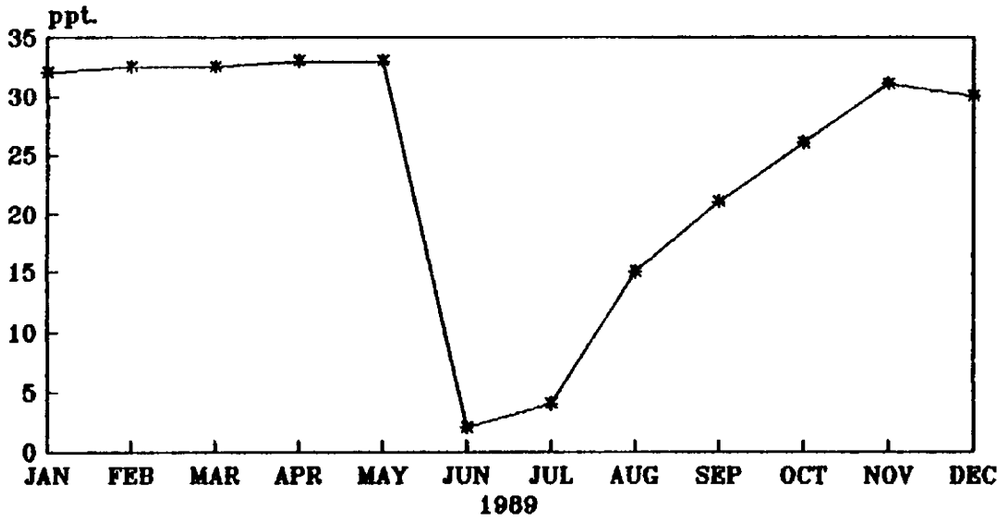

FIG. II.5

VARIATION OF SALINITY IN THE CLAM-BED

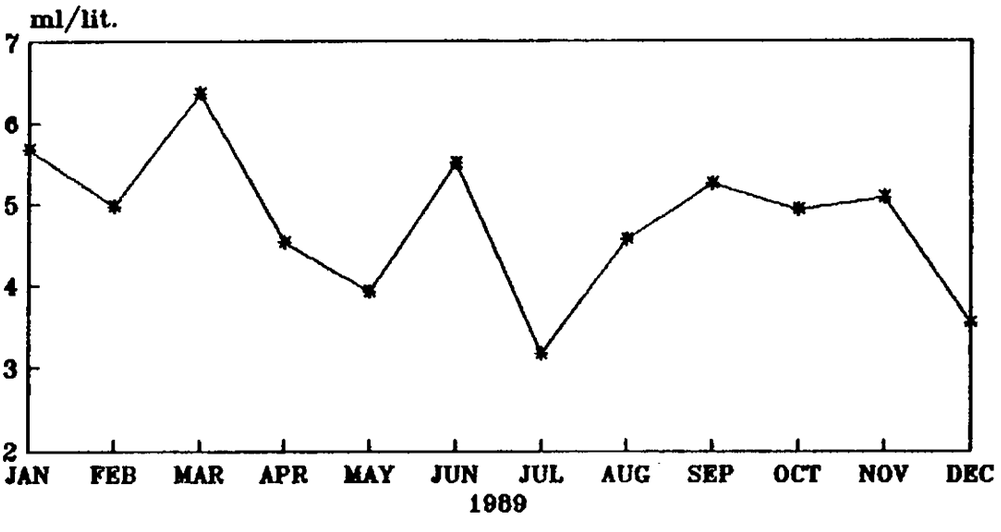

FIG. II.6

VARIATION OF DISSOLVED OXYGEN IN THE CLAM-BED

in the month of May (33 ppt.). Though water is more saline near the barmouth, generally, during the summer season it is seen to be more or less uniform in the surrounding parts also. The monsoon flood reduces the salinity considerably. A minimum salinity of 2 ppt. was recorded in the month of June. Details of the salinity recorded during this study period are presented in Table II. 1. The salinity was determined by using Knudson's argentometric titration method (Grasshoff, 1983) and the values given in the table are the average of salinity values recorded from 3 stations.

The barmouth is usually cut open by the local fishermen during the outbreak of monsoon. A scientific monitoring of the hydrographic parameters and the process of opening of the barmouth would be helpful in protecting the fishery resources to a large extent.

The quantity of dissolved oxygen in the vicinity of the clam-beds was estimated using Winkler method. The oxygen levels did not provide any clear seasonal variation in its distribution (Fig. II.6). Comparatively lower values of oxygen were recorded during the pre-monsoon, except during March,while it recorded high values in the post-monsoon period.

FISHERY

Kayamkulam Lake is endowed with finfish and shellfish resources. Majority of the local population in this area depends on the fishery, agricultural resources and coir industry. The clam resource forms the low cost and easily

TABLE II·1

VARIATIONS IN SALINITY AND DISSOLVED OXYGEN IN THE CLAM-BED.

No.	MONTH (1989)	SALINITY ppt	OXYGEN ml/l
1	JANUARY	32.0	5.67
2	FEBRUARY	32.5	4.97
3	MARCH	32.5	6.37
4	APRIL	33.0	4.53
5	MAY	33.0	3.92
6	JUNE	2.0	5.50
7	JULY	4.0	3.16
8	AUGUST	15.0	4.56
9	SEPTEMBER	21.0	5.24
10	OCTOBER	26.0	4.93
11	NOVEMBER	31.0	5.08
12	DECEMBER	30.0	4.54

TABLE II.2

CLAM LANDING

No.	MONTH (1983-84)	% MEAT WEIGHT	MONTHLY AVERAGE (Kg.)
1	MARCH	17.40	303750
2	APRIL	28.24	258750
3	MAY	17.80	292500
4	JUNE	16.12	492000
5	JULY	13.27	494000
6	AUGUST	13.46	475000
7	SEPTEMBER	15.82	361000
8	OCTOBER	--	455000
9	NOVEMBER	15.29	604500
10	DECEMBER	--	581150
11	JANUARY	23.62	581150
12	FEBRUARY	16.27	634750

(According to Appukuttan et al., 1985).

available food of the poor class of the community. The clams are locally called as "Poovan kakka" which has a better market, owing to their larger size, than the *Tapes* sp. and the *Meretrix casta*, which are locally called "Mona kakka" and "Eela kakka" respectively.

About 30 to 40 people are engaged in clam collection, which reaches upto 200 of them during peak season. Clams are collected in bamboo baskets by local fisher folk by hand-picking from the shallow regions of the lake. Hand operated dredges are used to collect clams from deeper region. The dredge consists of a long nylon net basket, attached to a circular metal ring at the end of a bamboo pole and operated by two men from a boat. This craft is locally known as "Kuthukoruvala". The dredge is driven into the substratum and dragged 1-2 meters with the help of long rope attached to the ring. The sediment collected along with the clams is washed off by shaking the net bag in water. The clams are emptied into the boat. About 50 to 300 Kg. of clams are collected by each clam picker per day according to availability. Maximum landing has been recorded during September to November.

The total landing of the clam from Ashtamudi and Kayamkulam lake in the year 1982-83, was 5436.5 tonnes, 95% of which was exported as frozen clam meat (Appukuttan *et al.*, 1985).

The clam dealers purchase the clams from the fisher folk. The cost of clams is about 20 to 40 paise per Kg. and that of clam meat is Rs. 3.50 to 7.00 per Kg depending on the

size. The live clams are left in cement tanks having wire-mesh bottom filled with filtered sea-water for a period of 18-24 h inorder to depurate. The clam meat is then separated from the shell by boiling in water, major share of which is selected for export and the rest is marketed locally. The monthly data of percentage meat weight and total landing of the clam, recorded during March 1982 to February 1983 (Table II.2) by Appukuttan *et al.* (1985) indicates that there has been a notable increase in the fishery from 2.58 lakh to 6.3 lakh Kg, per month, which have earned a substantial foreign exchange.

An increase in the landing of the clam was noticed during 1989, which could be attributed mainly to the increased demand of the clam in the export market. However there has been a decrease in the yield during the subsequent years. It is probably due to the unscientific exploitation, undesirable fluctuations in the environmental factors, effects of pollutants etc. Here an attempt is made to evaluate these factors.

Chapter III

REPRODUCTIVE BIOLOGY

INTRODUCTION

Reproduction is an important physiological process, for the sustenance of life. This is so in bivalves too. Although in most of the bivalves the reproductive system is simple, an immense quantity of energy is spent for this process. Sexes are separate in these molluscs and the gametes are discharged via gonadal ducts into the mantle cavity and from there into the surrounding water along with the exhalent water. After the external fertilization, the larval development takes place in the ambient media. Some bivalves exhibit special adaptations such as hermaphroditism, sex reversal, incubation of developing young etc. In species like *Marcia opima*, where the fertilization is external, chances are more for the interference of external factors. Failure in the reproductive activity may result in serious damage to the population structure and thereby productivity. An understanding of the biology of the organism is therefore essential for the proper management of fishery and the optimum exploitation of the resources.

The pattern of reproduction differs with species according to various intrinsic and extrinsic factors. Some exhibit year-round spawning and others spawn once, twice or thrice a year. The pattern of reproductive cycle was found to

be related to the geographic distribution of the bivalve. A comparative study in this respect has been made by Giese (1959) in polar, temperate and tropical bivalves.

The processes of reproduction in bivalves involve germ cell differentiation, gonad development, maturation, spawning, fertilization and larval development. Studies of gonadal smears, histological preparations of gonads, monitoring of spawning and larval development are the methods that can be followed to reveal the reproductive biology of bivalves.

REVIEW OF LITERATURE

A number of investigations has been reported on the reproductive biology of bivalves from India as well as from abroad. These include the earlier studies on *Crassostrea madrasensis* (Hornell, 1910), gonadal changes in *Venus mercenaria* (Loosanoff, 1937) and primary gonadal development in *Teredo navalis* (Coe, 1943).

Abraham (1953) made a detailed study on the biological aspects including growth, breeding habits, longevity and mortality of *Meretrix casta* in the Adayar backwaters. Chipperfield (1953) studied the breeding habits and settlement of spat of *Mytilus edulis*. A detailed investigation on the reproduction of Australian pearl oyster, *Pinctada albina* which included primary gonadal development, gametogenesis, breeding and sexuality which provide valuable information on the cytological aspects of reproduction was conducted by Tranter 1958 a, b and c). Mason (1958) reported the gonadal

development, spawning, fertilization, development of larvae and spat of *Pecten maximus*.

Loosanoff (1962) investigated the stages of gonadal development and spawning pattern of the oyster *Ostrea edulis*. Studies were conducted on the seasonal changes in the gonadal development and spawning in the clam *Meretrix casta* and that of *Crassostrea gryphoides* (Durve 1964; 1965). Alagarswami (1966) made a detailed study on the growth, reproduction and percentage edibility of the clam *Donax faba*. George and Nair (1973) reported the reproductive cycle of the mussel *Musculista arcuatula*. Giese and Pearse (1974) described the patterns of reproduction in bivalves.

A comparative study of reproductive cycle, growth and mortality and their influence on population structure of *Modiolus modiolus*, *Cerastoderma edule* and *Mytilus edulis* was presented by Seed and Brown (1975). Rao *et al.*(1975) reported the growth rate, breeding periodicity and larval abundance of the *Mytilus viridis* from Panaji and Vengurla and indicated the scope for its culture. Nagabhushanam and Mane (1975a) reported on the reproductive biology of *Katelysia opima* from Kalbadevi estuary. Rao *et al.* (1976) studied the spawning, fertilization and larval settlement of *Mytilus edulis*. Nagabhushanam and Dhamne (1977) studied the reproductive cycle of the clam *Paphia laterisulca*. Salih (1977) made a detailed study on the reproduction and environmental factors influencing the reproductive process of the clam *Meretrix casta* from Cochin. In the investigations done on the reproductive biology of the

wedge clam *Donax cuneatus*, Nagabhushanam and Talikhedkar (1977) found only one extended spawning cycle with no resting period.

Braley (1982) observed the reproductive periodicity in *Saccostrea cucullata*. Investigations on the gametogenic stages and detailed study on the reproductive cycle, spawning periodicity, spat fall, size at sexual maturity and sex ratio of the oyster *Crassostrea madrasensis* were conducted by Joseph and Madhyastha (1982 and 1984). Sloan and Robinson (1984) studied the age and gonad development of *Panope abrupta*, which had a century long reproductive period.

Wilson and Simons (1985) studied the gametogenesis and breeding of oyster, *Ostrea edulis* and they formulated an equation for predicting the onset of maximum ripeness of populations of oysters with the help of data obtained from histological preparations. The reproductive cycles of 3 bivalves *Meretrix meretrix*, *Meretrix casta* and *Katelysia opima* were traced by observing the degree of gonadal development and larval abundance by Jaybal and Kalyani (1986). Brousseau (1987) studied the reproductive cycles of three populations of *Mya arenaria*. Studies were conducted on the gametogenesis in the hard clam *Mercenaria mercenaria* and year round spawning with three annual peaks was recorded (Hefferman *et al.*, 1988). Morales-Alamo and Mann (1988), studied the ratio of gonad to body area in histological sections of *Crassostrea virginica*, investigated its reproductive cycle.

Knaub *et al.* (1988) found that *Mercenaria* stocks from two different habitats showed difference in spawning habits.

Hesselman *et al.*(1989) studied the reproductive cycle of the hard clams *Mercenaria* spp. Heffernan *et al.* (1989) reported the gametogenesis in *Crassostrea virginica* and found the female-male ratio as 3:1. Morton (1990) studied the reproductive cycle of *Saccostrea cucullata* and it was found to have a single extended spawning annually. Barkati and Ahmed (1990) studied the reproductive biology of *Mytilus edulis*. Shafee and Daoudi (1991) studied the gametogenesis and spawning in the carpet shell clam *Ruditapes decussatus*. Baron (1992) investigated the reproductive cycles of *Mactidea striata*, *Crafrarium timidum* and *Anadara scapha*. Ruiz *et al.* (1992) found that availability of food play important role in the reproduction of *Ostrea edulis*.

Bivalves exhibit gonochorism and hermaphroditism. Sex reversal is not a rare phenomenon. Mason (1958) reported hermaphroditism in *Pecten maximus*. Sex change by *Crassostrea gryphoides* from male to female during monsoon period has also been reported (Durve, 1965).

Role of environmental factors on reproductive cycle is often noticed. Nagabhushanam and Mane (1975b) envisaged the relationship of reproductive cycle of *Mytilus viridis* to salinity and temperature levels of the area. Influence of salinity on reproductive cycle and biochemical composition of *Crassostrea madrasensis* has been revealed (Stephen, 1981 a and b). Dinamani (1987) studied the reproductive patterns of two populations of *Crassostrea gigas* and related it to temperature. Sukumar and Joseph (1987) found that in *Saccostrea cucullata*

the gametogenesis was related to increase in salinity, whereas decrease in salinity favoured spawning.

Robinson (1989) suggested optimum temperature of 26°C and optimum salinity of 25 ppt for the larval rearing of Kumamoto oyster *Crassostrea gigas* after studying the reproductive cycle and conditioning trials of the oyster. Newell *et al.*(1989) investigated the factors regulating the reproduction and recruitment of American oyster, *Crassostrea virginica* and found that food availability had little influence on the larval settlement but low salinity had an adverse effect on survival rate.

Sphigel (1989) found that gametogenesis in European flat oyster, *Ostrea edulis* occurred in salinity as high as 41 ppt. Gauthier and Soniat (1989) compared the gonad/body ratio of Louisiana oysters and ambient temperature from four sites and significant positive correlation was recorded. Sprung (1991) found that increase in temperature can cause spawning in the bivalve, *Dreissena polymorpha*. Brey and Hains (1992) recorded single reproductive cycle in *Lissarca notorcadensis*, from Antarctic, but egg and embryonic development required two years period.

M. opima is of considerable importance in the clam fishery of Kerala. Taxapositioned with reporting the species from Kayamkulam lake, this study has also been made to find out its reproductive biology. However more emphasis has been given to the studies of gonadal smears and histological preparations. This is so because detailed study on the larval development was

hindered due to the occurrence of minor populations of *Meretrix casta*, *Tapes* sp., and *Villorita cyprinoides* along with *M.opima* in the natural population and therefore monitoring was difficult as differentiating the species during the early stages was impossible. The histological studies conducted for a period of two consecutive years reveal the reproductive pattern of *M. opima* in the area.

MATERIALS AND METHODS

The studies on the reproductive biology of *M. opima* were conducted for a period of two years from January 1989. The specimens were collected during the second week of every month. The clams were hand picked from the shallow regions of the clam-bed and were carefully transported to the laboratory in polyeurethene containers filled with water collected from the sampling site.

The clams were maintained in the laboratory in filtered sea water collected from the sampling site for a duration upto 24 h. About 30 clams falling under 20 - 26 mm size groups were sacrificed each month for histological studies on gonads. Care was taken to include equal proportions of males and females in each set of sample. Sexes were distinguished by observing fresh gonadal smears. The shells were opened with a scalpel and then the gonadal region was cut open and smeared on a clean slide with a few drops of sea water. The size of the ova was observed under a light microscope. Ova of 45 µm were considered as matured and those

below 45 μm as developing. The male-female ratio was recorded each month and chi-square tests were conducted.

Serial sections of a part (5 mm^2 approximate) of the large gonads were used for histological preparations which represent the reproductive stage of the clam, as the development being uniform in different regions of the gonads. The gonadal region close to the digestive diverticula was cut and fixed in Bouin's fixative for 24 h. The tissue was then washed for 5 minutes in running water, dehydrated in ethanol and embedded in paraffin wax (melting point 58-62°C). Serial sections of 7μ were made, and spread on slides smeared with Mayer's albumen, stained in Mayer's Haematoxylene, counter-stained in eosine and mounted in the D.P.X. mounting media. At least two slides were made for each clam and observed under light microscope.

The developmental stages of gonads were categorised according to the classification followed by Joseph and Madhyastha (1984). The indeterminate clams are grouped under 'I'. The male gonadal developmental stages were classified as follows 1) Early gametogenesis (MD1), 2) Late gametogenesis (MD2), 3) Mature (MD3), 4) Early regression (MR1) and 5) Late regression (MR2). Similarly the female gonadal developmental stages have been classified as 1) Early oogenesis (FD1), 2) Pre-vitellogenesis (FD2), 3) Vitellogenesis (FD3), 4) Mature (FR1), 5) Early regression (FR1) and 6) Late regression (FR2).

To determine the size at which the onset of first sexual maturity, clams upto 46 mm were collected and classified into different size groups with 2 mm class intervals.The gonadal development of each size group was determined using histological sections. Clams of ripe and regression stages were considered mature.

RESULTS

The outer wall of the soft body of *M. opima* is thin and translucent. Immediately below this is a thin muscular layer through which the colour variation of ripe male and female gonads can be noticed. The ripe male gonads appeared milky white, while that of the female is brownish cream in colour. Mature gonads are found to be the most conspicuous organs in the ripe clam. The follicles of gonads are highly branched tubules and they fully occupy the interior of the visceral mass between the viscera and outer wall. Connective tissues are found to occupy this region during the resting stage.

M. opima was found to be gonochoristic with no sign of sexual dimorphism. No case of hermaphroditism was noticed during the period of study. The clam underwent a resting phase in between the reproductive cycles, during which the reduction in gonadal size took place and the gonadal region was replaced by connective tissue. These clams which could not be separated into males and females are termed hereafter as indeterminate clams.

Size at first sexual maturity was 18 - 20 mm as 50% of the clams were found to reach maturity at this size (Table III.1). Gonadal appearance was not detected in the juvenile clams. Primary gonadal follicles appeared when the clams were at 14 to 16 mm in size and ripe gonads were seen in clams of 16 mm and above (Fig. III.1). Each follicle was bound by an outer follicular wall, where the gametogenesis occurred. In the early stages of development, all regions of the gonads looked similar, as the gross anatomy of gonadal follicles of both the sexes was similar. Later, as development progressed, mature gametes got concentrated towards the centre of the follicle, while younger developing ones remained attached to the follicular wall. The younger ová were elongated, while the mature ones were ovate to spherical in shape. In the ripe stage of gonads almost all the gametes were mature.

In the sex ratio estimated during the first year of this study (1988-89), out of the 288 samples analysed 129 were males, 134 were females and 25 were of indeterminate sex. In the succeeding year, 1989-90, out of the 391 samples 197 were males, 165 females and 29 indeterminates. Indeterminates were only few in number as they occur in the samples collected only during the resting stage after spawning.

In mature *M. opima*, the follicles were found to develop from the region of the digestive diverticula and spread all over substituting the inter follicular connective tissue and gametes of both the sexes developed from large resting cells dispersed around the follicular wall. The outer wall of

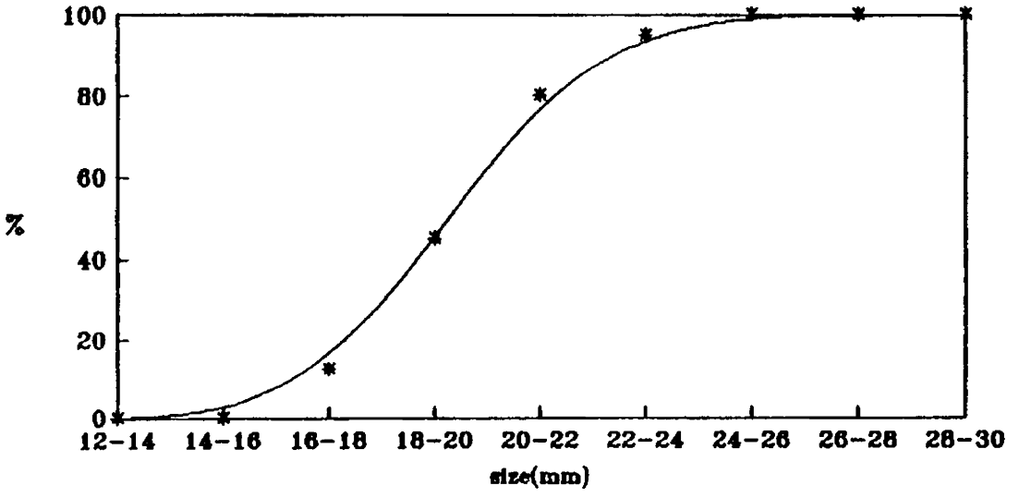

FIG. III.1
SIZE AT SEXUAL MATURITY

TABLE III.1
SIZE AT SEXUAL MATURITY

SIZE (mm)	IMMATURE	MATURE	% MATURE
12 - 14	10	00	0
14 - 16	10	00	0
16 - 18	14	02	13
18 - 20	08	07	47
20 - 22	02	13	87
22 - 24	01	17	94
24 - 26	00	16	100
26 - 28	00	10	100
28 - 30	00	10	100

the body was thin and translucent and the thin muscular layer immediately below this help in the contraction of gonadal follicles for the release of gametes.

Spawning was found to occur twice a year, a major spawning period was observed during November-January and a minor one during May-June. The stages of gonadal development also were seen to be repeated accordingly. The stages of development were determined from the observations made on the monthly samples analysed.

The stages of male gonadal development and spawning are classified under five divisions.

EARLY GAMETOGENESIS (MD1)

Gametogenesis started after the proliferation of follicles. Gametes started to develop from the stem cells present in the follicle walls. Most of the spermatids were found to form lightly staining centripetal bands inside the gonadal follicles along with a few free spermatids. Fully developed spermatozoa were not seen (Fig. III.2).

LATE GAMETOGENESIS (MD2)

In late gametogenic stage, most of the spermatocytes were observed to have developed into spermatids which, in turn, were in the process of development into spermatozoa (Fig. III. 3). Spermatozoa which were developed, assembled at the center of the gonadal follicles.

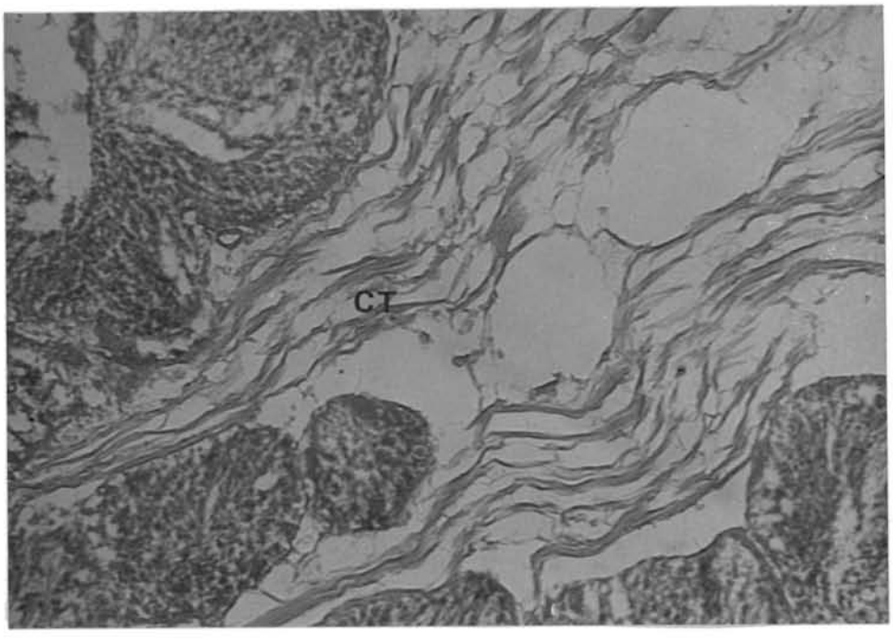

FIG. III. 2

EARLY GAMETOGENESIS (MD1) CT - Connective tissue

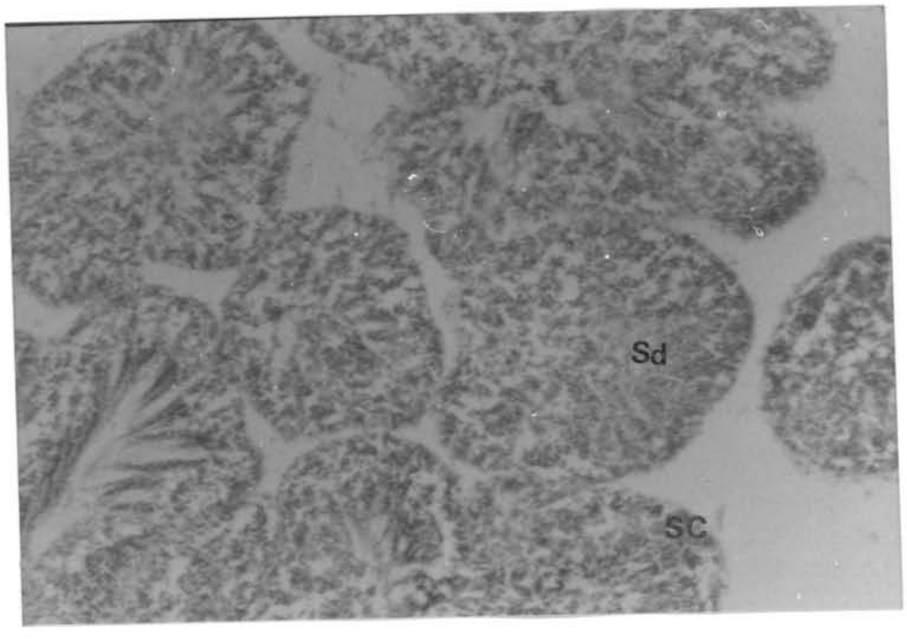

FIG. III. 3

LATE GAMETOGENESIS (MD2) SC - Spermatocyte, Sd - Spermatid

MATURE (MD3)

Almost all the spermatids subsequently developed into spermatozoa. No spermatocytes were seen during this stage, when gonads were filled with spermatids (Fig. III. 4). Clams existed in the ready-to-spawn condition at this stage.

EARLY REGRESSION (MR1)

Spermatozoa were seen being released through the gonadal ducts. Distal parts of the follicles were empty. Spermatozoa tended to concentrate towards the center of the gonads, forming thick bands (Fig. III. 5).

LATE REGRESSION (MR2)

Spawning was almost complete and follicles were empty except for a few residual gametes and were filled with a watery fluid. Phagocytic cells were present and reabsorption of residual gametes was in progress (Fig. III. 6).

RESTING (I)

Connective tissue was seen in the area that was occupied by the gonadal follicles in the earlier stages. Accumulation of reserve food materials took place. During this stage, sex of the clams was not distinguishable. In the resting stage between the spawning cycles, the follicles appear shrunken with no gametes retained. The connective tissue filled the follicles and inter follicular space. Gametes of

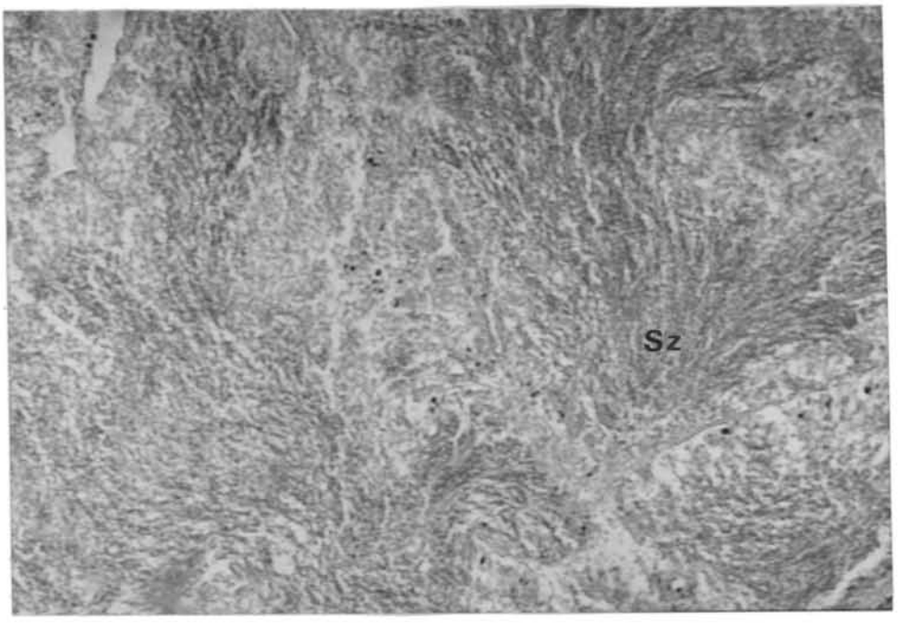

FIG. III. 4
MATURE (MD3) Sz - Spermatozoa

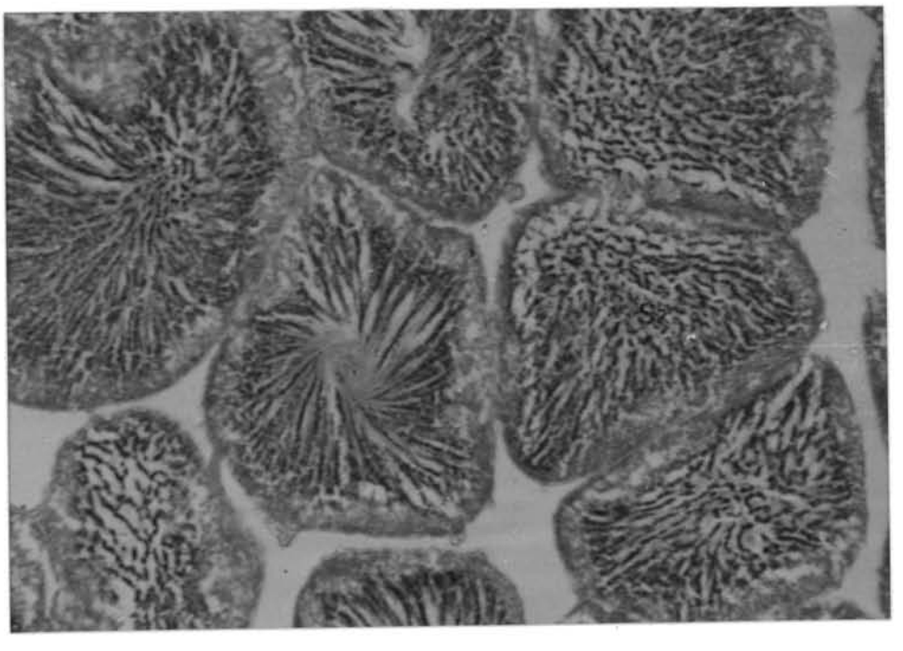

FIG. III. 5
EARLY REGRESSION (MR1) Sz - Spermatozoa

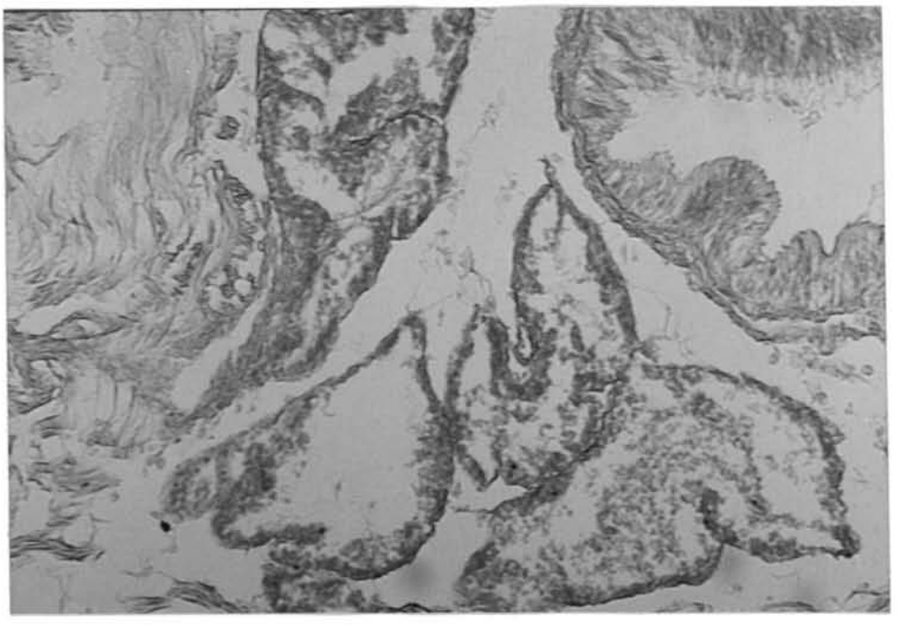

FIG. III. 6
LATE REGRESSION (MR2)

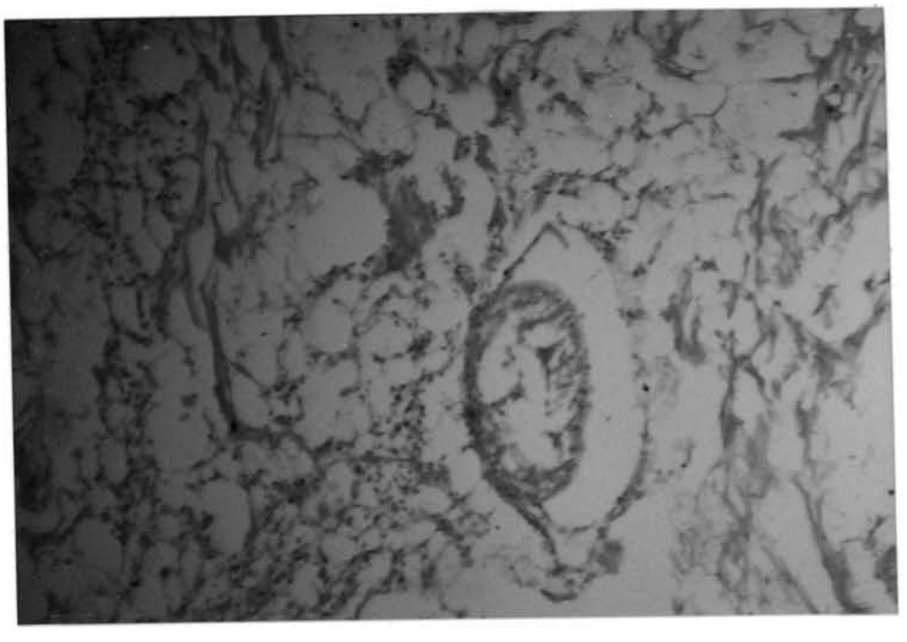

FIG. III. 7
RESTING (I)

both sexes were developed from large resting cells dispersed around the follicle wall (Fig. III. 7).

In the month of January, the male clams with spawning, spent and resting stages were recorded. 69 % of this was in the spawning stage, 24.1 % in the spent and 6.9 % in resting the stage. During February, the number of spawning clams went down to about 15 % marking the end of the spawning. 20 % was in the spent stage, while major share (35 %) was found to be in the resting stage, during which phagocytic reabsorption of cellular debris was completed and reduction in the size of gonads was observed. Gonads were found to be substituted by the connective tissue and some clams (30 %) having initial stages of gametogenesis were also observed.

The gametogenesis was continued in the next month and most of the clams (64.5 %) were in the late gametogenic stage. 12.9 % of the clams attained maturity while the remaining 22.6-% was in the early gametogenic condition. By April, most of them (71.9 %) were mature, 15.6 % was in the late gametogenesis and some of the clams (12.5 %) were spawning. Active spawning occurred in June with 72.4 % clams in the spawning and 27.6 % in the spent condition. The spawning continued and 80.6 % of the clams obtained in the month of May were in their spawning stage, which continued in June and some (9.7 %) had spent gonads while 9.7 % was in the mature state. Spawning almost seized and only 6.7 % of the clams in the spawning condition and 30 % in the spent stage was obtained in July. Almost 56.7-% of them was in the resting stage and 6.7 % in early

gametogenesis. There are chances of spent phase of male gonadal development being considered as resting stages as residual spermatozoa cannot be easily detected as in the case of ova due to their smaller size.

In August, gametogenesis started for the next reproductive cycle. 66.7 % of the clams was in their early gametogenic phase, 25 % in late gametogenic and the rest (8.3-%) had resting gonads. Gametogenesis proceeded and most of the (56.7 %) clams were mature in September. 23.3 % had early gametogenic and 20 % with late gametogenic gonads. In October, the clams (71.4 %) were in the ripe condition and 17.1 % was found to have started spawning while 11.4 % was in their late gametogenesis. The spawning continued in November (89.7 %) and no gametogenic follicles were observed and the rest (10.3 %) were spent. Some of the clams (37.5 %) were in the spent stage in December, while others (62.5 %) exhibited active spawning phase. The second spawning was found to be the major spawning and it continued in the month of January also.

The stages of female gametogenesis were divided into four divisions as followed by Joseph and Madhyastha (1984). Since the ova were considerably larger than spermatozoa, stages of development were easier to distinguish.

EARLY OOGENESIS (FD1)

Oogenesis started from the stem cells of the developing follicle walls. Being larger, early development was more easily distinguishable than in the case of males. Oocytes

had a diameter of 5 μm. Darkly staining chromosomes were also seen (Fig. III. 8). Developmental changes were rapid.

PRE-VITELLOGENESIS (FD2)

Pre-vitellogenic oocytes were found to develop in the follicle before attachment with the follicle wall. It had an average diameter of 11 μm and cytoplasm was basophilic (Fig.III. 9).

VITELLOGENESIS (FD3)

Oocytes got attached to the follicle walls by means of a broad base and they attained a pear shape with a size of 46 μm. Oocytes were seen to increase in size as yolk accumulated. Cytoplasm lost the basophilic nature at this stage (Fig. III. 10).

MATURE (FD4)

Follicles fully developed filling the whole lumen and were completely filled with mature oocytes of maximum size (Fig. III. 11).

EARLY REGRESSION (FR1)

Release of oocytes through the gonadal ducts was observed. As the release of oocytes continued, free space appeared towards the distal parts of the tightly packed follicles (Fig. III. 12).

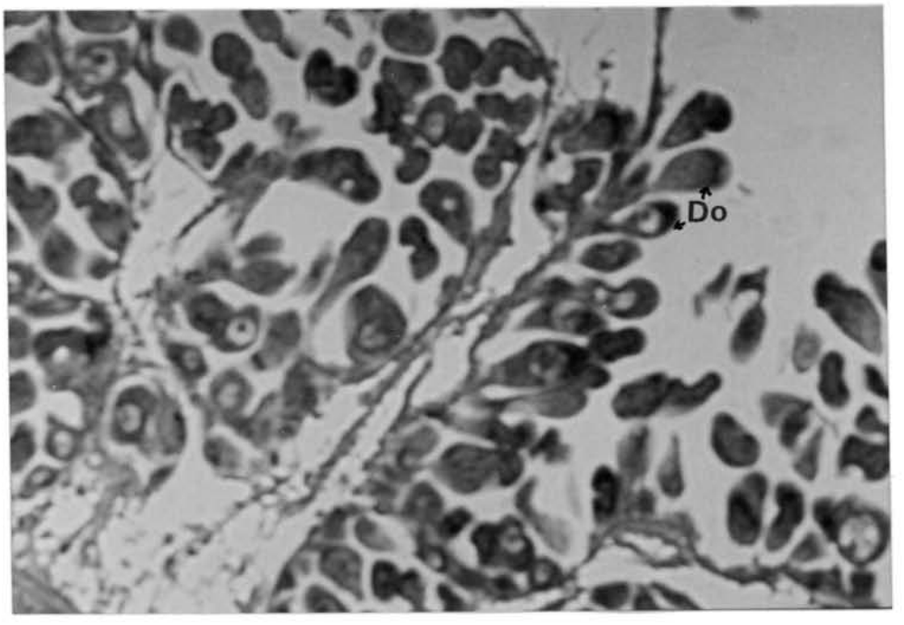

FIG. III. 8

EARLY OOGENESIS (FD1) Do - Developing Oocytes

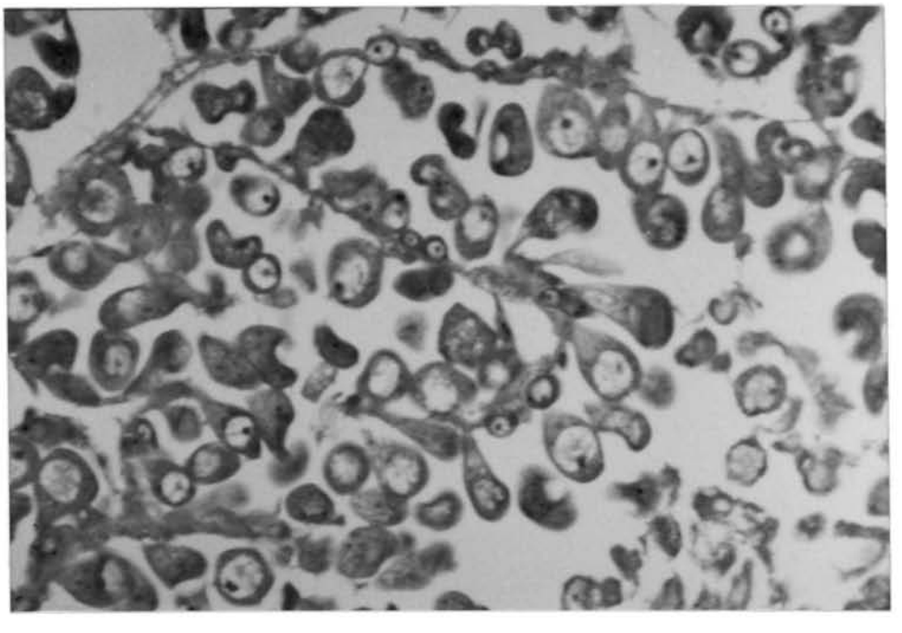

FIG. III. 9

PRE-VITELLOGENESIS (FD2)

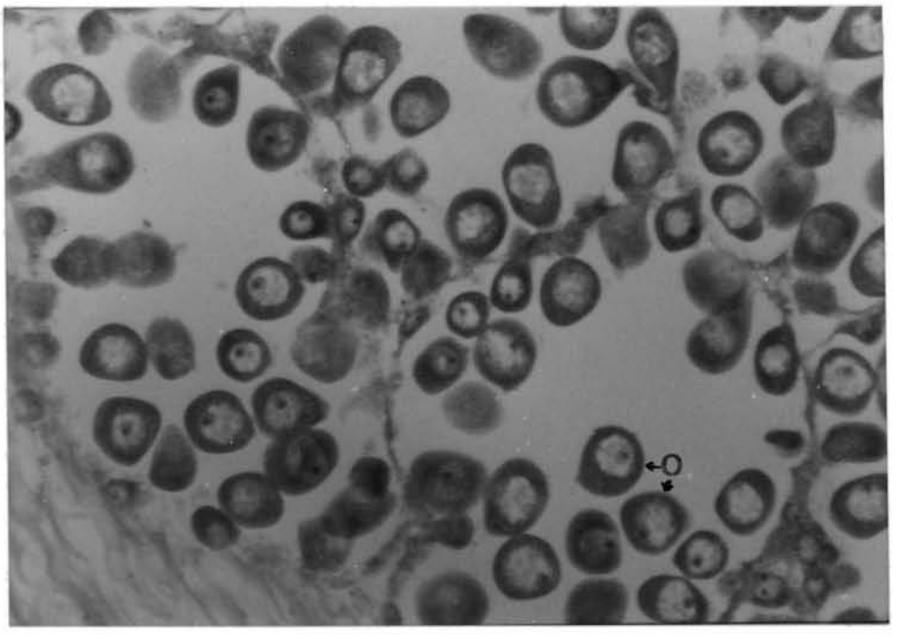

FIG. III. 10

VITELLOGENESIS (FD3) O - Oocytes

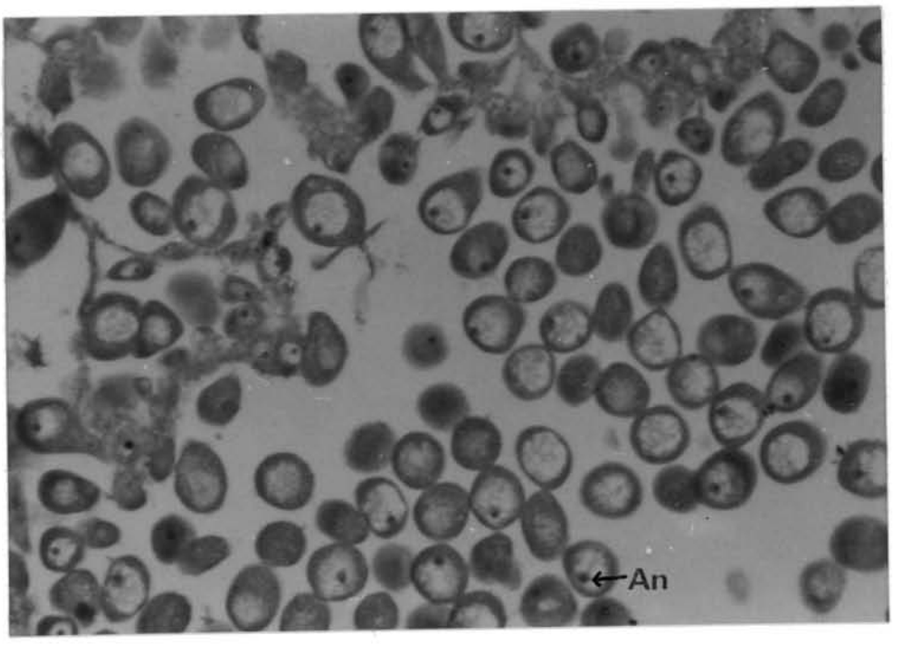

FIG. III. 11

MATURE (FD4) An - Amphinucleus

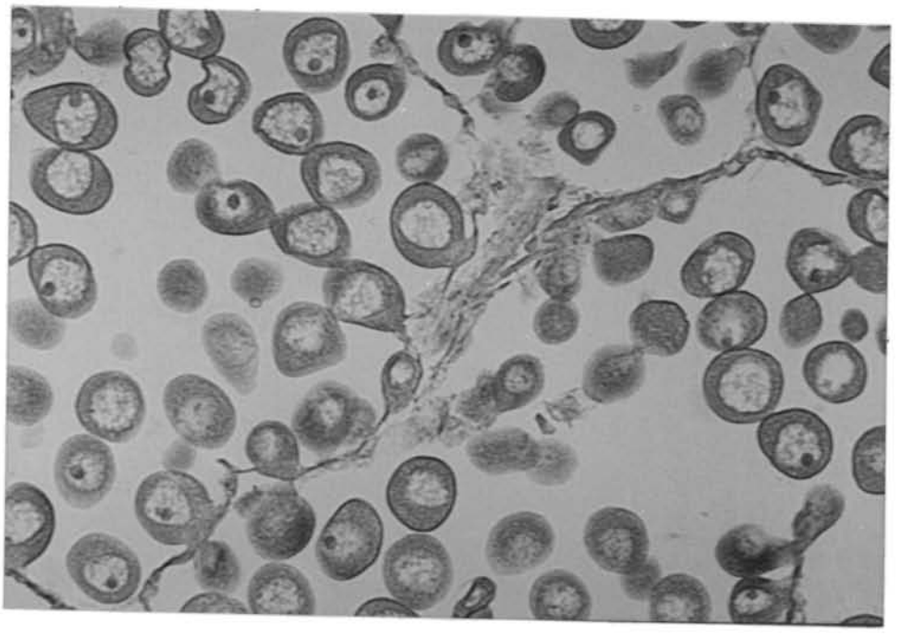

FIG. III. 12
EARLY REGRESSION (FR1)

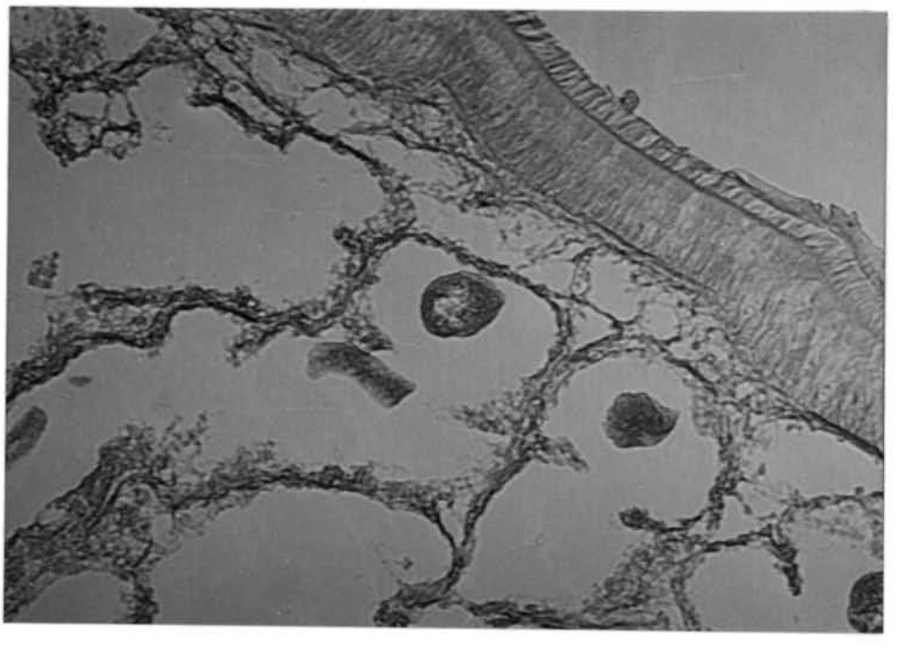

FIG. III. 13
LATE REGRESSION (FR2)

LATE REGRESSION (FR2)

Almost all oocytes were found to be released and, instead the follicles contained a watery fluid and few residual oocytes which were found to be in the process of reabsorption. Phagocytic cells were seen scattered in the follicles, which were involved in the process of reabsorption of the residual oocytes (Fig. III.13).

In January, a major share (74.2 %) of the female *M. opima* were in the spawning stage, 19.4 % had spent and 6.5 % had resting gonadal condition. It was the end of the spawning peak as the number of spawning clams were reduced to a low level (9.7 %) in February, when most of the clams (58 %) were in the spent stage. At this period, the residual gametes were reabsorbed as large number of phagocytic cells were found all over the gonads. In some (32.3 %) the reabsorption was completed to reach the resting phase of reproductive cycle. During this period, the gonads were found to be retreated and the area was substituted by connective tissue. No resting stage was observed in March.

In March, all the four developing stages were observed. Rapid gonadal development occurred with 10.7 % early oogenesis, 28.6 % in pre-vitellogenesis, 39.3 % of clams was in the vitellogenesis and 21.4 % was mature clams. Some of the clams (38.7 %) in April were in the mature stage of development and 51.6 % was found to be spawning. All the ova in this stage were free, having spherical to oval shape, packed in the lumen

of the follicle. However, 9.7 % clams with the vitellogenic stage was also obtained.

83.3 % of the clams was in the spawning stage in May. A few (16.7 %) of the clams of vitellogenic stage were also recorded. The spawning continued to the next month also as 10 % of the clams was seen in this stage. The development was found to be completed and no clams were found undergoing gametogenesis as 90 % of them was observed to be in the spent condition with some residual ova retained in the gonadal follicles in the process of reabsorption. The phagocytes were found to be scattered in the fluid filled follicles with some residual gametes.

In the month of July, spent and resting stages of gonads existed. 42.9 % of the clams was found to have completed spawning and others (57.1 %) completed reabsorption of cellular debris and entered resting phase. The gonadal zone was found to be replaced by connective tissue. In August, rapid gametogenesis occurred and was indicated by the presence of clams in their resting (11.5 %), oogenic (53.9 %), pre-vitellogenic (19.2 %) and vitellogenic stages (15.4 %). A larger proportion was in the oogenic stage.

Vitellogenic stage dominated (44 %) during September, closely followed by pre-vitellogenic stages (36 %). Early oogenic (12 %) and mature clams (8 %) were also recorded. During October, spawning was commenced with a small share (10.7-%) of clams were found in the spawning stage. The clams were ripe in this stage as most of them (46.4 %) had gonads in the

mature stage, with ova getting separated from the follicle wall and concentrated towards the center of the follicle. Clams with oogenic (10.7 %) and vitellogenic stages of gonads were also present (32.1 %).

The gonadal development in the second spawning cycle was in a slower pace, when compared to the first spawning. In November, 63.3 % of the clams was in this spawning stage and the rest (36.7 %) in the mature condition. The spawning continued in December and 77.4 % was in the spawning condition, rest being mature (12.9 %) and spent (9.7 %). As mentioned earlier, the clams continued spawning in the month of January and this was the major spawning phase of the clams.

Though males started spawning first, it was closely followed by spawning in females. Presence of spermatozoa in the ambient media, may be the factor triggering spawning in females and thus synchronous spawning.

ANNUAL REPRODUCTIVE CYCLE

The relative occurrence of different stages of male and female clams during different months studied are presented in Table III.2 and graphically represented in Fig.III.14 and Fig.III.15 respectively. The annual reproductive cycle showed that a minor spawning occurred in May - June period and a major spawning in September to November. Two spawnings were demarked by the short resting periods which occurred between them. The spawning peaks are graphically represented in Fig. III. 16

ABLE III. 2

ONTHLY VARIATION IN STAGES OF GONADAL DEVELOPMENT (1989 & 1990)

MONTHS	I	MALE					FEMALE					
		D1	D2	D3	R1	R2	D1	D2	D3	D4	R1	R2
JANUARY	4				20	7					23	6
FEBRUARY	17	6			3	4					3	18
MARCH		7	20	4			3	8	11	6		
APRIL			5	23	4				3	12	16	
MAY				3	25	3			4		20	
JUNE					21	8					2	18
JULY	33	2			2	9						12
AUGUST	5	16	6				14	5	4			
SEPTEMBER		7	6	17			3	9	11	2		
OCTOBER			4	25	6		3		9	13	3	
NOVEMBER					26	3				11	14	
DECEMBER					20	12				4	24	3

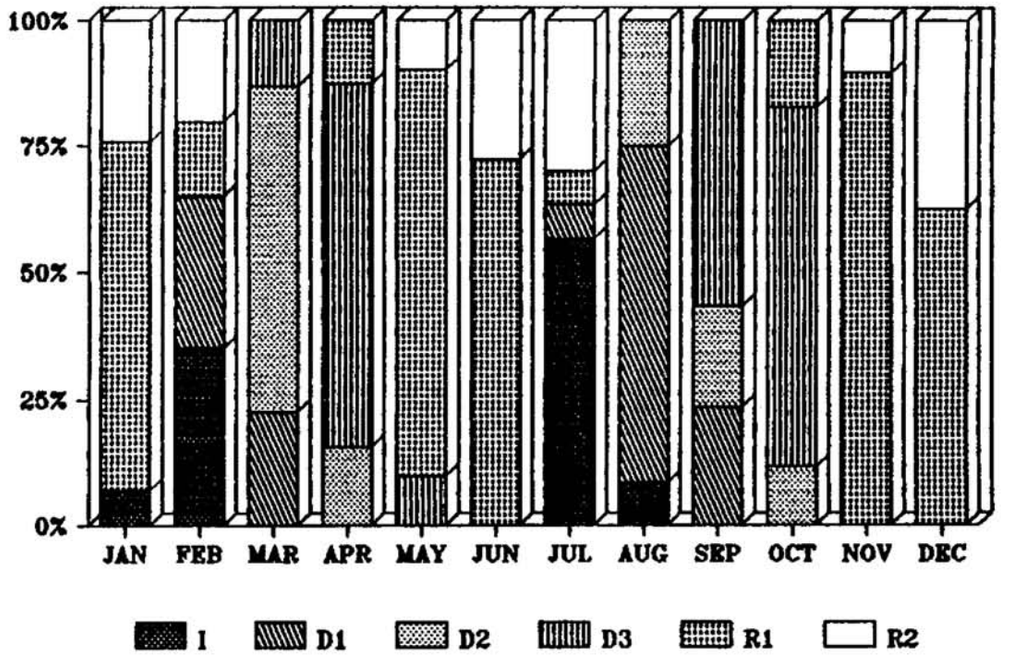

FIG. III.14
STAGES OF MALE GONADAL DEVELOPMENT

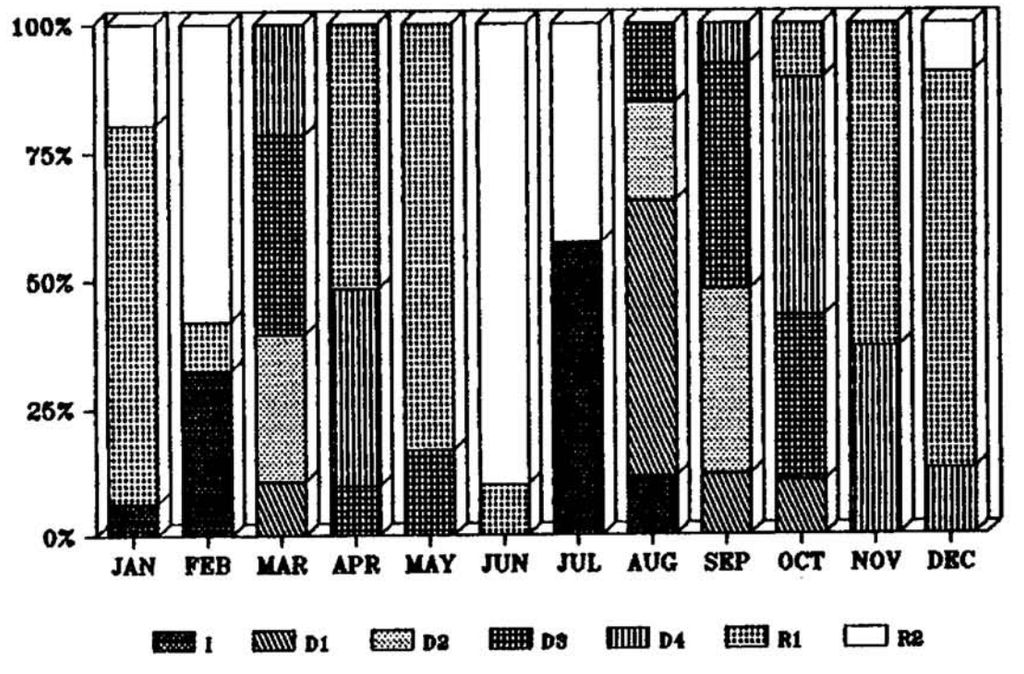

FIG. III.15
STAGES OF FEMALE GONADAL DEVELOPMENT

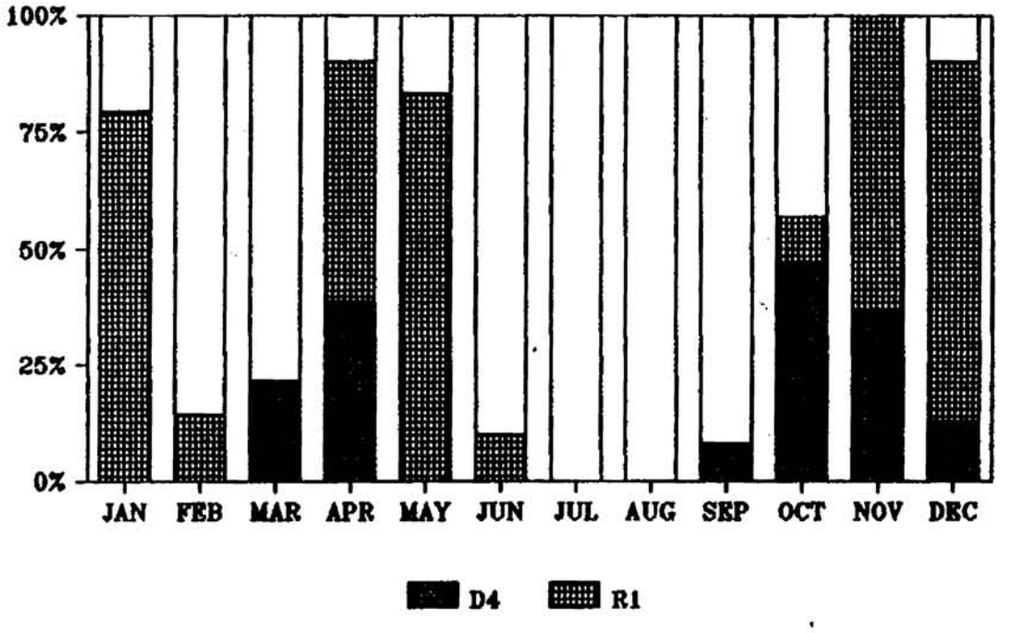

FIG. III.16
PERCENTAGE OF MATURE AND SPAWNING FEMALES

using the number of female clams in the mature and early regression stage in each month.

The average temperature recorded from the clam-bed was 28°C. Seasonally temperature was high during January to April and low in May to September. Higher temperature (31°C) was recorded in the shallow isolated pockets of the clam-bed.

DISCUSSION

M. opima was found to be gonochoristic and no case of hermaphroditism was recorded during the period of this study.Bivalves exhibit gonochorism and hermaphroditism. Sexual dimorphism is absent in bivalves and some exhibit sex reversal (Mackie, 1985). According to Nagabhushanam and Dhamne (1977), rapid rate of growth and fluctuations in environmental conditions are the major reasons for the sex changes in bivalves.

The ratio of different sexes of *M. opima* was estimated. During the first year of study, out of the 288 specimen studied, 45 % was males, 46 % females and 9 % indeterminates. While in the second year, the ratio was 50 %, 42 % and 8 % of males, females and indeterminates respectively. Chi square test indicate (Table III.3) that the deviation from 1:1 male female ratio is insignificant.

Environmental factors are found to influence the sex ratio of the clams. Notable increase in the percentage of males in *Perna viridis* population near coconut husk retting grounds was reported (Ajithakumar, 1984). Similarly, in

ɜLE III.3.

ı-SQUARE TEST FOR 1:1 MALE - FEMALE RATIO.

	1989		CHI - SQUARE*	1990		CHI - SQUARE*
	MALE	FEMALE		MALE	FEMALE	
JANUARY	13	15	0.143	14	14	0.000
FEBRUARY	7	9	0.250	8	12	0.800
MARCH	13	8	1.190	18	20	0.105
APRIL	14	16	0.133	17	15	0.125
MAY	13	11	0.167	18	13	0.806
JUNE	15	10	1.000	15	10	1.000
JULY	5	5	0.000	10	7	0.529
AUGUST	9	10	0.053	18	13	0.806
SEPTEMBER	8	12	0.800	17	13	0.533
OCTOBER	11	9	0.200	24	16	1.600
NOVEMBER	9	15	1.500	18	15	0.273
DECEMBER	12	14	0.154	20	17	0.243

INSIGNIFICANT.

Saccostrea cucullata inhabiting mangroves of Hong Kong also reported to have a heavily male biased population (Morton, 1990). Though increased organic load can be noted as a common factor in both the cases for male dominant population, it was not seen in *M. opima* does not support such a conclusion as the male female ratio retained the 1:1 proportion.

The classification of sexual stages were undertaken according to the type of gonadal development which different bivalves are undergoing. In *Meretrix casta*, the resting period extended from later part of October to early January during which the clams were sexually indistinguishable (Salih, 1977). In *Sunetta scripta* the interval between spawning and next gametogenesis was short and no resting period was noticed. Gametogenesis started again when the residual gametes were being reabsorbed (Katticaran, 1988).

Tranter (1958b) in his detailed studies on the reproduction in pearl oyster, classified stages of male gonadal development into five stages of development MD1, MD2, MD3, MD4 and MD5 along with three stages of spawning MR1, MR2 and MR3. Similarly female gonadal stages into FD1, FD2, FD3, FD4 and FD5 and three stages of spawning, FR1, FR2 and FR3. The oyster exhibited only one reproductive cycle per annum and progress of gonadal development were rather slow and hence the possibility of division into eight stages for males as well as females.

Nagabhushanam and Mane (1975b) classified the reproductive stages of *Mytilus viridis* into four stages in both males and females. 1) Recovery/Regression : follicles with few

residual gametes and connective tissue appearing, which is the stage following spent condition of gonads. 2) Early growing / early gametogenesis : the gonads with stem cells and gametes in early stages of development. 3) Late growing/ Mature : the gonads filled with mature gametes 4) Spent follicles empty with some residual gametes.

Nagabhushanam and Dhamne (1977) divided the stages of gonadal development of *Paphia laterisulca* into 1) Immature, where gonads are in the early stages of development with connective tissue and early stages of gamete development. 2) Maturing gametes, in the later stages of development with some mature gametes. 3) Mature, gonadal follicles filled with mature gametes. 4) Partly spent, follicles empty, except gametes concentrated in the lumen. 5) Spent gonadal follicles collapsed and almost empty with few gametes. 6) Regression, follicles empty except few residual gametes. Dinamani (1987) classified the gonadal stages of *Crassostrea gigas* into 1) inactive, 2) early active, 3) late active, 4) mature and 5) fully ripe. The reproductive stages of *Mytilus edulis* was divided into Resting\Spent, two developing stages,D1 and D2, ripe stage R, and two spawning stages S1 and S2 by Barkati (1990).

Even though the patterns of gonadal development were more or less similar, differences in the reports are to be expected as the mode of classification of stages of gonadal development were different. The number of spawning per annum,

however varied depending on many parameters such as species, geographic distribution, food availability, hydrographic conditions, etc.

Out of the different patterns of classification of bivalve reproductive stages, that adopted by Joseph and Madhyastha (1984) was found to be the best suited for *M. opima*, which is having two spawning peaks per annum. Therefore, this method was used and further division of stages, is difficult as development is rapid. As described in detail in the results, male reproductive cycle of *M. opima* were divided into 1. Early gametogenesis (MD1), 2. Late gametogenesis (MD2), 3. Mature (MD3), 4. Early regression (MR1) and 5. Late regression (MR2). Similarly, female gonadal development classified into 1. Early oogenesis (FD1), 2. Pre-vitellogenesis (FD2), 3. Vitellogenesis (FD3), 4. Mature (MD4), 5. Early regression (FR1) and 6. Late regression (FR2). The sexually indeterminate resting phase (I) is also recorded.

The pattern of reproduction of *M. opima* with two spawning peaks per annum is exhibited by other tropical bivalves like *Donax faba* (Alagarswami, 1966), *Crassostrea madrasensis* (Stephen, 1980a; Rajapandian and Rajan, 1983), *Katelysia opima* and *Mytilus viridis* (Nagabhushanam and Mane, 1975a and 1975b), *Mytilus edulis planulatus* (Dix and Feruson, 1984) *Mercenaria mercenaria* (Manzi *et al.*, 1985), *Saccostrea cucullata* (Sukumar and Joseph, 1987), *Ostrea angasi* (Hickman *et al.*,1988), *Mercenaria* spp. (Hesselman *et al.*, 1989), *Ruditapes*

decussatus (Shafee and Daoudi, 1991), and *Villorita cyprinoides* (Modassir, 1991).

Two to three spawning peaks were noticed in *Crassostrea madrasensis* (Joseph and Madhyastha, 1984) and two to four peaks in *Anadara granosa* (Narasimham, 1988). Three peaks in *Mercenaria mercenaria* (Hefferman and Walker, 1988) have also been reported while single extended annual reproductive cycle is exhibited by some bivalves like *Pinctada albina* (Tranter, 1958b), *Crassostrea gryphoides* (Durve, 1965) *Panope abrupta* (Sloan and Robinson, 1984), *Polymesoda (geloina) erosa* (Morton, 1985), *Mactra chinensis philippi* (Cheng *et al.*, 1987), *Crassostrea virginica* (Hefferman *et al.*, 1989), *Saccostrea cucullata* (Morton, 1990), *Mytilus edulis* (Barkati and Ahmed, 1990), *Mactidea striata*, *Crafrarium timidum* and *Anadara scapha* (Baron, 1992), *Ostrea edulis* (Ruiz, et al., 1992).

Geographic distribution is found to have influence in the reproductive pattern. *Mercenaria mercenaria* population was found to exhibit 3 peaks in Massachusetts while only one peak in Carolina per year (Knaub *et al.*, 1988). Brousseau (1987), on the studies conducted on three populations of *Mya arenaria* found that two populations exhibited single spawning cycle while one population had two peaks per year.

In the case of *M. opima* synchronous spawning of the males and females occur. Where eggs and sperms are broadcast and fertilization is the result of random contact in the sea water, there is some measure of co-ordination, so that large

numbers of individuals, of both sexes spawn at the same time (Purchon, 1968). Extrinsic and intrinsic factors play important role in the reproductive pattern of bivalves as discussed.

Synchronous spawning is important in external fertilizers, and delay in spawning of either male or female can adversely affect the reproductive success. Studies conducted on *Mercenaria mercenaria* showed that a higher concentration of sperms yielded higher percentage of fertilization in all delayed fertilizations. Similarly in the case of time, fertilization of eggs stored for three hours was severely reduced and no live embryos resulted from the eggs held for 24-h (Godsell and Eversole, 1989). Changes have been noticed in the neurosecretory cells of clams in association with reproductive stage (Mane, 1974).

Of all the factors influencing reproduction, temperature was found to play an important role in the reproduction of bivalves, especially in the temperate region. Maturation of gametes and spawning is cued by annual temperature fluctuation or threshold temperature (Mackie, 1974). Significant positive correlation in *C. virginica* between the temperature and gonad/body ratio was found by Gauthier and Soniat (1989). Conditioning trials done on *Crassostrea gigas* at optimum temperature resulted in accelerated production of gametes and conditioning time was shortest in May and June indicating the influence of season

more than that of temperature in gametogenesis of the oysters (Robinson, 1989).

Temperature is not constant in the clam beds of *M. opima* and is found to fluctuate according to the tidal action. It ranged from 27 to 30°C. Temperature is not much important in tropical bivalves as in temperate ones. This is due to the fact that under tropical conditions like those in India, water temperature is relatively high throughout the year, and generally the temperature does not fall below the optimum requirements of many molluscs, and salinity is seen to be more important than temperature (Nagabhushanam and Mane, 1975b).

Food availability is another important factor that influences reproduction indirectly. Close association between gonad and digestive system is well documented. The digestive system and gonads of *M. opima* were found to have a direct contact. The gonadal follicles were found to envelope the whole alimentary canal during the ripe stage. The narrow range of variation in the nutrient reserves when compared to that of temperate clams (discussed in Chapter IV) indicated that *M. opima* is likely to depend on ingested food for the energy demands during reproduction. Tropical bivalves like *M. opima* does not have to overcome a period of food scarcity as experienced by the clams of temperate regions. The studies conducted by Newell *et al.* (1989) on the factors regulating the reproduction and recruitment in *Crassostrea virginica*, it has been found that food availability was not important but salinity played an important role.

According to Hornell (1910), salinity determined the spawning activity in Indian oysters rather than temperature. Stephen (1980a) after studying the gametogenesis patterns of *Crassostrea madrasensis* and the salinity, supported the relationship between reproductive cycle and salinity as clearly defined by Hornell and proposes it to be termed "Hornell's rule". Sukumar and Joseph (1987) found that progress in gametogenesis coinciding with increase in salinity and its decline initiating spawning in *Saccostrea cucullata*. But very high salinity can cause retardation in sexual activity (Durve, 1964).

In the present study, salinity was found to have a role in the reproductive activity; resting period of gonad coincided with low salinity, gametogenesis with increasing salinity and maturation at maximum salinity level. Low salinity has some serious adverse effects on *M. opima* in Kayamkulam lake. During post-spawning period salinity falls as low as to 2.8 ppt due to high influx of flood water from rivers and nearby areas which cause considerable damage to the pelagic larvae of the clam, and this may be the reason for the wide variation in the annual population density of the clam and the resultant decline in fishery.

A number of reports on the detrimental effects of low salinity on pelagic larval forms are available. Giese and Pearse (1974) have indicated that the salinity requirements for reproduction may reflect salinity requirements for spawning and development of the larvae rather than tolerance of adults.

Some larval forms are found not to tolerate wide ranges of salinity in contrast to the adults. Similarly, salinity may affect reproduction decisively in areas where pronounced variation in salinity occurs. Extensive salinity fluctuations or extremely low salinities may cause a number of benthic invertebrate species with pelagic larvae to decrease and the number of species with non pelagic larvae to increase (Kinne, 1971). Studies on fouling oysters have recorded that no settlement occurred in oligohaline condition (Menon *et al.*, 1977). Low salinity was highly correlated with low spat fall in *Crassostrea virginica* (Newell *et al.*, 1989).

The fresh water flooding during monsoon while the barmouth remains closed, is the reason for the marked decrease in salinity, which is havimg a serious impact on the reproduction and thereby on the population density of the clam. A scientific monitoring of salinity and timely opening of the barmouth to prevent the drastic decrease in salinity can contribute substantially for protecting and enhancing the fishery of the area.

The chapter depicts the stages of gonadal development, spawning and annual reproductive cycle of *M.opima*. The clam was found to be gonochoristic with a rather quick gonadal development. The stages of male gonadal development are classified under five divisions and stages of female gonadal development under six divisions. Size of the clam at sexual maturity was found to be 18 - 20 mm. The clam was found to undergo two complete reproductive cycles per year.

A minor spawning from April to June and a major one commeccing from October and extending upto February of next year. Each reproductive cycle was followed by a short sexually resting phase in both the sexes. The wide fluctuation in salinity in the region during monsoon due to fresh water flooding when the barmouth is remaining closed, have adverse effect on the productivity, as it coincides with the spawning and early stages of gonadal development of the first spawning cycle. The study concludes with a suggestion for scientific monitoring and exploitation of the clam for optimum usage of the natural resource, for transplanting the resource to viable areas, as well as for the aquaculture.

Chapter IV

BIOCHEMICAL COMPOSITION

INTRODUCTION

The estuarine territories are reservoirs of highly nutritive seafood, a bulk of which is centered around the sentinel organisms — the bivalves. However, a lion share of this contribution to the human diet is left unexploited mainly due to the ignorance with regard to its nutritive value. The importance of sea food as protein rich food for human consumption can not be overemphasised. In a country like India, where about 30% of the population are under malnutrition, efforts should be made to increase the availability of nutritious food. Studies on the seasonal variation in nutritive value and steps taken for fishery enhancement is having a significant role in the prospects of fulfilling the nutritive demands of the growing population.

Presently only 10 gram of fish per day per head is consumed in India, which is very low when compared to the 275 gram per day per head available in Japan. Sea food contains rich proteins with well balanced amino acids and the proteins consumed are also better utilized at high level when compared to the same consumed in the form of pulses, grains or wheat. Recent trends in the development of aquaculture in India, in order to meet the growing demands is appreciable. However,

aquaculture of bivalves is yet to be recognized which is an efficient way in the conversion of primary production to highly nutritive sea food within a short period of time.

The major biochemical constituents namely proteins, carbohydrates and lipids show annual variation in concentration in relation to various intrinsic and extrinsic factors, like growth, reproduction, availability of food, favourable environmental factors, etc. Among these, reproductive cycle and availability of food are found to be of utmost significance.

REVEIW OF LITERATURE

Changes in the biochemical composition during the gametogenic cycle are profound in animals that show an annual reproductive cycle (Giese and Pearse, 1974). However, variation is less comparable in the case of those with two or more annual reproductive cycles (Giese, 1969). In organisms for which food is available throughout the year, less variation in biochemical composition is seen, while those which have to overcome periods of food scarcity, annually depend on reserve materials during the period and show wider variation in biochemical composition. Both these factors are influenced by geographic distribution. Temperate species have to overcome the unfavourable winter periods while it does not prevail in tropical conditions. Most of the tropical bivalves exhibit reproductive pattern with more than one annual spawning peaks (Alagarswami, 1966; Nagabhushanam and Mane, 1975a,b; Salih,

1977; Rajapandian and Rajan, 1983; Joseph and Madhyastha, 1984; Sukumar and Joseph, 1987; Narasimham, 1988). Majority of the temperate bivalves are observed to have single reproductive cycle per annum (Tranter, 1958b; Sloan and Robinson, 1984; Morton, 1985;1990; Chung *et al.*, 1987; Hefferman *et al.*, 1989). This difference is reflected in their corresponding biochemical cycles also.

A major share of the biochemical studies conducted on bivalves to-date inferred that the reproductive cycle is the most important factor influencing the biochemical levels as observed in *Donax cuneatus* by Nagabhushanam and Talikhedkar (1977), in *Mytilus viridis* by Nagabhushanam and Mane (1975b) and Waffar *et al.* (1976) and in *Chlamys opercularis* by Taylor and Venn (1979). Studies conducted on the seasonal variation in weight and biochemical constituents of *Chlamys opercularis* showed that it was related to the reproductive cycle (Taylor and Venn, 1979). They further noticed that the adductor muscle was found to be the storage site of glycogen and protein. Ansell (1974a,b,c) showed that in the bivalve *Abra alba* nutrient build up occurred during gametogenesis and decreased during spawning and in winter. It was further given that in *Chlamys septemradiata*, female gonad was found to have a greater proportion of lipid than male. But males contained a higher nitrogen level, with increased lipid content in the females of *Nucula sulcata*. However, the biochemical changes were found to be independent of shell growth (Ansell, 1974 c). High values of lipids and calorific content was also observed in *Villorita*

cyprinoides var *cochinensis* which coincided with gonad development and low values were recorded during the spawning (Nair and Shynamma, 1975).

Protein and lipid were found to be maximum in male and carbohydrate concentration was maximum in females in *Tellina angulata* according to Kumari and Nair (1988). Increased contents in the gonad during gametogenesis were recorded by Gabbot (1976). Similar increase in water content and protein value during breeding activity and high glycogen content in the resting period was recorded by Suryanarayanan and Nair (1976). Bressen and Marin (1985) reported the seasonal biochemical variation and condition index and its relation with reproductive cycle, temperature and phytoplankton availability. Ansell (1972) studied seasonal biochemical composition of the bivalve *Donax vittatus* and found an increase in nutrient reserves during spring season with diatom peak, and a subsequent decrease in nutrient reserves was observed during winter and spawning period. In *Meretrix meretrix* concentration of protein, lipid, and carbohydrate was found to be primarily influenced by food availability (Jaybal and Kalyani, 1986).

Deslous-Paoli and Heral (1988) studied the protein, ash, lipid, and carbohydrate content of *Crassostrea gigas* and found that poor trophic conditions of environment resulted in the use of glycogen, protein and lipids to a large extent. Pridmore *et al.* (1990) studied the variation in composition and its relation to the pollution level in the pacific oyster *Crassostrea gigas*.

Protein, which is the important structural material of aquatic organisms, forms the major organic component. Somatic protein has been reported to form the predominant energy substrate during gametogenesis in bivalves (Gabbot, 1975; Adachi, 1979; Barber and Blake, 1981). The dependence on protein was found to be prominent during the periods of nutritive stress (Gabbot and Bayne, 1973; Benninger and Lucas, 1984). According to Gabbot (1976), much of the increase in gonad weight during gametogenesis was associated with an increase in the protein and lipid of the gonad, which are known to be important constituents of bivalve gametes. Protein forms the major organic component of bivalve oocytes (Holland, 1978). Both glycogen and lipid are mobilized from different tissues to form the oocytes. However, oocyte proteins are synthesized *de novo* (Holland and Honnant, 1978).

In most of the bivalves, the annual variation in protein level was more or less narrow year round when compared to the concentration of glycogen and lipid which showed a wider fluctuation in relation to the reproductive cycle. During sex change from male to female, protein level was found to remain high and during female to male change it was low (Nagabhushanam and Mane, 1978). The monthly variation of the protein in the gill, adductor muscle and midgut gland of the clam *Tapes philippinarum* was observed by Adachi (1979). Protein content in the gill and midgut gland was found to be relatively constant and that of adductor muscle fluctuated in relation to reproductive cycle. Similar studies were done by Walne and

Mann (1975) in *Ostrea edulis* and *Crassostrea gigas* and Ansari *et al.* (1981) in *Villorita cyprinoides*.

Glycogen is the ready source of easily available energy and it is able to yield to the energy requirement even under anaerobic conditions. Metabolism in lamellibranchs is hinged on a glycogen economy (Giese, 1966). Carbohydrates in bivalves, comprise mainly of glycogen (Gabbot and Bayne, 1973), are stored in this form which is used up during the stages of metabolic demands like spawning and starvation. In the studies on *Crassostrea madrasensis*, Stephen (1980b) observed that carbohydrate is the main reserve nutrient to be stored primarily in the gonads before the commencement of the first gametogenic cycle. In the second gametogenic cycle, it was found to depend directly on nutrients derived from food sources.

In bivalves generally the carbohydrate reserves may be rapidly utilised under unfavourable conditions and the wide variation found in the tissue indicates that the level of mobilisable carbohydrate reserves may fluctuate widely and rapidly in response to fluctuations in conditions affecting the nutrition of the animal (Ansell *et al.*, 1973). The seasonal cycle of storage and utilization of glycogen reserves reflects the complex interaction between food supply, growth and reproduction.

Loss of glycogen was found to be synchronous with vitellogenesis in *Mytilus edulis* (Gabbot, 1975) and in *Mytilus viridis* (Nagabhushanam and Mane, 1975b) as glycogen levels were

higher in immature gonads. Castro and Mattio (1987) conducted studies on *Ostrea puelchana* and observed the glycogen index to be a suitable indicator of physiological state of the organism and suggested that the best condition in the quality of flesh with high energy value was detected in the post spawning period.

Of the biochemical components, the consumption of lipid can be ranked only after protein and carbohydrate. Lipids in bivalve molluscs contain sterols, fatty acids, phospholipids, lipids containing ether bonds and glycolipids (Voogt, 1983). Nagabhushanam and Mane (1978) attributed the low content of lipid in several bivalves to their sedentary way of living and for their capacity to survive anaerobic conditions. The lipid concentration in bivalves exhibits seasonal variation which has been related to the physiological state of the organism. Lipid concentration in *Crassostrea virginica* was found to be more related to the reproductive stage than food lipid supply Chu *et al.* (1990).

The lipid metabolism in bivalves is indicated to be oriented to glycogen metabolism (Voogt, 1983). According to Zandee *et al.* (1980), lipids are the main source of energy production in growing mussels. In *Crassostrea gigas*, lipids were found to be the important nutrient reserve during starvation (Riley, 1976). Lipids are therefore found to be multi functional, functions varying according to requirements like reproduction, starvation etc. The concentrations of lipid is also found to vary with species.

The extensive literature available regarding the biochemical studies on bivalves, summarises that many factors like reproductive cycle, food availability, environmental quality etc. influence the biochemical composition of bivalves.

Studies on the seasonal changes in the calorific content, organic carbon and lipids in *Mytilus viridis* (Waffar *et al.*, 1976), estimation of the calorific value, organic carbon and lipid content and their seasonal biochemical variation in male, female and indeterminate sex of *Donax incarnatus* (Balasubramanian *et al.*, 1979), marked fluctuation in the lipid content in mantle with gill, heart, and adductor muscle of *Crassostrea virginica* (Chu *et al.*, 1988), are some of the works which stress the relation of biochemical constituents with reproduction.

Comparative studies were conducted on the seasonal biochemical changes, variation in condition and reproductive activity of indigenous and introduced species of clams *Tapes decussatus* and *T. philippinarum* by Benninger and Lucas (1984).

Thus it has become clear that in order to understand the nutritive value of any organism which is exploited by the human community it is vital to study its biochemical composition. Here an attempt is made to evaluate the nutritive status of *Marcia opima*, its seasonal variation and the relation of biochemical composition to reproductive cycle of the species. Simultaneously a comparison of the biochemical composition in relation to sexes is also made. The monthly variations of biochemical constituents- protein, glycogen and

lipid were studied for a two year period. As this species is widely exploited by the local population, it has become vital to study its nutritive value. The analyses of the various biochemical compositions, thus, become an unavoidable aspect in the study.

MATERIALS AND METHODS

Biochemical studies were conducted on the clam for a period of two years. The monthly samples collected were left overnight in the laboratory in filtered sea water for clearance of mud and fecal matter. Ten clams of the size range 25-35 mm were selected each time for biochemical studies. During the first year of study, estimations were made on pooled soft tissue and in the second year separate analyses were made for males, females and sexually indeterminate forms.

The clams were opened with a scalpel, washed with minimum quantity of glass distilled water and blotted dry. The soft tissue was weighed immediately after separating from the shell. It was then oven dried at 65°C to a constant weight. Percentage of water content of soft tissue was calculated from the fresh weight and dry weight. The biochemical composition was estimated from the finely powdered dry soft tissue.

Protein level in the soft tissues was determined by the method of Lowry *et al.*, (1951). A weighed sample of the dried soft tissue was extracted with alkali and warmed in a water-bath. The extract was treated first with an alkaline

solution of copper sulphate and then with Folin cieocalteu reagent. The intensity of the blue colour of the resulting solution was measured spectrophotometrically at 750 nm. The concentration was calculated from the absorbence values with a standard curve prepared using Bovine Serum Albumin.

For estimating the total glycogen levels, weighed soft tissue samples were extracted with 5% trichloroacetic acid containing 0.1% silver sulphate. The extract was warmed with concentrated sulphuric acid and the rose coloured furfural formed was estimated spectrophotometrically at 520nm. This method was proposed by Kemp and vanKitz (1954). The standard curve was prepared using glucose.

The method of Barnes and Blackstock (1973) was used to estimate lipid levels. Weighed soft tissue samples were extracted with 2 : 1 chloroform-methanol mixture and the lipid extract was treated with sulphuric acid, phosphoric acid and vanillin. The absorbence of the red coloured complex was estimated at 520 nm. Cholesterol was used for the preparation of standard curve.

Concentrations of biochemical constituents were calculated from the absorbence measured using the Spectrophotometer (Hitachi —Model 200-20).

The monthly variation in calorific value was calculated using calorific equivalents 5.61, 4.10 and 9.45 Kcal/g for protein, carbohydrate and lipid respectively (Brody, 1945).

RESULTS

The monthly variation of water content in the soft tissue of *M. opima* has been presented in Fig. IV.1 Minimum water content of 71.21% was recorded in May and maximum level of 82.71% was observed in June. Apart from the values recorded in May, June and July, the water level was within a narrow range (72.66% to 78.29%).

The monthly average values of protein in the pooled tissue recorded during the two years of study has been presented in Fig. IV.2 Highest value of 375.31 mg/g was recorded in the month of August (Table IV.1) and lowest value of 347.48 mg/g in June.

Protein content in male, female and indeterminate clams did not show wide variation. Males and females exhibited more or less a similar pattern of accumulation (Fig. IV.3) while the indeterminates recorded a lower value. A maximum value in males were recorded in August (404.09 mg/g) and a minimum value in January (351.29 mg/g). Highest protein content in the females were observed in September (397.03 mg/g). Lowest value was recorded in June (362.24 mg/g) as presented in Table IV. 2. The protein concentration in indeterminates ranged from 352.53 to 358.26 mg/g.

Monthly values of glycogen concentration recorded, showed a wide fluctuation (Fig. IV. 4). The maximum value of 411.21 mg/g of glycogen in July and minimum value of 147.67 mg/g in September (Table IV. 3).

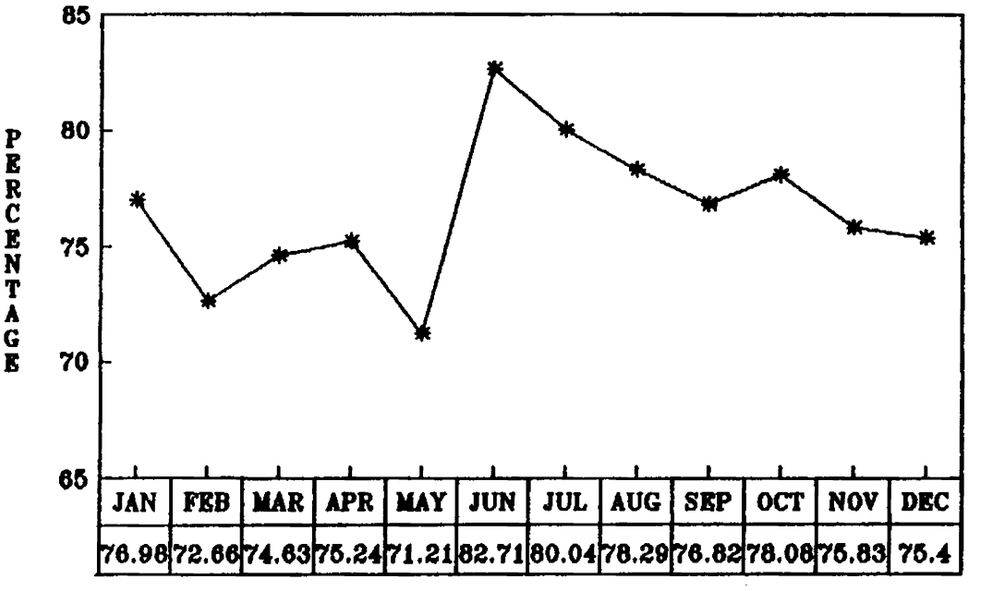

FIG. IV.1
MONTHLY VARIATION IN WATER CONTENT

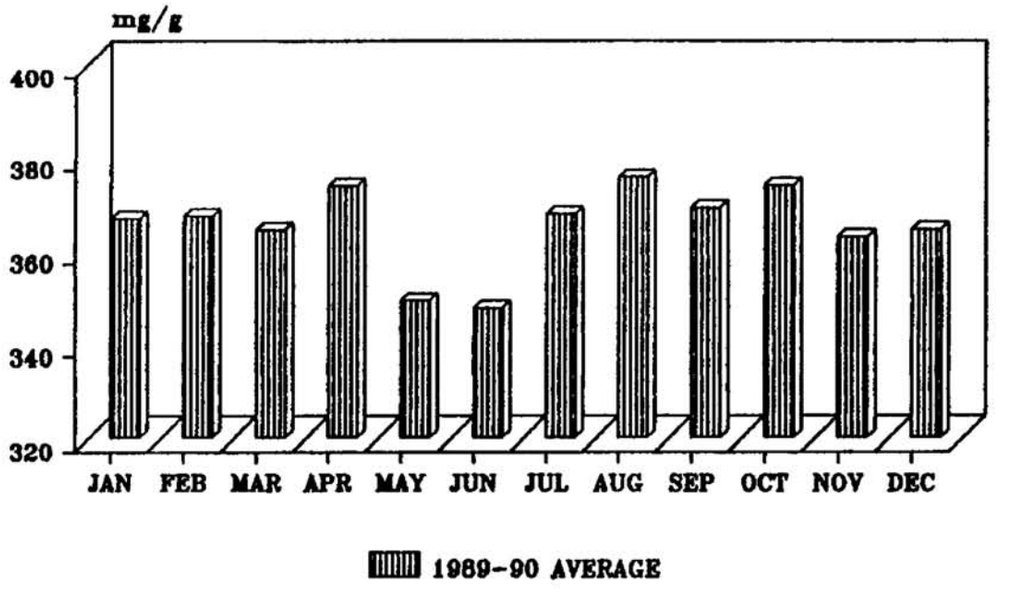

FIG. IV.2
MONTHLY VARIATION IN PROTEIN (POOLED SAMPLE)

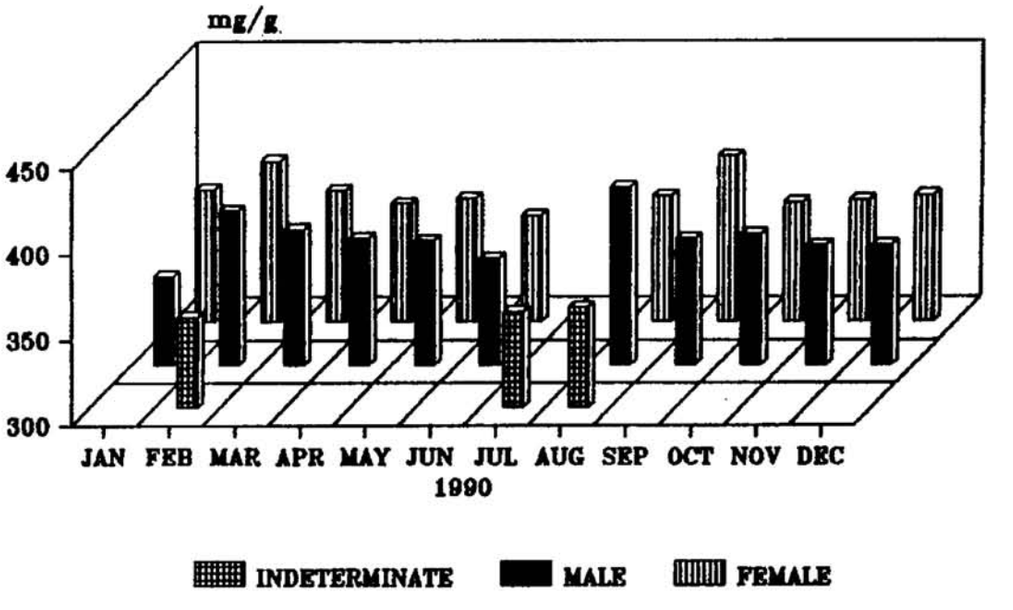

FIG. IV.3
MONTHLY VARIATION IN PROTEIN (SEXES SEPARATED)

TABLE IV.1

MONTHLY VARIATION IN PROTEIN (POOLED TISSUE)

No.	MONTH	PROTEIN (mg/g)		
		1989	1990	AVERAGE
1	JANUARY	368.03	364.49	366.26
2	FEBRUARY	354.51	378.96	366.73
3	MARCH	350.00	378.01	364.01
4	APRIL	375.11	371.57	373.34
5	MAY	325.54	372.54	349.04
6	JUNE	329.40	365.56	347.48
7	JULY	378.97	355.47	367.22
8	AUGUST	371.89	378.73	375.31
9	SEPTEMBER	351.94	385.42	368.68
10	OCTOBER	373.83	373.25	373.54
11	NOVEMBER	354.51	370.61	362.56
12	DECEMBER	355.80	372.38	364.09

TABLE IV.2

MONTHLY VARIATION IN PROTEIN (SEXES SEPARATED)

No.	1990	PROTEIN (mg/g)		
		MALE	FEMALE	INDETERMINATE
1	JANUARY	351.29	377.69	
2	FEBRUARY	389.92	394.43	352.53
3	MARCH	378.68	377.05	
4	APRIL	373.83	369.32	
5	MAY	372.54	372.54	
6	JUNE	362.24	362.24	
7	JULY			355.48
8	AUGUST	404.09	373.86	358.26
9	SEPTEMBER	373.83	397.03	
10	OCTOBER	376.26	370.24	
11	NOVEMBER	369.76	371.47	
12	DECEMBER	370.12	374.63	

MONTHLY VARIATION IN PROTEIN (POOLED TISSUE)

No.	MONTH	PROTEIN (mg/g)		
		1989	1990	AVERAGE
1	JANUARY	368.03	364.49	366.26
2	FEBRUARY	354.51	378.96	366.73
3	MARCH	350.00	378.01	364.01
4	APRIL	375.11	371.57	373.34
5	MAY	325.54	372.54	349.04
6	JUNE	329.40	365.56	347.48
7	JULY	378.97	355.47	367.22
8	AUGUST	371.89	378.73	375.31
9	SEPTEMBER	351.94	385.42	368.68
10	OCTOBER	373.83	373.25	373.54
11	NOVEMBER	354.51	370.61	362.56
12	DECEMBER	355.80	372.38	364.09

TABLE IV.2

MONTHLY VARIATION IN PROTEIN (SEXES SEPARATED)

No.	1990	PROTEIN (mg/g)		
		MALE	FEMALE	INDETERMINATE
1	JANUARY	351.29	377.69	
2	FEBRUARY	389.92	394.43	352.53
3	MARCH	378.68	377.05	
4	APRIL	373.83	369.32	
5	MAY	372.54	372.54	
6	JUNE	362.24	362.24	
7	JULY			355.48
8	AUGUST	404.09	373.86	358.26
9	SEPTEMBER	373.83	397.03	
10	OCTOBER	376.26	370.24	
11	NOVEMBER	369.76	371.47	
12	DECEMBER	370.12	374.63	

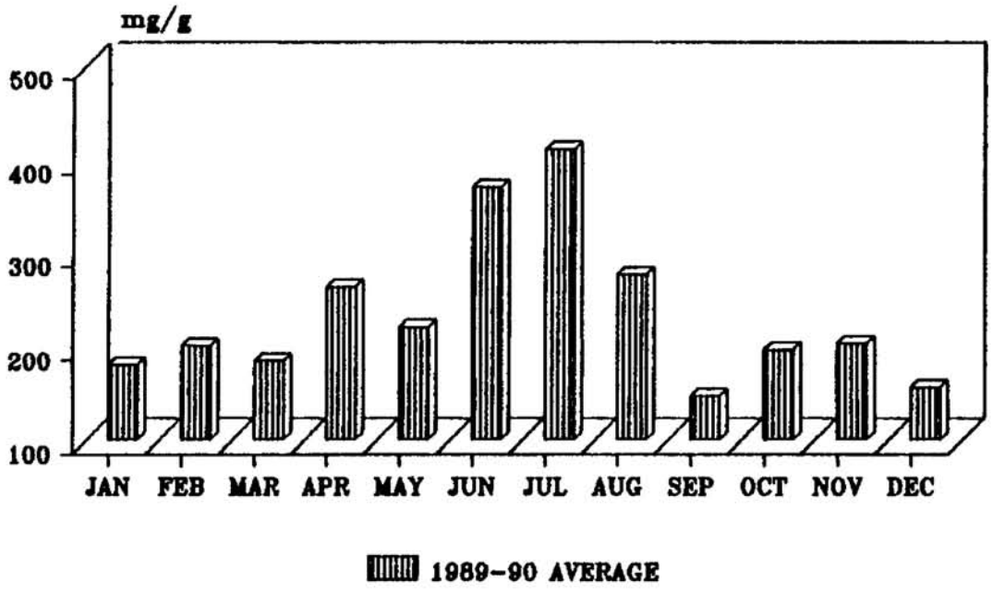

FIG. IV.4
MONTHLY VARIATION IN GLYCOGEN (POOLED SAMPLE)

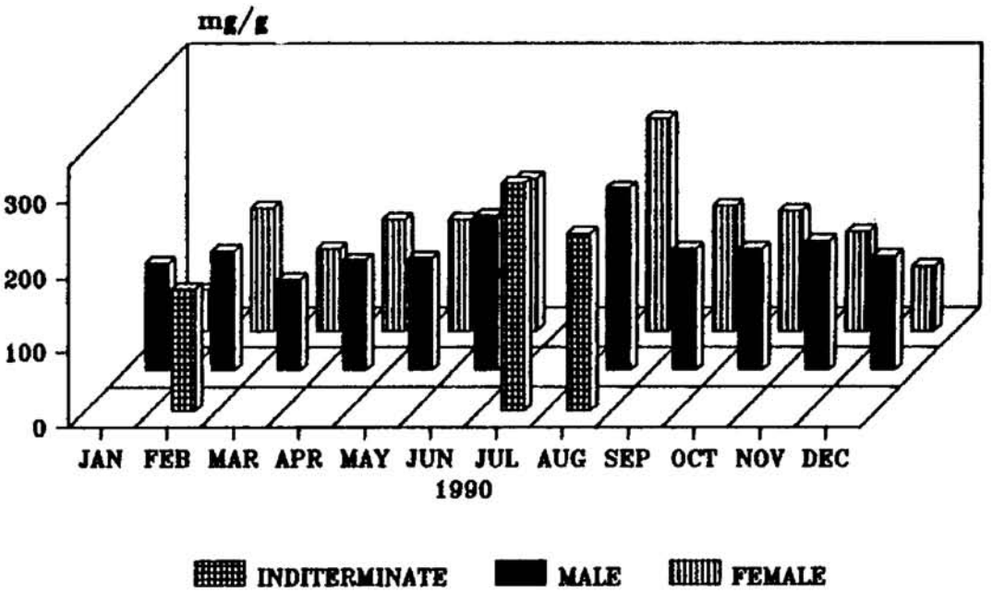

FIG. IV.5
MONTHLY VARIATION IN GLYCOGEN (SEXES SEPARATED)

TABLE. IV.3
MONTHLY VARIATION IN GLYCOGEN (POOLED TISSUE).

No.	MONTH	GLYCOGEN mg/g		
		1989	1990	AVERAGE
1	JANUARY	271.02	90.34	180.68
2	FEBRUARY	238.87	162.29	200.55
3	MARCH	251.49	117.20	184.35
4	APRIL	378.86	148.74	263.80
5	MAY	291.37	149.96	220.67
6	JUNE	537.16	205.30	371.23
7	JULY	518.44	303.98	411.21
8	AUGUST	299.10	255.03	277.07
9	SEPTEMBER	130.22	165.11	147.67
10	OCTOBER	232.36	162.34	197.35
11	NOVEMBER	247.83	158.85	203.34
12	DECEMBER	159.11	120.60	157.46

TABLE. IV.4
MONTHLY VARIATION IN GLYCOGEN (SEXES SEPARATED).

No.	1990	GLYCOGEN (mg/g)		
		MALE	FEMALE	INDETERMINATE
1	JANUARY	144.87	55.81	
2	FEBRUARY	161.04	164.70	164.12
3	MARCH	123.30	111.09	
4	APRIL	148.94	148.53	
5	MAY	151.41	148.50	
6	JUNE	207.23	203.37	
7	JULY			303.98
8	AUGUST	244.16	283.33	237.57
9	SEPTEMBER	163.71	166.51	
10	OCTOBER	164.24	160.43	
11	NOVEMBER	174.42	133.27	
12	DECEMBER	154.11	87.08	

The annual trend of glycogen recorded in the pooled tissue samples was replicated in the case of male, female and indeterminate also (Fig. IV. 5). All the three sets exhibited variation in glycogen concentration uniformly without notable variation in accumulation between sexes (Table IV. 4). Males recorded a maximum value of 244.16 mg/g in August and minimum values of 123.3 mg/g in March and a maximum value of 283.33 mg/g in August and minimum of 55.81 mg/g in January in the females were recorded. Indeterminates had a concentrations of 164.12 mg/g, 303.98 mg/g and 237.57 mg/g in February, July and August respectively .

Lipid levels showed a clear seasonal variation annually (Fig. IV. 6). A maximum value of 60.73 mg/g was recorded in December and minimum value of 29.75 mg/g in February. Excepting the odd value recorded in March, gradual increase from the minimum value in February to 53.53 in June was noticed. It fell to 31.07 mg/g in July which then gradually increased to a maximum of 60.73 mg/g in December (Table IV. 5). Lipid concentrations were the lowest in concentration among all the organic components analysed.

The variation in the lipid concentration of different sexes of *M. opima* also followed the trend observed in the case of pooled tissue sample (Fig. IV.7). Males recorded a low concentration of lipid when compared to females. Males recorded a maximum value of 44.48 mg/g in November and minimum value of 17.58 mg/g in April. The maximum concentration (88.79 mg/g) was recorded in January and minimum (42.3 mg/g) during

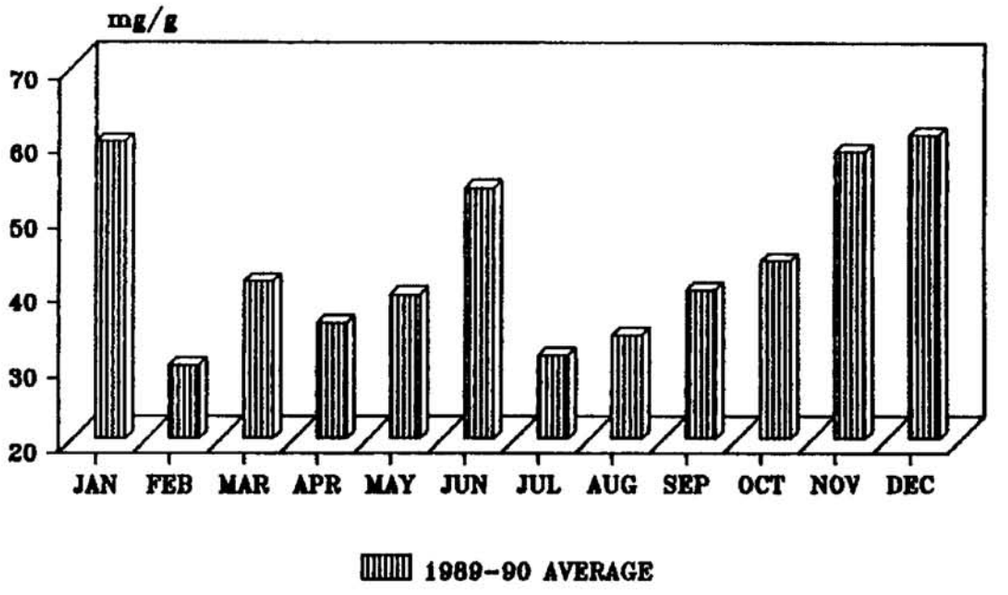

FIG. IV.6
MONTHLY VARIATION IN LIPID (POOLED SAMPLE)

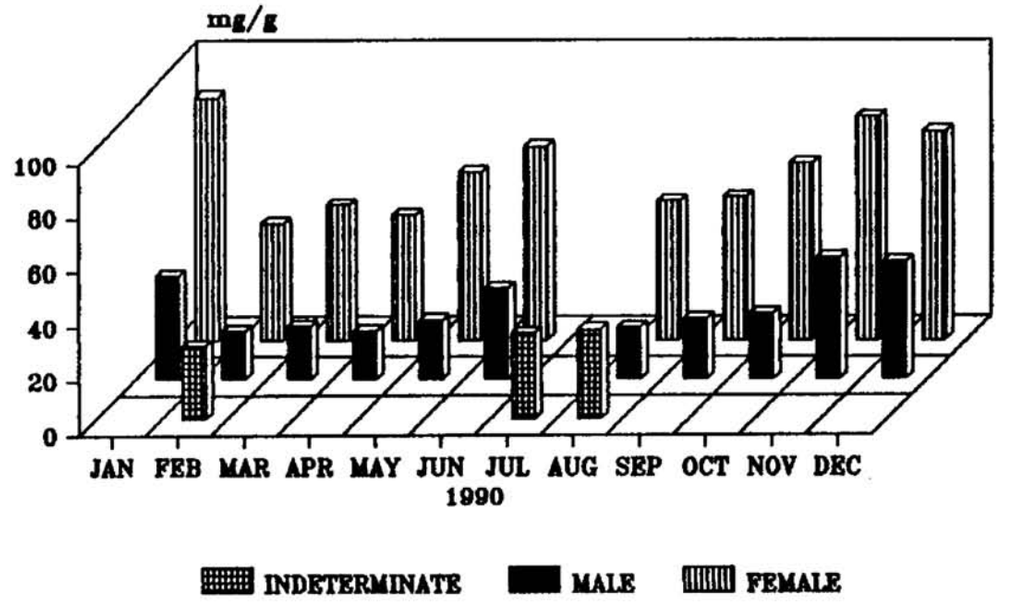

FIG. IV.7
MONTHLY VARIATION IN LIPID (SEXES SEPARATED)

BLE IV.5

NTHLY VARIATION IN LIPID (POOLED TISSUE)

No.	MONTH	LIPID (mg/g)		
		1989	1990	AVERAGE
1	JANUARY	56.21	63.42	59.82
2	FEBRUARY	30.55	28.95	29.75
3	MARCH	47.44	34.41	40.92
4	APRIL	39.12	31.47	35.29
5	MAY	36.79	41.58	39.19
6	JUNE	54.73	52.33	53.53
7	JULY	30.47	31.67	31.07
8	AUGUST	33.83	33.59	33.71
9	SEPTEMBER	42.34	37.17	39.76
10	OCTOBER	42.86	44.47	43.67
11	NOVEMBER	53.72	63.10	58.41
12	DECEMBER	61.83	59.64	60.73

BLE IV.6

NTHLY VARIATION IN LIPID (SEXES SEPARATED)

No.	1990	LIPID (mg/g)		
		MALE	FEMALE	INDETERMINATE
1	JANUARY	38.05	88.79	
2	FEBRUARY	17.70	42.30	26.84
3	MARCH	19.65	49.16	
4	APRIL	17.58	45.35	
5	MAY	21.61	61.55	
6	JUNE	33.49	71.16	
7	JULY			31.67
8	AUGUST	19.12	50.54	33.12
9	SEPTEMBER	22.23	52.11	
10	OCTOBER	24.14	64.80	
11	NOVEMBER	44.48	81.71	
12	DECEMBER	42.94	76.34	

February in females. Indeterminates had a moderate level with 26.84 to 33.12 mg/g lipid (Table IV. 6).

The calorific value indicated a clear seasonal variation (Fig. IV.8). Pooled tissue showed a maximum calorific value of 4.05 kcal/g in July and minimum of 3.06 Kcal/g in September (Table IV. 7). An annual average of 3.44 Kcal was calculated.

Calorific value in different sexes exhibited well defined variation. (Fig. IV.9). Males recorded a maximum value of 3.46 Kcal in August and minimum value of 2.83 Kcal/g in March and females recorded maximum value of 3.75 Kcal/g in August and minimum 3.05 Kcal in April.

DISCUSSION

Biochemical composition and the corresponding seasonal variations indicated a clear relationship to the reproductive status of the clam, *M. opima*. Gonad development involves intense metabolic activity. The accumulation of egg reserves may take place at the expense of stored reserves in the body tissue or from directly ingested food or both (Gabbot, 1975). However the fluctuation in the nutrient levels was within a narrow range indicating that it was not much dependent on the reserve food materials. This is probably due to the high nutrient levels in the shallow tropical waters which is more than ample to meet the metabolic requirements of the clam as noticed by Katticaran (1988) in the case of *Sunetta scripta* from Cochin.

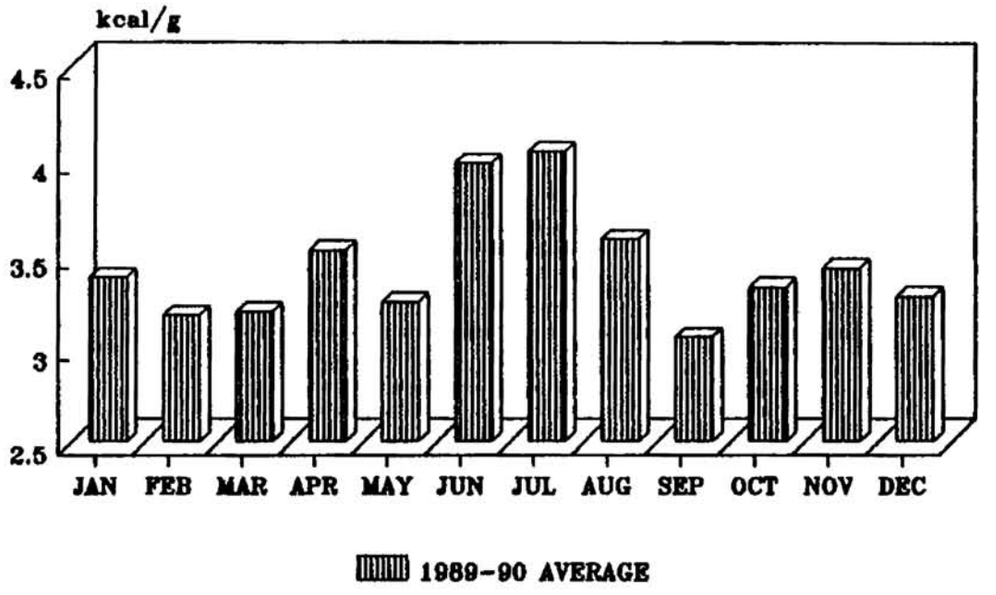

FIG. IV.8
MONTHLY VARIATION IN CALORIFIC VALUE (POOLED SAMPLE)

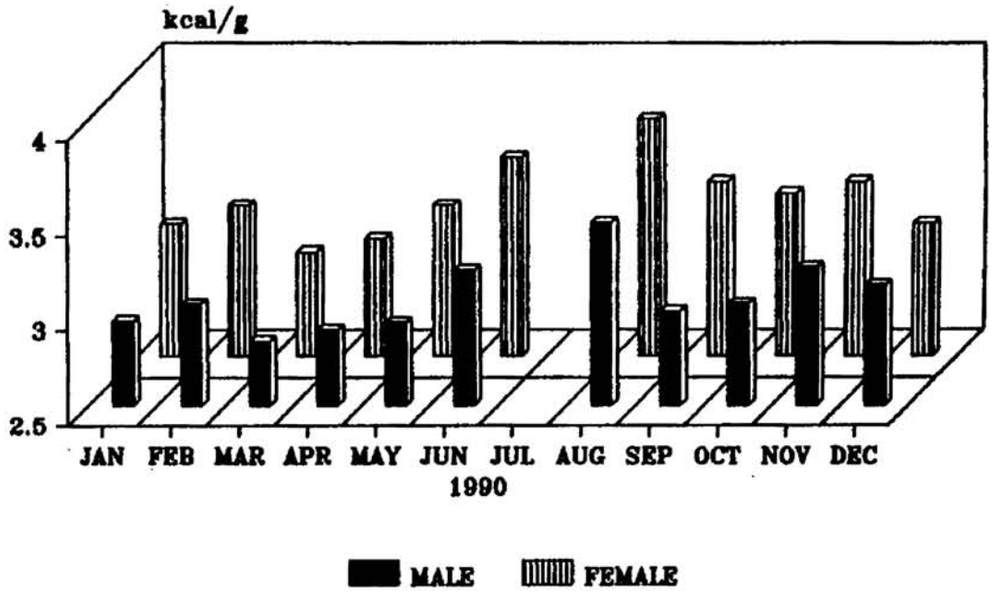

FIG. IV.9
MONTHLY VARIATION IN CALORIFIC VALUE (SEXES SEPARATED)

TABLE. IV.7
MONTHLY VARIATION IN CALORIFIC CONTENT

No.	MONTH	CALORIFIC VALUE (kcal/g).		
		MALE*	FEMALE*	POOLED TISSUE**
1	JANUARY	2.94	3.20	3.38
2	FEBRUARY	3.03	3.30	3.18
3	MARCH	2.83	3.05	3.20
4	APRIL	2.89	3.12	3.52
5	MAY	2.93	3.30	3.25
6	JUNE	3.21	3.55	3.99
7	JULY			4.05
8	AUGUST	3.46	3.75	3.58
9	SEPTEMBER	2.99	3.42	3.06
10	OCTOBER	3.03	3.36	3.33
11	NOVEMBER	3.22	3.42	3.43
12	DECEMBER	3.13	3.20	3.28

* 1989

** 1989-1990 AVERAGE

A clear difference can be noticed in the biochemical cycle of tropical and temperate bivalves. In temperate bivalves the seasonal cycle of gross biochemical composition follow a regular pattern with a period of inactivity in the winter months during which gametogenesis may proceed slowly and when reserves stored in various tissues may be drawn upon to supply reduced metabolic demands. This is followed by a short period in the spring when reserves are renewed, growth recommences and there is rapid gametogenesis and gonad proliferation, and a reproductive period during summer when the temperature rises above a certain minimum and body growth, gonad growth and spawning may proceed together in environmental changes especially in the availability of food (Ansell and Travellion, 1967).

In the tropical bivalves, the reproduction occurs year round with two to four spawning peaks per year. Feeding and accumulation of nutrients occurs throughout the year so that metabolic energy requirements are not much dependent on reserve nutrients. This is probably due to the uniform environmental conditions throughout the year and does not have to overcome a winter season of unfavourable conditions like low temperature, scarcity of food etc. as in the case of temperate bivalves. The influence of geographical distribution of biochemical composition is indirect, linked by the reproductive process, food availability, etc. which is reflected in the annual reproductive cycle.

The soft tissue of *M. opima* had a water content in the range of 71.2 to 82.71 % . The water content is within a narrow range of 78.3 to 75.8% from August to April. The decrease in water content coincided with high saline condition in May and increase in water content during June July can be correlated with the decreased salinity of the ambient media and spent gonadal condition.

Castro and Mattio (1987) recorded 69 to 76 % of water in *Ostrea puelchana*. Nagabhushanam and Talikhedkar (1977) recorded 75.91 to 85.17 % of water in *Donax cuneatus* with high water content during monsoon (July - September). A maximum water content was reported after spawning in *Mytilus viridis* (Mane and Nagabhushanam, 1975), and it coincided with the monsoon season. Similar observations were also made in *Meretrix meretrix* by Nagabhushanam and Deshmukh (1974). An increase in water level in populations of *Tapes decussatus* and *T. philippinarum* during spring which was maintained throughout winter was also noted (Benninger and Lucas, 1984). Increase in soft tissue water content during low food availability was also recorded (Ansell, 1975; Taylor and Venn, 1979).

Inverse relationships between water content and organic content was pointed out by Durve and Bal (1961) in *Crassostrea gryphoides*, Saraswathi and Nair (1969) in *Nausitora hedleyi*, Salih (1977) in *Meretrix casta* and Stephen (1980b) in *Crassostrea madrasensis*. Ansell (1972) found the body weight of the clam, *Donax vittatus* to be inversely proportional to

the water content and that spawning was accompanied by an increase in body water content.

During the process of spawning, the gametes filled in the gonadal follicles get substituted with a watery fluid. The decrease in organic contents at this condition accounts for its inverse relation with water content. Usually water content recorded high level during the period of decreased salinity, ie. coinciding with monsoon season (Mane and Nagabhushanam, 1975; Salih, 1977). In most of the tropic bivalves one of the spawning occurred in the premonsoon or early monsoon period and low saline condition coincided with spent gonadal stage. The altered osmotic state and watery fluid which fill the spent gonadal follicles synergistically cause the increased water content. It is evidenced by the higher water content in the spent condition in monsoon while it is comparatively lower in the spent gonadal condition other than that in the monsoon.

Protein recorded minimum range of annual fluctuation out of the three energy reserves (protein, glycogen and lipid) studied in *M. opima*. High values of protein concentrations (37.5%) were recorded in August and low concentrations (34.7%) in June. Deslous-Paoli and Heral (1988) recorded 29 to 52% dry weight protein in *C. gigas* and Kumari and Nair (1988) noted 39.04 to 75.72% dry weight in *Tellina angulata*. Bressen and Marin (1985) recorded 31 to 52% protein in *Mytilus galloprovincialis* which exhibited no seasonal trend.

Nagabhushanam and Deshmukh (1974) found high protein values through out the year in *Meretrix meretrix*. Low values

coincided with spawning. Similar observation was recorded in *Villorita cyprinoides* also (Ansari *et al.*, 1981). Walne and Mann (1975) estimated an average of 54% protein in *Ostrea edulis* and 55% in *Crassostrea gigas*. They opined that the decline in protein and carbohydrate during summer was not due to release of gametes but may be associated with gonad maturation. The high value of the protein content during the ripe stage was to be expected as protein constitutes the major organic compound of bivalve oocytes (Holland, 1978).

The reproductive cycle of *M. opima* was found to influence the protein concentration of the clam. The higher values were recorded during gametogenesis 37.3 and 37.5% while decrease in concentration was noticed in the spawning period. The decrease was found to be prominent during the spawning gonadal condition which took place in May-June (14.9% and 14.7%). Though a decrease in concentration was observed during the second spawning period also, it was comparatively less. The stages of reabsorption of residual gametes of previous spawning, sexually indeterminate resting period and gametogenic stages show an increasing trend of protein concentration, owing to the accumulation of nutrients.

Proteins play an important role in the supply of energy during the conditions of metabolic need particularly during reproduction. In *Mytilus edulis*, protein reserve is used during periods of increased energy demand and low glycogen availability (Gabbot and Bayne, 1973). Similarly, utilisation of somatic protein reserves during gametogenesis has been

reported in *Tapes philippinarum* (Adachi, 1979; Benninger and Lucas, 1984) and in *T decussatus* (Benninger and Lucas, 1984).

In *Crassostrea madrasensis*, a major conversion of reserve nutrients into gamete material was noticed before the first rapid gametogenic cycle. However in the second gametogenic cycle gonad development was rather slow. It has been inferred that the major share of nutrients must come directly from feeding during the gonadal development (Stephen, 1980b). In *Tapes philippinarum* protein was observed to have accumulated in the adductor muscle after spawning period of which appeared to be used up during gonad maturation. It was not repeated in the second spawning which may be due to the difference in the metabolic .status and food availability (Adachi, 1979).

Nagabhushanam and Mane (1978) recorded 52 to 71% protein in *Mytilus viridis*. The highest level was noted in mature and low level in spent stage of reproduction. In *Donax cuneatus* protein content was relatively high (56.59% to 68.31%) throughout the year with the decrease during breeding and maturation period (Nagabhushanam and Talikhedkar, 1977). The annual average value of protein content in *M. opima* (36.8%) is found to be less than that recorded in other bivalves. Nagabhushanam and Mane (1978) observed a clear positive correlation of protein level with different stages of gametogenesis and negative correlation with spawning in *Mytilus viridis*. The trend was found to be repeated in the second spawning period also. Castro and Mattio (1975) recorded

increase of protein in gonads at ripe condition and decline to normal level after spawning in *Ostrea puelchana*. Jaybal and Kalyani (1986) observed high values of protein during maturation phase and very low protein levels during the active spawning period of *Meretrix meretrix*. According to Benninger and Lucas (1984) somatic protein formed the predominant energy substrate during gametogenesis. The observed maximum protein values were during the spawning periods of two populations of *Tapes decussatus* and *T. philippinarum*.

In *M. opima* The protein concentration in different sexes was similar to that of the pooled tissue. Both males and females showed uniform variation. The males recorded a maximum of 40% and minimum of 35% in August and January respectively, while it was 40% and 36% in September and June in the case of females. Indeterminates recorded a low level of protein concentration between 35% and 36% . The almost uniform levels of protein content indicate that sex difference has no influence in the concentration of protein. The low protein concentration in the indeterminates has been due to the low protein concentrations in the post spawning stage.

Glycogen form another important biochemical component. Average values of the pooled tissue indicated notable variation in glycogen concentration in *M.opima*. The values recorded a wide fluctuation from 14.8% in September to 41.1% recorded in July. Varying levels of glycogen concentration have been reported in different bivalves. Bressen and Marin (1985) recorded 2 to 32 % of carbohydrate in

Mytilus galloprovincialis with minimum in unfavourable period of winter and maximum in summer. In *Crassostrea gigas*, 26.7% to 69.9% dry weight of glycogen was recorded (Deslous-Paoli and Heral, 1988). Kumari and Nair (1988) recorded 4% to 24% of carbohydrate in *Tellina angulata*. In *Donax cuneatus* 11.14% to 25.85% of glycogen was estimated and it was found to be related to the breeding behaviour (Nagabhushanam and Talikhedkar, 1977). An average carbohydrate content of 13% for *Ostrea edulis* and 12% for *Crassostrea gigas* was estimated by Walne and Mann (1975). In *M.opima*, glycogen, the ready sources of energy reserve, was observed to have widely fluctuated. However, the concentration recorded a range similar to that recorded in other bivalves.

It has long been evident that the seasonal cycle of storage and utilisation of glycogen reserves in adult bivalves is related to the annual reproductive cycle in bivalves. Nagabhushanam and Mane (1978) found maximum glycogen accumulation before spawning which declined with spawning in both the spawning periods of *Mytilus viridis*. In *Tellina*, high level of carbohydrate build up was noticed during the favourable season of spring which was not utilised for spawning, however, it was found to be used during winter. On the other hand *D. villatus* from the same location was found to use carbohydrates during spawning which was again made up before being used in winter (Ansell, 1972). The utilisation of reserve glycogen is therefore found not to be restricted to

reproductive process alone, but open for other situations of energy demand also.

Some parts of the tissue were reported to be sites of glycogen storage in bivalves. These include adductor muscle in *Sunetta scripta* (Katticaran, 1988) and in scallop *Chlamys opercularis* (Taylor and Venn, 1979), digestive gland in *Agropecten irradians concentricus* (Barber and Blake, 1981), gonad in *Mytilus edulis* (Sastry, 1976) and mantle in *Ostrea edulis* (Walne, 1970;Gabbot and Bayne, 1973).

In both the sexes of *M. opima*, lower glycogen content was recorded during the gametogenic stages which increased during the post spawning stage. Indeterminates recorded slightly higher levels. Both males and females showed almost a similar pattern of glycogen concentration. In the oyster *Crassostrea rizophorae* glycogen was not found to vary with sex. Minimum concentration of 12.3% was recorded in males during March and a maximum of 24.4% in August. Females recorded a minimum of 55.8% in January and Maximum of 28.3% in August(Littlewood and Gordon, 1988). In the glycogen estimation conducted on raft cultivated *Crassostrea rizophorae*, 0.13 to 23.86 mg/g glycogen was found, however, males and females did not show significant variation (Littlewood and Gordon, 1988).

Carbohydrate was found to decrease in both male and female *Crassostrea madrasensis* with the advancement of gametogenesis — 13.2-6 % in male, 13.2-7.8% in female and 12.3% in indeterminate (Stephen, 1980b). The higher level of carbohydrate in females has been attributed to the fulfillment

of the biochemically expensive egg production process. Kumari and Nair (1988) found maximum carbohydrate in female clam *Tellina* in contrast to protein and lipid content. Gabbot (1975) has proposed a metabolic pathway by which glycogen in the mussels may be converted into lipid reserves in the developing eggs.

The role of lipid as an energy reserve has been well documented. The lipid level in *M.opima* exhibited a clear annual variation ranging from 3 % in February to 6.1% in December. Excepting the odd value recorded in March it exhibited a clear correlation with the reproductive cycle with accumulation during gametogenesis and depletion with spawning.

Nair and Shynamma (1975) recorded 2.6 to 8.7 % of lipid in *Villorita cyprinoides* var *cochinensis*. In *Mytilus galloprovincialis* lipid pattern was characterized by the presence of two peaks one in early spring (March, 12-14%) and one in summer (August 8-11%) both followed by a decrease. The variation is likely to be related to spawning (Bressen and Marin, 1985). 6 to 20 % lipid was recorded in *Crassostrea gigas* by Deslous-Paoli and Heral (1988). Kumari and Nair (1988) recorded 7.41 to 19.81 % dry weight of lipid in *Tellina*. The lipid concentration in *M.opima* was found to be similar to that a recorded in other tropical bivalves.

The concentration in lipid indicated clear relation to the reproductive cycle of the clam. Low values were recorded in the post spawning stage (2.96 % in February and 3.11 % in July). The values gradually increased with

gametogenesis and reached maximum in the spawning condition (5.35 % in June and 6.07 % in December).

Lubet (1959) also found that lipid accumulation coincides with the end of reproductive phase and beginning of sexual resting phase. In *Donax vittatus* a distinct cycle of change was recorded in the lipid content of the body soft tissue. The percentage of lipid increased in the pre-spawning period and decreased with spawning. This was followed by a period of accumulation and a depletion in the winter months. Similar changes in relation to the reproductive cycle was observed in *Abra alba* also (Ansell, 1972, 1974a). Lipid content in *Ostrea edulis* and *Crassostrea gigas* was observed parallel to that of carbohydrate except in a short period. However, loss of lipid was recorded in *Ostrea edulis* during spawning (Walne and Mann, 1975).

In *Donax cuneatus* fat content ranged from 4.56 to 7.15 %. A maximum fat level coincided with ripe and minimum with spent condition (Nagabhushanam and Talikhedkar, 1977). During the stages of gonadal development, the lipid stored in soft tissues other than gonads is transferred to the developing gonad making no difference in total lipid level which declines with the release of gametes (Taylor and Venn, 1979). Jaybal and Kalyani (1986) found that the biochemical cycle in *Meretrix meretrix* is more influenced by the reproductive cycle, than food availability.

Studies on *Ostrea puelchana* by Castro and Mattio (1987) revealed that lipid concentrations diminished during

winter season, during which the food available was maximum and increased during spring and summer seasons. They found that the lipids stored in the digestive gland-gonad complex exhibited variation in relation to spawning while those of other body components remained unvaried. If food in the environment is insufficient, glycogen may be changed into lipids during the reproductive period and be used for making sexual products (Goddard and Martius, 1966).

In tropic bivalves, the major use of lipids were found to be during the reproductive process while in temperate bivalves, the lipid stores were used up during the unfavourable conditions (winter). Observations are reported from tropical regions where the clams were found to depend on available food during favourable season and on nutrient reserves when food is scarce. In the second spawning in the same year adductor muscles and digestive diverticula are reported to be the major sites of nutrient storage in bivalves.

A clear variation in lipid concentration can be noticed in different sexes of the clam. Both male and female specimens of *M.opima*, recorded a clear difference in lipid accumulation with higher accumulation in females. Indeterminates recorded a low level as they appeared after the spawning stage. The higher level of lipid in females is due to higher biochemical budget for egg production since the larvae are lecithotrophic. In the eggs and larvae, there are clear advantages to be gained from storing fat. First it being a more concentrated energy form and secondly due to an increase

in buoyancy pertaining to the lower density than carbohydrate or protein (Gabbot, 1975).

In the bivalve *Chlamys septemradiata* the females had greater lipid content with ripe female gonad containing twice as much lipid as males. In another clam *Nucula sulcata* there was a great variation in lipid content of male and females and lipid accumulation was observed in ripe clams which decrease after spawning (Ansell, 1972, 1974a,b,c). The higher level of carbohydrate and lipid in females can be due to a higher biochemical budget for egg production, since the larvae are lecithotrophic.

Use of 74.6 % of glycogen, 44 % of lipids and 36 % of proteins to meet more than 50 % of the requirement for basal metabolism was recorded in *Crassostrea gigas* due to poor trophic condition of the environment (Deslous-Paoli and Heral,1988). Pridmore *et al.* (1990) found marked increase in glycogen content in *Crassostrea gigas* down the pollution gradient while that of protein was found to be insignificant.

Calorific values indicated annual variation in relation with the reproductive cycle of the clam. A Maximum value of 4.05 Kcal /g was recorded in July while minimum value of 3.06 Kcal /g was recorded in September with an annual average of 3.44 Kcal /g. Females recorded a higher calorific value than males with an annual average of 3.06 Kcal/g while it was 2.81 Kcal in males. The nutrient reserve of the egg, which is evident in the lipid concentration, accounts for the increased calorific value of females.

The clam meat is gaining importance as a nutritive food enriched with better assimilable forms of protein and glycogen. Thus, with the clam meat gaining much importance as a nutritive food and the increasing need to assess the quality of the bivalve tissue, the present part of the investigation has evaluated the importance of the bivalve *M.opima* from the point of view of human nutrition. It has been demonstrated that the mature condition of gonads occurring in the months of May-June and September-October are the periods of maximum nutritive value. However, over exploitation should be checked during this period inorder to maintain enough brood stock in the natural system which may otherwise harm the fishery potential.

Chapter V

METAL BIOCONCENTRATION

INTRODUCTION

Animal food products form an important source of intoxicating levels of harmful metals for human beings. Though heavy metals are not man-made, as in the case of synthetic organic materials, the redistribution by man plays an important role in the environmental pollution problem. The heavy metals are released into the natural environment, for dilution and deposition of waste heavy metal products. It is possible for the contaminants to enter man's food through various routes, especially fishery products. Determination of metal levels in marine products, bivalves which can accumulate large quantities of metals, is an important step in the surveillance.

The increasing tendency to alter the natural environment and to replace with artificial environment by human being for fulfilling his needs is giving rise to changes in exposure to trace metals both essential and nonessential in increasing quantities.Toxic heavy metals, which are persistent, are being constantly discharged into rivers and estuaries in substantial amounts. Major sources of these heavy metal pollution are industrial effluents and agro-chemicals. It has been our experience that such changes can adversely affect living systems due to toxic effects.

Living organism contains many chemical elements in various molecular forms and in greatly varying concentrations. Most elements occur in low concentrations. Only a few like hydrogen, oxygen, carbon, nitrogen and phosphorus constitute the main composition of living organism. Amongst the trace elements (concentration below 100 µg/g), a certain number of trace elements are recognised as being essential for life processes. Compared with the total number of trace elements present, the definite biological significance of most of the trace elements may be unknown. Trace elements in living organisms are present in concentrations considerably higher than those found in their natural environment.

Most molluscs are known to take up and concentrate tissues zinc, lead, nickel, cobalt, manganese, copper, chromium and cadmium in their body apparently without any ill effects. However some metals even in concentrations as low as few parts per billion are lethal to the larvae of these organisms. The Arabian sea is reported to contain 2.5 µg/l of zinc and 4.9 µg/l of copper which is high when compared to the concentration of that in the open ocean (Senguptha *et al.*, 1978).

The metal absorptive process include absorption from solutions, suspended particles, sediments and food (Bryan, 1979). The ability of marine organisms, particularly shellfish to concentrate heavy metals in several orders of magnitude over the amounts found in sea water, has long been recognised (Brooks and Rumsby, 1965: Bowen, 1966: Riley and Chester, 1971).

Seasonal fluctuation in the distribution of trace metals in the sedentary organism in an estuary, is controlled by an array of extrinsic and intrinsic factors such as the extent of pollutant delivered into the estuary and the associated *in situ* dilution, interaction of pollutants, changes in the physiological and biochemical state of the organism, weight of soft tissue and the direct effects of temperature, salinity and other water quality parameters which show seasonal variations. The seasonal profiles of trace metal availability may vary according to the estuarine position and also the locality of occurrence of the animal (Nair and Nair, 1986).

REVEIW OF LITERATURE

Bivalves have received extensive treatment in literature owing to their reported ability to reflect environmental levels of trace metal contaminants in marine and estuarine environments. Most of the studies deal with the accumulation and depletion, and its effects on the organism. These include Mathew and Menon (1986) and Laxmanan and Nambisan (1979) on *Perna viridis*, Rajendran and Kurien (1986) on *Crassostrea madrasensis*, Baby and Menon (1987), Kumar *et al.* (1987) on *Perna indica*, Abraham *et al.* (1986) and Sathyanathan *et al.*(1988) on *Villorita cyprinoides var cochinensis* Mathew and Menon (1992) in *Donax indicus* and Katticaran and Salih (1992) on *Sunetta scripta*.

Numerous studies on concentration of metals in bivalves have been reported. Segar *et al.* (1971) studied the

distribution of six major and thirteen trace elements in two bivalves. Bryan (1973) studied the seasonal variation of trace metals in scallops. Mackay *et al.* (1975) estimated the concentration of copper, zinc, cadmium, lead and arsenic in cultivated oysters. Bryan and Hammerstone (1978) reported the heavy metal concentration in the bivalve, *Scorbicularia plana*. Seasonal variation of trace metal content in *Mytilus edulis* was recorded by Boalch *et al.* (1981). Phillips and Muttarasin (1985) studied the concentration of trace metals like cadmium, chromium, copper, iron, lead, nickel, mercury and zinc in bivalves from Thailand. Kumari and Nair (1990) studied the metal concentration in two molluscs. Works were also carried out by Coimbra *et al.* (1991) and Berrow (1991) in *Mytilus edulis*, Paez-Osuna *et al.* (1991) in *Chione* sp., *Saccostrea cucullata* and *Crassostrea corteziensis* and Berkman and Negro (1992) in scallops.

Concentration of the metals copper, zinc, manganese (minor elements) and calcium (major element) in the soft tissue of *M. opima* were analysed. All the metals analysed are biologically essential (Spaargaren, 1985). No report is available on the heavy metal concentration or bioaccumulation of metals from Kayamkulam lake.

Zinc plays an important role in the metabolism of marine organisms. Many of the best studied and earliest identified enzymes which are relatively abundant, including alcohol dehydrogenase, aminopeptidase and carboxypeptidase have later been shown to be associated with metals, particularly

zinc. Carbonic anhydrase, for example, is a zinc containing protein that plays a major part in the laying down of calcium carbonate in molluscan shells and crustacean exoskeleton (Bundy, 1977).

Copper, being a biologically essential trace metal, performs many functions such as forming a part of oxygen binding pigment in crustaceans and molluscs, though it is absent in most bivalves (Morton, 1958). Copper represents the metal fraction in one metalloenzyme cytochrome oxydase of *Crassostrea virginica* (Chambers *et al.*, 1975) and luciferase of *Pholas dactylus* (Henry *et al.*, 1975). Copper containing cytochrome systems have been reported from the mitochondria of bivalves (Kawai, 1975). Bowen (1979) reported 30 copper associated enzymes. Similarly, Dixon and Webb (1979) listed a total number of 2137 enzymes in marine organisms, of which 20 contained copper. From this data, White and Rainbow (1985) estimated 17.5 μg/g dry weight of copper requirement. A histological study on *Crassostrea gigas* and *C. virginica* has suggested that oyster basophils contain high concentrations of both zinc and copper and that there is a correlation between the number of basophils in the tissue (Ruddell and Rains, 1975).

Manganese exhibits a preference for particulate association rather than remaining in solution (Nair and Nair, 1986). Therefore food forms the major route of manganese accumulation in filter feeding bivalves. Molluscs generally accumulate more manganese, particularly in the gills and

mantle, than other invertebrates and it offers protection effect against many toxic metals, likely to be owing to the competition for uptake or binding sites.

Manganese metal is of low ionic toxicity but has high biological significance. It is having an important role in enzyme activation (Smith, 1951; Mounter and Chanurin, 1953) Presence of manganese in the metalloenzyme pyruvate kinase in *Crassostrea gigas* was also noticed (Hochacuka and Mustafa, 1972).

Calcium, being the most important component of the calcareous shell, plays an important role in the metabolism of the clam. The mantle tissue which is the outermost part of the soft tissue of the clam is the organ which secretes the shell and it is likely to contain a very large quantity of calcium. The uptake of calcium from the surrounding media and its conversion into the calcareous matter is a speedy process as the growth of the shell indicates.

Clams, as sentinel organisms integrate the pollutant level over time, thereby giving a true indication of pollutant status when sampling can only be conducted monthly or yearly, and thus become a representative of well defined areas. The purpose of this study was to analyse both major and trace metal representatives in *M.opima* from Kayamklam lake for a period of two years inorder to provide a measure of their bio availability and persistence in the organism. Thus the bioavailability of metals in clam would highlight not only the general indication of the potential for food chain

biomagnification of metals, but also supplement the traditional water and sediment ambient monitoring programme.

In the present study the annual variation in the metal content of *M. opima* is made on a monthly basis. Males, females and indeterminates were treated separately in order to find variations in accumulation, if any. The study of the metal content of the clam tissue is of concern, since *M. opima* is exploited on a large scale for human consumption.

MATERIALS AND METHODS

The specimen samples were collected from the clam-beds of the Kayamkulam lake (methods in Chapter III). Clams of size 20 to 40 mm were kept in filtered sea water for 24 h for clearing the gut contents. Pooled samples of male, female and indeterminate clams were used for analyses during the two years of study, while separate analyses were conducted according to the sexual condition in the second year. Ten clams were sampled for each set of analysis. Pooling of individuals permits processing of large number of clams in a given period of time, resulting in reliable means and hence more accurate values. Secondly, it increases the amount of tissue available for analyses. The results give a general picture of the metal accumulation which is a mean value, thereby avoiding the inter sample variability.

All the glass wares used for trace metal analyses were washed with 10% nitric acid and rinsed with glass distilled water. The clams were opened with a stainless steel

scalpel, tissues were washed in minimum quantity of glass distilled water, dried at 65°C for 24 h and crushed to fine powder. A known weight of the tissue sample was digested with perchloric acid and nitric acid mixture (1:4) in a kjeldahl flask until solution was clear. The extract was diluted with distilled water and the metal concentrations were determined by using atomic absorption spectrophotometer (Perkin-Elmer Model 2380) at appropriate wave lengths (Copper ; 324.7 nm, manganese ; 279.5 nm and zinc ; 213.9 nm). Calcium was determined by using flame photometer (Model Elico C 22 D). Standard solutions were prepared using metallic copper, metallic zinc, manganese sulphate and calcium carbonate for copper, zinc, manganese and calcium respectively. The whole digestion and analysis were conducted for each sample in duplicate and the mean values were used for subsequent data analysis.

Seasonal variability in copper, zinc, manganese, and calcium content in the soft tissue of *M. opima* was estimated. Metal content of the pooled tissue samples was determined during the two consecutive years of study. Apart from the pooled samples male, female and indeterminate clams were also analysed separately in the second year of study. Since indeterminate clams appeared during the resting gonadal condition alone, only three sets of samples were analysed during the year. In the case of pooled tissue samples, the mean values of the consecutive years are considered and discussed, in order to get a general trend.

The quantities of metals present in the clam soft tissue were determined and the values are presented in microgram per gram dry weight of the tissue. The metals were detected in the clam tissue in varying concentrations.

RESULTS

The general trend in the concentration of zinc is presented in the Fig. V.1. Zinc was not found to follow a definite pattern annually. The maximum value was recorded in February and April (55 µg/g) and minimum (18 µg/g) value in March (Table V. 1). Whereas all other values recorded, were within a narrow range of 34 µg/g to 48 µg/g.

The concentrations of zinc in the soft tissue of different sexes showed similarity to that of the pooled tissue (Fig. V. 2). Males recorded high value of 75 µg/g in February and a minimum value of 4 µg/g in March. A high value of 90 µg/g was recorded in female in the month of April and zinc was not present in detectable levels in March. Indeterminates recorded 62, 52 and 44 µg/g in February, July and August respectively (Table V. 2).

Copper was present in the tissue of *M. opima* throughout the year in detectable quantities. The monthly mean values of the copper content obtained during the two years of study are presented in Fig. V. 3. The level of copper in *M. opima* showed a marked monthly variation (Table V. 3). The maximum average value of 34 µg/g was recorded in August. Whereas the lowest value of 12 µg/g was recorded during April.

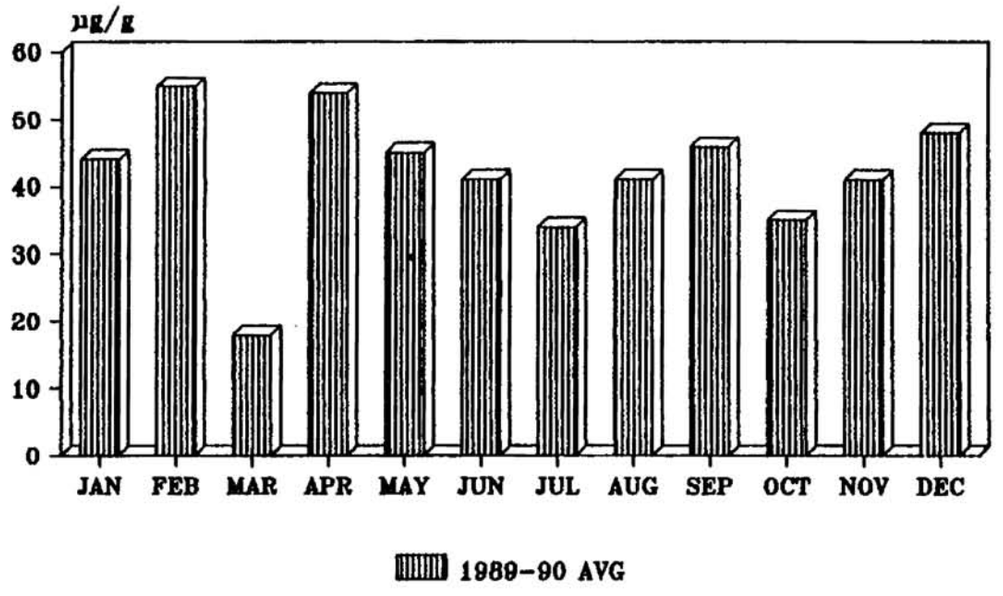

FIG. V.1
MONTHLY VARIATION IN ZINC (POOLED SAMPLE)

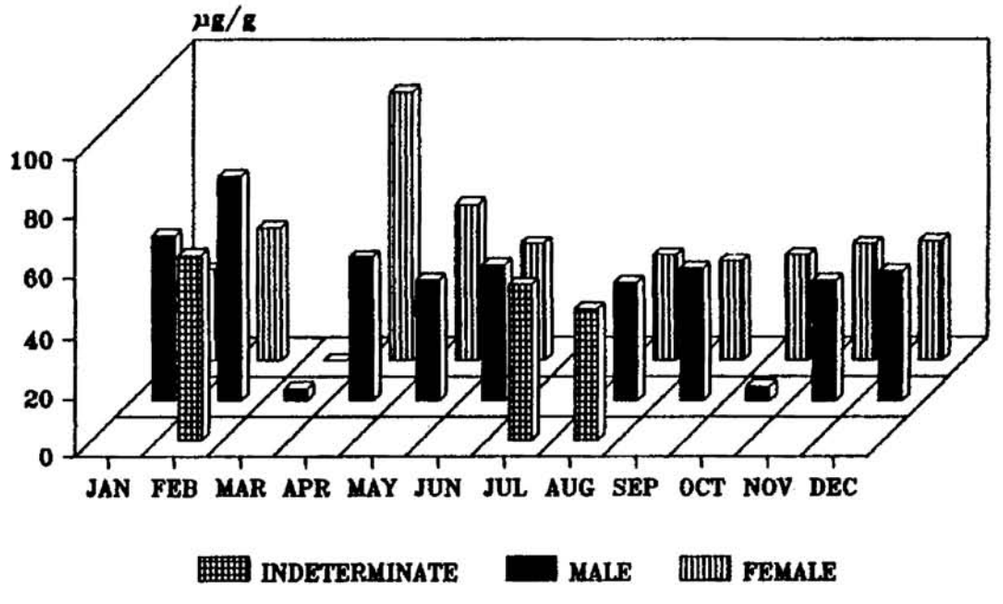

FIG. V.2
MONTHLY VARIATION IN ZINC (SEXES SEPARATED)

TABLE. V.1

MONTHLY VARIATION IN ZINC (POOLED TISSUE)

No.	MONTH	ZINC (µg/g)		
		1989	1990	AVERAGE
1	JANUARY	46	42	44
2	FEBRUARY	50	60	55
3	MARCH	35	02	18
4	APRIL	40	69	55
5	MAY	43	46	45
6	JUNE	40	42	41
7	JULY	40	52	34
8	AUGUST	42	40	41
9	SEPTEMBER	54	39	46
10	OCTOBER	50	20	35
11	NOVEMBER	43	40	41
12	DECEMBER	55	41	48

TABLE. V.2

MONTHLY VARIATION IN ZINC (SEXES SEPARATED)

No.	1990	ZINC (µg/g)		
		MALE	FEMALE	INDETERMINATE
1	JANUARY	55	30	
2	FEBRUARY	75	44	62
3	MARCH	04	ND	
4	APRIL	48	90	
5	MAY	40	52	
6	JUNE	45	39	
7	JULY			52
8	AUGUST	39	35	44
9	SEPTEMBER	44	33	
10	OCTOBER	05	35	
11	NOVEMBER	40	39	
12	DECEMBER	43	40	

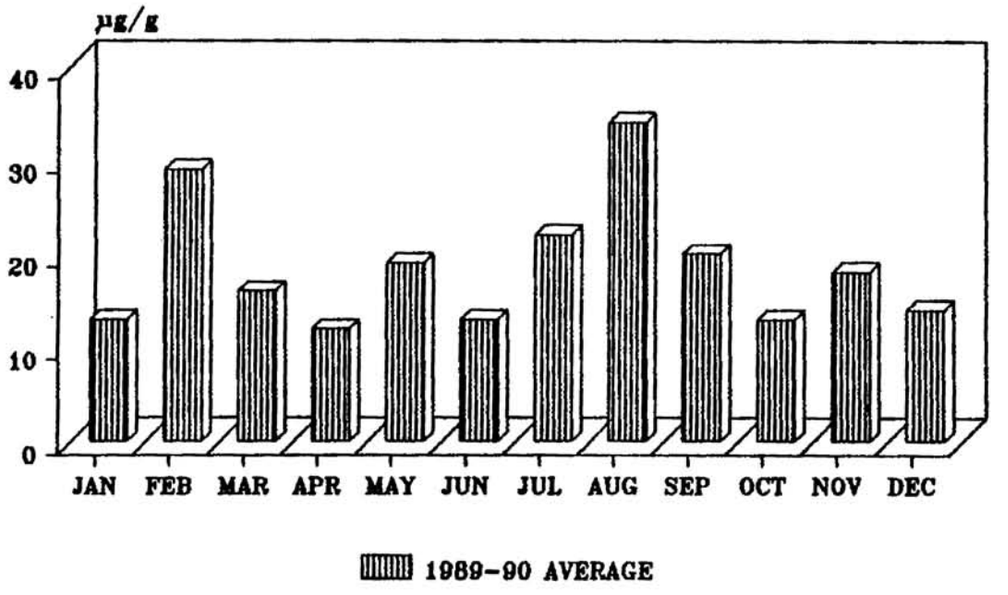

FIG. V.3
MONTHLY VARIATION IN COPPER (POOLED SAMPLE)

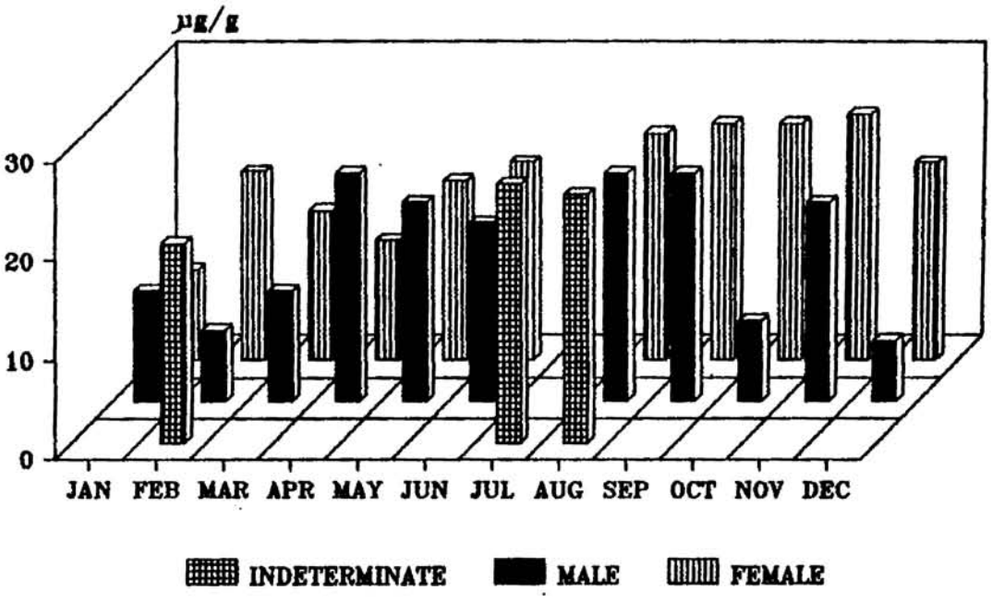

FIG. V.4
MONTHLY VARIATION IN COPPER (SEXES SEPARATED)

ABLE V.3
ONTHLY VARIATION IN COPPER (POOLED TISSUE)

No.	MONTH	COPPER (µg/g)		
		1989	1990	AVERAGE
1	JANUARY	16	10	13
2	FEBRUARY	43	15	29
3	MARCH	19	13	16
4	APRIL	06	18	12
5	MAY	19	19	19
6	JUNE	07	19	13
7	JULY	18	26	22
8	AUGUST	44	24	34
9	SEPTEMBER	16	24	20
10	OCTOBER	10	16	13
11	NOVEMBER	13	23	18
12	DECEMBER	15	13	14

ABLE V.4
NTHLY VARIATION IN COPPER (SEXES SEPARATED)

No.	MONTH	COPPER (µg/g)			
		MALE	FEMALE	INDETERMINATE	F/M RATIO
1	JANUARY	11	09		0.818
2	FEBRUARY	07	19	20	2.714
3	MARCH	11	15		1.364
4	APRIL	23	12		0.522
5	MAY	20	18		0.090
6	JUNE	18	20		1.111
7	JULY			26	
8	AUGUST	23	23	25	1.000
9	SEPTEMBER	23	24		1.043
10	OCTOBER	08	24		1.250
11	NOVEMBER	20	25		1.250
12	DECEMBER	06	20		3.333

Difference in the concentration of copper content in male, female and indeterminate clams was also observed (Fig. V. 4). The annual fluctuation was prominent in males. Males recorded high value of 23 μg/g in the months of April, August and September and minimum value of 7 μg/g in February. Minimum value of 9 μg/g in January and maximum value of 25 μg/g in November was recorded by females. Indeterminates recorded high values of 20, 26 and 25 μg/g respectively in February, July and August (Table V. 4).

The monthly mean concentration of manganese in the pooled clam tissue is presented in Fig. V. 5. Manganese was present in all the samples collected during the first year of study, 1989, while it was not present in detectable quantities in March and April during the second year of investigation (Table V. 5). The level of manganese showed a wide annual fluctuation. The lowest value was recorded in March (02 μg/g) and the highest value in January (29 μg/g).

Fig. V. 6 presents the monthly variation of manganese content in male, female and indeterminate clams. Manganese was not present in detectable quantities in February and August in indeterminates; April, September and October in males and in March, April and December in females. Females recorded a slightly higher values in the accumulation of manganese than males. Maximum value of 15 μg/g was recorded in males during February whereas it was 11 μg/g in females during February (Table V. 6.). Out of the four metals analysed, concentration of manganese was found to be the lowest.

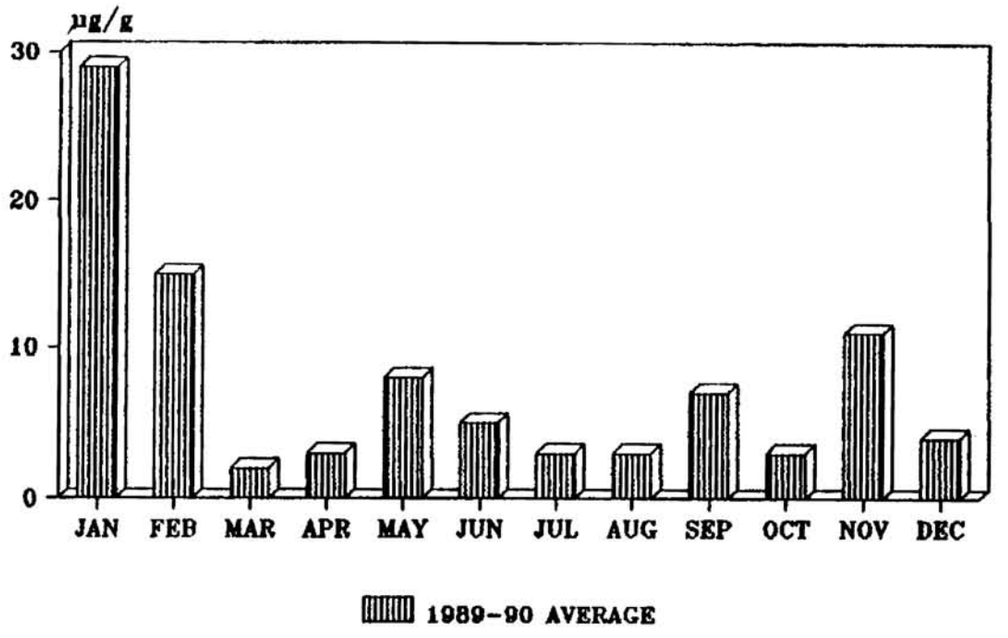

FIG. V.5
MONTHLY VARIATION IN MANGANESE (POOLED SAMPLE)

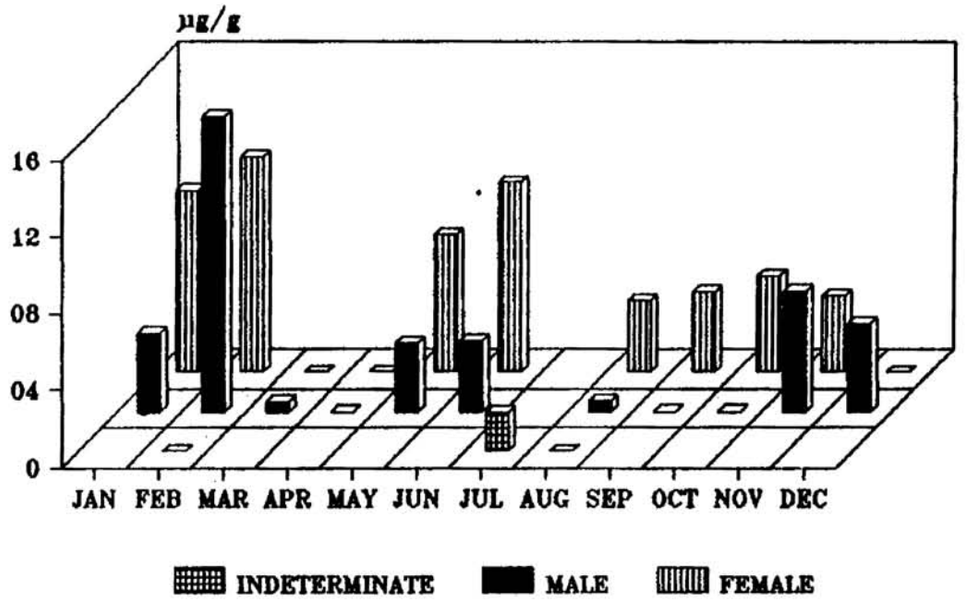

FIG. V.6
MONTHLY VARIATION IN MANGANESE (SEXES SEPARATED)

ABLE V.5

ONTHLY VARIATION IN MANGANESE (POOLED TISSUE)

No.	MONTH	MANGANESE (µg/g)		
		1989	1990	AVERAGE
1	JANUARY	52	07	29
2	FEBRUARY	21	09	15
3	MARCH	04	ND	02
4	APRIL	06	ND	03
5	MAY	10	05	08
6	JUNE	04	07	05
7	JULY	04	02	03
8	AUGUST	05	01	03
9	SEPTEMBER	11	02	07
10	OCTOBER	04	03	03
11	NOVEMBER	17	05	11
12	DECEMBER	06	02	04

ABLE V.6

ONTHLY VARIATION IN MANGANESE (SEXES SEPERATED)

No.	1990	MANGANESE (µg/g)		
		MALE	FEMALE	INDETERMINATE
1	JANUARY	04	10	
2	FEBRUARY	15	11	ND
3	MARCH	01	ND	
4	APRIL	ND	ND	
5	MAY	04	07	
6	JUNE	04	10	
7	JULY			02
8	AUGUST	01	04	ND
9	SEPTEMBER	ND	04	
10	OCTOBER	ND	05	
11	NOVEMBER	06	04	
12	DECEMBER	05	ND	

D - NOT DETECTABLE

Calcium, being a major essential element studied, recorded high values out of all the metals analysed. The maximum and minimum values of calcium observed in the pooled tissue sample of *M. opima* were 26.36 mg/g and 18.43 mg/g respectively (Table V.7.). High values were recorded during February and December and low values in July and October (Fig. V. 7). Accumulation of calcium in the tissue did not show significant relation to sex (Fig. V.8). Male, female and indeterminate clams exhibited almost uniform level of calcium concentration throughout the year of study (Table V. 8), except the slight increasing concentration exhibited by females.

DISCUSSION

Accumulation of metals is dependent on the bioavailability, which in turn is influenced by various other factors. The trace metal content in an estuarine organism could either increase or decrease in quantity at any particular time. An increase in the trace metal content in the tissue of a benthic bivalve is usually controlled by the increased availability of the trace metal in the bottom sediments, although a decrease in the trace metal levels in the tissue depends on the biological half life of the metal in the organism (Nair and Nair, 1986). Waldichuk (1985) studied the different factors influencing the biological availability of metals to marine organisms. According to Spaargaren (1985), the essential elements like zinc, copper, and manganese may

TABLE V.7
MONTHLY VARIATION IN CALCIUM (POOLED TISSUE)

No.	MONTH	CALCIUM (mg/g)		
		1989	1990	AVERAGE
1	JANUARY	18.137	24.956	21.547
2	FEBRUARY	27.964	24.755	26.360
3	MARCH	24.376	20.025	22.213
4	APRIL	25.421	25.103	25.262
5	MAY	24.148	25.878	25.013
6	JUNE	12.224	24.734	18.479
7	JULY	18.748	24.882	21.815
8	AUGUST	20.028	19.873	19.951
9	SEPTEMBER	25.455	19.589	22.522
10	OCTOBER	17.056	19.804	18.430
11	NOVEMBER	21.317	17.102	19.210
12	DECEMBER	26.145	26.055	26.100

TABLE V.8
MONTHLY VARIATION IN CALCIUM (SEXES SEPERATED)

No.	1990	CALCIUM (mg/g)		
		MALE	FEMALE	INDETERMINATE
1	JANUARY	28.009	21.902	
2	FEBRUARY	25.542	22.300	26.422
3	MARCH	20.050	20.050	
4	APRIL	22.691	27.515	
5	MAY	26.218	25.537	
6	JUNE	25.483	23.984	
7	JULY			24.882
8	AUGUST	19.638	19.862	20.120
9	SEPTEMBER	18.743	20.434	
10	OCTOBER	18.413	21.195	
11	NOVEMBER	15.595	18.609	
12	DECEMBER	27.242	24.867	

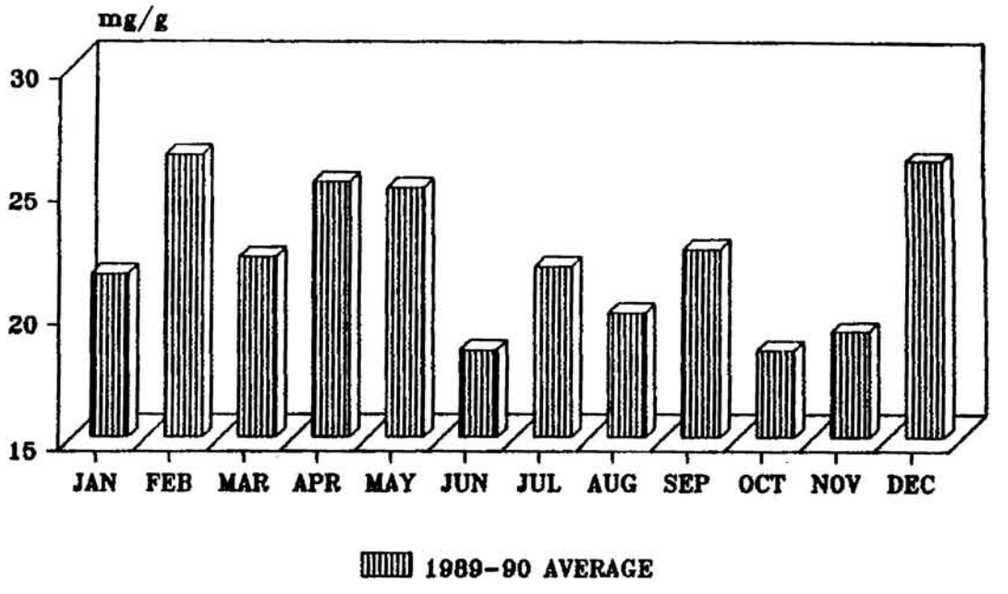

FIG. V.7
MONTHLY VARIATION IN CALCIUM (POOLED SAMPLE)

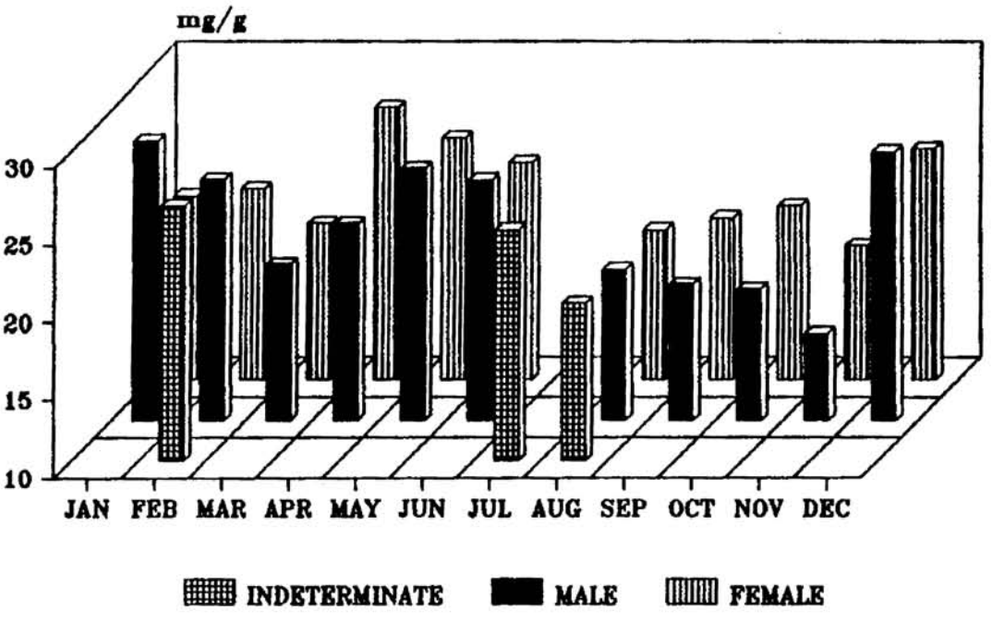

FIG. V.8
MONTHLY VARIATION IN CALCIUM (SEXES SEPARATED)

show larger variation in biological concentration than elements with doubtful biological significance.

The most important source of bioaccumulation of heavy metals in bivalve mollusc is suspended particles in the case of suspension feeders and sediment in deposit feeders. Major share of the evidences for absorption of heavy metals and their radionucleotide from solution seems to involve passive diffusion of the metal probably as unchanged soluble complexes down the gradients created by adsorption at the surface and binding by constituents of the surface cells, body fluids and external organs (Bryan, 1976; 1979; Simkiss, 1983). It is likely that even relatively small increases in ambient metal concentrations due to pollution will be reflected in measurable increases in metal concentrations in the clam.

Bivalves and barnacles have higher concentrations of accumulated metal, when compared to other groups of molluscs and crustaceans. Possible explanations for the anomalously high concentrations are that these filter feeding organisms pass large quantities of water across permeable body surfaces throughout their lives, which invariably leads to the considerable uptake of heavy metals (Bryan, 1979); oysters and barnacles feed on phytoplankton which are typically metal-rich (Knauer and Martin, 1973); and physiology of zinc and copper in both bivalves and barnacles, particularly involves detoxification by laying down of concentrated metaliferous granules (Walker, 1977; George *et al.*, 1978).

High concentrations of copper and zinc are reported in many estuaries, in such conditions bivalves can remain as active and can interact with the medium. So that any determination of copper and zinc within the tissues will reflect the availabilities of metals in the environment. At times when the environmental concentration exceeds the sublethal tolerance levels of the animal, it will result in the trigger of behavioral avoidance mechanism (Akberali and Trueman, 1985).

Copper and zinc can limit growth of the organisms, if present in insufficient quantities, but can be toxic if present at elevated concentrations. Once trace metals have been accumulated, metabolic pathways must exist either to utilize, eliminate or sequester these metals depending upon their nutritional value or toxic potential (Engel and Brouwer, 1984).

Changes in physiological state of the organism may have influence on the trace metal content of which reproductive cycle is of utmost importance as far as bivalves are concerned. However, Fowler and Orengii (1976) found no evidence of the involvement of reproductive cycle in the seasonal variation of metals in the mussel *Mytilus galloprovincialis* and suggested the increased bioavailability to be the reason for the increase.

Some molluscs have the ability to regulate their internal concentration of copper and zinc over a range of external metal concentration (Bryan, 1968; 1976; White and Rainbow, 1982). A regulatory mechanism exists in bivalves so

that the rate at which the metals enter the organism is proportonal to the level in the ambient media (Nambisan *et al.*, 1977; D'silva and Quasim, 1979).

Viarengo *et al.* (1982) reported significant decrease in protein synthesis in various tissues of *Mytilus gallonrovincialis* collected from metal polluted area. White and Rainbow (1985) reported the metabolic requirement of copper and zinc in molluscs.

In marine biota, highest concentrations of zinc were found in molluscs. It is likely that zinc does not limit the normal molluscan life process and is therefore accumulated in excess of organism's needs. According to Eisler (1981), bioconcentration of zinc is influenced by many factors such as season of collection, geographic location, specific sites of accumulation etc.

The zinc concentration in the soft tissue of *M. opima* showed an average value of 40 ± 9 µg/g out of the two year monthly samples studied. This value is low when compared to the zinc concentrations recorded earlier in other bivalve tissues.

Segar *et al.* (1971) estimated 320 to 530 ppm of zinc in the soft tissue of *Modiolus modiolus*, 120 ppm in *Glycemeris glycemeris*, 94 ppm in *Mercenaria mercenaria*, 230 ppm in *Pecten maeims*, 120 ppm in *Anodonta* sp. and 130 ppm in *Cardium edulae*. Lobel and Wright (1982) recorded 1.6 to 6.8 µ mol/g zinc in *Mytilus edulis*. Romeo and Gnassia-Barelli (1988) recorded 105 µg/g in *Donax trunculus* and 58 µg/g in *Venus verrucosa*.

Kumari and Nair (1990) recorded zinc concentration as high as 4522 µg/g in *Saccostrea cucullata*. *Mytilus edulis* contained 323 µg/g to 765 µg/g of zinc (Berrow, 1991). Paez-Osuna *et al.* (1991) recorded 118 µg/g, 1190 µg/g and 509 µg/g in *Chione* sp, *Saccostrea cucullata* and *Crassostrea corteziensis* respectively. Coimbra *et al.* (1991) reported 203.6 µg/g in *M. edulis*. Berkman and Negro (1992), estimated 80.7 µg/g to 147.3 µg/g zinc in scallops.

The annual variation in zinc concentration in *M. opima* did not show any regular pattern of accumulation. Except the low value (18 µg/g) recorded in March, all the values in pooled were within narrow range (34 to 55 µg/g), zinc accumulation did not provide any relation to the reproductive cycle of the clam.

The levels of zinc recorded in male, female and indeterminate clams further clarified that sexes have no influence on the accumulation of zinc in *M. opima*. Except few odd values recorded, males, females and indeterminates showed a similar pattern of zinc accumulation. Observations with *Mytilus edulis* indicated that seasonal dynamics of zinc was not dependent on the germinal tissue but to the somatic growth (Lobel and Wright, 1982). Zinc concentration was found to be 60% higher in the testes of *Mytilus californianus* than that in its ovarian tissue with no sex related difference in muscle or digestive gland zinc content (Watling and Watling, 1976).

In contrast reports are also available on sex related difference in the accumulation of zinc. According to Waldichuk

(1985), for any species, there may be a difference between male and female in the rate of uptake and release of metals. Watling and Watling (1976) estimated high zinc concentration in the soft tissue of female *Choromytilus meridionalis* than that in males. Similar observations were also made by Romeo and Gnassia-Barelli (1988) in *Donax* sp and *Venus* sp.

Bioaccumulation of a metal is also dependent on the quantity of the metal already accumulated by the organism also. Bryan (1973) with the studies conducted on the accumulation of ^{65}zinc in *Pecten maximus* and *Chlamys opercularis*, found that the concentration factor of ^{65}Zn vary according to the concentration of stable zinc in each tissue. The proportion of zinc absorbed was found to have a definite relation with the concentration of zinc in the water.

In literature, there are few reports of zinc concentrations in molluscs or crustaceans of less than 50 µg/g, even in those species collected from open oceans, and hence this may approximate to a minimum metabolic requirement (White and Rainbow, 1985). Dixon and Webb (1979) listed a total number of 2137 enzymes in marine organisms, of which 37 enzymes contained zinc and Bowen (1979) reported 80 zinc associated enzymes. White and Rainbow (1985) estimated the metabolic requirements of zinc to be 34.5 µg/g in the tissue of marine organisms. The zinc concentration estimated in *M. optima* (40 µg/g) is well within the range of the estimated requirement and it indicates the low bioavailability of the metal in Kayamkulam lake and hence the low metal pollution.

High biomagnification of copper from sea water was the rule among molluscs; bivalves from Greek waters contained 4,000 to 15,000 times more copper than the ambient media (Papadopoulu, 1973). Copper uptake among mussels was found to be erratic due to the influence of available concentration of zinc, cadmium and lead salts (Phillips, 1976). Similarly toxicity of copper was found to vary when combined with other heavy metals in *Perna indica* and *Donax incarnatus* (Mathew and Menon, 1992)

The annual average concentration of copper recorded in the soft tissue of *M. opima* was 19 µg/g. The variation in the copper content of exhibited a relation to its reproductive cycle as evident from the comparative chart (Fig. V. 9) of copper and the spawning levels. The level of copper is low (13 µg/g) in the month of January, which coincided with the spawning peak of the second reproductive cycle. The copper concentration increased to a high value (55 µg/g) in the resting phase during April and decreased as next gametogenesis occurred. The low and high values coincided with the spawning peak and the post spawning period of the second reproductive cycle respectively. The repetition of the trend in the accumulation of copper is a clear evidence for the relation of accumulation to the reproductive cycle of *M. opima*.

Several studies have been reported on the level of the copper accumulation in bivalve tissues and the concentration was found to show wide variation. Segar *et al.* (1971) estimated 44 ppm of copper in the soft tissue of *Modiolus*

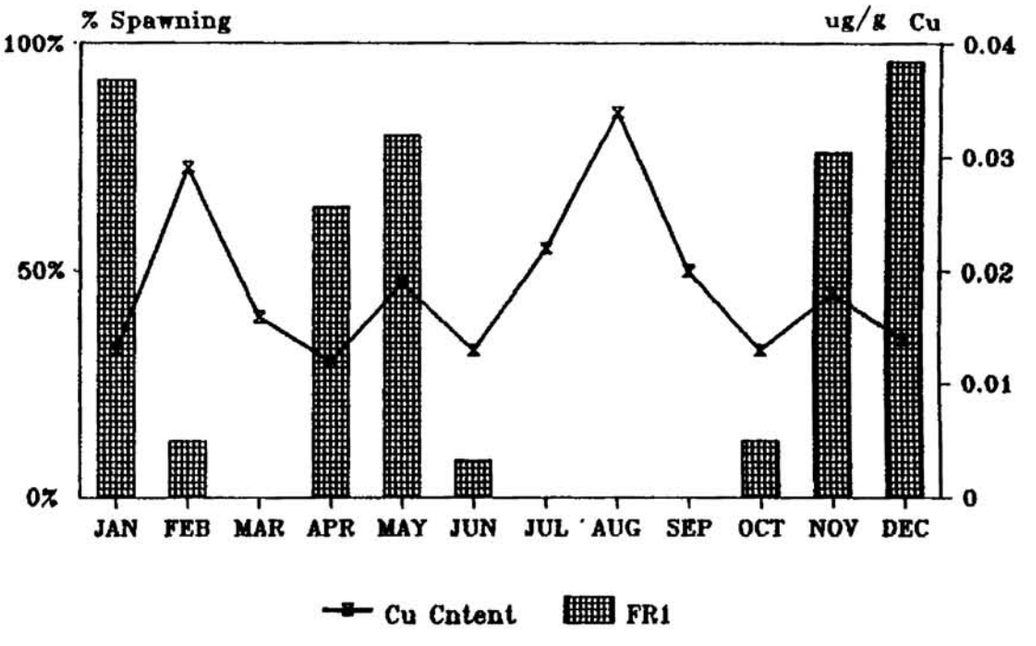

FIG. V.9
SPAWNING FREQUENCY AND COPPER

modiolus, 5.7 ppm in *Glycemeris glycemeris*, 25 ppm in *Mercenaria mercenaria*, 3.3 ppm in *Pecten maximus*, 9.6 ppm in *Mytilus edulis*, 3 ppm in *Anodonta* sp. and 11 ppm in *Cardium edulae*. Concentration of copper as high as 450 µg/g and 21,000 µg/g (wet weight) has been reported in oysters from the Derwent river estuary Australia by Thrower and Eustace (1973). Salih (1977) reported 0.018 % to 0.02 % of copper in the soft tissue of *Meretrix casta*.

Sankaranarayanan *et al.* (1978) reported 70-203 µg/g of copper in *Crassostrea madrasensis* from Cochin harbour area and Romeo and Gnassia-Barelli (1988) recorded 11.8 µg/g in *Donax trunculus* and 4.1 µg/g in *Venus verrucosa*. 420 µg/g in *S. cucullata* was reported by Kumari and Nair (1990). Berrow (1991) recorded upto 88.3 µg/g in *M. edulis*. Paez-Osuna *et al.* (1991) determined copper concentration to be 13.2 µg/g in *Chione* sp., 104 µg/g in *S. palmula* and 67.4 µg/g in *Crassostrea corteziensis*. Babukutty (1991), estimated 26.97 µg/g in *Villorita cyprinoides* and Coimbra *et al.* (1991) reported 9.05 µg/g in *M. edulis*. Berkman Negro (1992) recorded 5.6 and 13.8 µg/g of copper in scallops from two stations in Antarctic.

In *M. opima* the general trend observed in the seasonal variability of copper content in pooled tissue samples was found to be followed by males and females also. The concentration of copper in females exhibited a fluctuation during the first spawning, while it was maintaining a high value during the second spawning period. The ratio of copper accumulation in female to male indicated upto 3.33 times higher

accumulation efficiency of females (Table V. 1). Differential accumulation efficiency of copper in male and female bivalves has also been reported. In *Choromytilus meridionalis*, females contained more copper in whole soft parts than males (Watling and Watling, 1976). Female gonads of *Mytilus californianus* are reported to contain higher copper content than testes. However, no sex related difference in copper accumulation was noticed in digestive gland or muscle tissue (Alexander and Young, 1976).

Large quantities of manganese are transported by the rivers to the oceans. Of all Municipal waste water is the first source, followed by dumping of sewage sludge, smelting and refining and metal manufacturing process (Moore, 1991). Food was found to be the major route for manganese accumulation in mussels. Soluble chemical species of manganese were taken up more rapidly than particulate forms by mussels (Pentreath, 1973). According to Phillips (1977) chemical availability of manganese is secondary to that of bioavailability and bioavailability is significantly influenced by the presence of other metals in solution as well as by water salinity, pH, temperature etc.

Presence of manganese was detected in all the samples of*M.opima* collected during the first year of study (1989) while it was not detected in eight (sexes separated) samples during the second year of the study. The mean values indicated a notable seasonal fluctuation. From the trend, it can be inferred that the level of concentration of manganese is found to be related to the reproductive cycle of the clam. The high

values were recorded in the spawning clams, while it is very low in the post spawning stage. Females indicated higher concentration than males. Watling and Watling (1976) also found females of *Choromytilus meridionalis* were reported to contain significantly higher manganese than males.

Manganese levels in the soft tissue of *Pinna nobilis* was found to be 180,000 times higher than that in sea water and low concentration factors were exhibited by four species of bivalves from the same area (Papadopoulu, 1973). Shah *et al.* (1973) recorded 441 ppm of manganese in *Tunetta dosinia* and 168 ppm in *Meretrix meretrix*. Segar *et al.* (1971) estimated 150 ppm of manganese in the soft tissue of *Modiolus modiolus*, 34 ppm in *Glycemeris glycemeris*, 18 ppm in *Mercenaria mercenaria*, 140 ppm in *Pecten maximus*, 3.5 ppm in *Mytilus edulis*, 2100 ppm in *Anodonta* sp. and 6.3 ppm in *Cardium edulae*. The wide range of 3.5 ppm in *Mytilus edulis* to 2100 ppm in *Anodonta* sp. indicates the property of variation in accumulation of the metal between different genus of bivalves. *Crassostrea cucullata* contained 3.2 to 17.5 ppm manganese (Zingde *et al.*, 1976). Paez-Osuna *et al.* (1991) recorded 23.2 µg/g manganese in *Chione* sp, 4.8 µg/g in *P.palmula* and 7.2 µg/g in *Crassostrea corteziensis*. Berkman and Negro (1992) recorded 4.4 to 7.5 µg/g in scallops.

Decrease in concentration of manganese in *M. opima* was noticed during the monsoon period during which low salinity was observed and the decrease was maximum in males. Decrease in manganese during the period of low salinity was also

observed by Sankaranarayanan *et al.* (1978) in *Crassostrea madrasensis* and opined that the decrease in manganese during May to December is due to the influx of freshwater during this period and reduction in salinity to be the reason for the reduced availability of these metal ions.

Calcium recorded maximum value of all the metals analysed (22240 µg/g). Pooled tissue analyses showed a well defined monthly variation. The high values were recorded in June and October-December. The tissue concentration did not exhibit any relation to the reproductive cycle. The decreased calcium content in the monsoon and post monsoon period is probably due to the decreased availability.

No indication of variation was noticeable in different sexes. Male, Female and indeterminate clams exhibited a uniform level throughout the year except a slight increase exhibited by females.

Salih (1977) reported 0.106 % to 0.67 % calcium in the soft tissue of *Meretrix casta*. Segar *et al.* (1971) found wide variations in calcium content in different bivalves. *Modiolus modiolus* contained 3400 ppm, 4000 ppm in *Glycemeris glycemeris* 2600 ppm in *Pecten maximus*, 3200 ppm in *Mercenaria mercenaria*, 3200 ppm in *Mytilus edulis*, 70000 ppm in *Anodonta* sp. and 3300 ppm in *Cardium edulae*.

Ionic calcium is likely to be the important source of the metal. Fox and Coe (1943) found in *Mytilus californianus* that the amount of calcium obtained from organic food alone was not sufficient to account for the amount deposited in the

shell. Rao and Goldberg (1954) in the studies of calcium uptake by *Mytilus californianus* using radioactive calcium found a measurable intake of the cation from the media into the tissues within two hours. Autoradiography of mantle margin recorded the maximum calcium content and extremely high in the shell regenerating areas of the mantle. Viarengo *et al.* (1982) found that the concentrations of copper in the media can alter the calcium homeostasis. Calcium ions offer protection to the bivalve from intoxication due to other heavy metals (Moore, 1991).

The major thrust of this trace metal analysis is to define possible toxicant threats to public health since the clam is used for human consumption. In this context, the present results indicate that the pollution is not beyond the level to endanger public health on consumption of the clam meat even though they accumulate the heavy metal toxicants from the surrounding media.

Chapter VI

TOXICOLOGY

INTRODUCTION

Aquatic environment is perhaps the ultimate receptacle of all pollutants. The impact of pollution is more pronounced in rivers, estuaries and backwaters. The increasing pollution of the water by industrial and agricultural runoff results in serious ecological damages, adversely affecting the fauna and flora of any region. Damages caused on the aquatic fishery resources, there by retardation in fishery potential and transfer of toxic organic and inorganic substances to human being, form part of the serious consequences of the increasing pollution.

Kayamkulam lake receives effluents from the industries, chemical contaminants from agricultural practices. However, the major deterioration of the lake is brought about by the age long retting activities. Retting of coconut husk is the basic process that is involved in the production of coir and coir goods which is one of the most popular traditional occupations in the coastal areas of Kerala. Abundance in supply of coconut husk and the unique retting conditions available in the lakes and estuaries of coastal belt of Kerala have led the coir industry into one of the largest small scale industries in the state. The retting grounds are seen all

along Kerala coast. The extensive backwaters located between the Arabian sea and the mid land of Kerala are the sites for retting coconut husk. Most of the retting areas are concentrated in the backwaters of Trivandrum, Quilon, Alleppey, Ernakulam, Trichur and Calicut districts. In Kerala about 233.9 lakhs of coconut husk are retted annually (Department of Economics and Statistics).

The stress imparted on the fishery resources by the pollutants from coconut husk retting grounds was found to cause serious damage to the population structure of the clams which in turn affect the fishery potential. According to Bayne (1975), "Stress is a measurable alteration of physiological steady state which is induced by an environmental change and which render the individuals (or population) more vulnerable to further environmental change." Unlike the case of pelagic organisms, bivalves, being sedentary, are incapable of escaping undesirable conditions of the environment. Bivalves have a wider tolerance to environmental changes and very drastic changes may be tolerated only temporarily but would ultimately result in death of the organism. Between the limits of tolerance and resistance to environmental stressors, a region of sublethal response occurs whereby the capability for survival may be reduced as a consequence of the stressor (Akberali and Trueman, 1985). Sublethal responses include behavioural abnormalities, altered rate functions, modifications or damage the cytological structures.

Reports on coconut husk retting include those of Bhat (1974); Bhat *et al.* (1973 and 1975); etc. who studied the various aspects of microbial action involved in the process of husk retting. Vijayan *et al.* (1975) and Unnithan *et al.* (1975), studied the organic pollution in Cochin backwaters and Remani *et al.* (1981), studied the sediment composition of a retting yard. Some important works relating to the influence of pollutants on the crustacean plankton, meiofauna and fishery and the impact of toxicants from retting yards on the ecology were conducted by Azis and Nair (1982, 1983 and 1986).

Reports are available on the microbial action and chemical changes involved in the process of retting, chemical composition of the water from the retting grounds, and sediment composition. However studies on the biological effects of the toxicants are sparse. Ajithakumar (1988) noticed male dominance in *Perna viridis* population near coconut husk retting yards. Gopakumar (1992) recorded the damages caused by the high sulphate content of Kayamkulam lake on the survival of *Penaeus indicus*. In the context of the importance given to the development of aquaculture in our country, studies on pollution of the aquatic environment have assumed greater significance.

Lethal toxicity studies help to evaluate the relative lethality of the toxicant. The experiment is designed to determine the concentration of toxicants sufficient to kill a fixed 50% of the organisms in a limited period of time. This method is the standard procedure used for the toxicity studies of various toxicants based on pharmacological assay. The ret

liquor is a complex substance and the toxic effect induced by a combination of factors. The bacterial breakdown of the husk releases a large number of organic compounds like lignin, tannin, polyphenols etc. to the surrounding environment (Jayashanker, 1966) and the high biological oxygen demand is produced by the reducing environment.

Toxicity studies of various pollutants on bivalves mainly look with lethal. Reports on cellular level changes due to contaminants are also available comparatively rare and out of the available studies, most of them are conducted on fishes rather than shellfishes. Only limited number of toxicants can cause lesions in aquatic species, which may be useful for diagnostic purposes, although none are pathognomonic (Mayers and Hendricks, 1982).

The chapter deals with the studies conducted on some effects of "rett liquor" on the survival of the clam *M. opima* from the Kayamkulam lake. It is hoped that the results highlighted herein would enhance the current awareness on the nature of the pollution due to retting and the damage caused on the clam fishery

MATERIALS AND METHODS

The large coconut husk retting yard near Aayiramthengu was selected for the study. The samples were collected during July 1992. Temperature was recorded using a thermometer and pH using an electronic pH meter. Salinity estimations were done by argentometric titrations (Mohr-

Knudson Method) and dissolved oxygen was estimated using Winkler method (Grasshoff, 1983). Phosphate content was determined using Murphy and Relay (1962) method, by treating a known volume of rett liquor with mixed reagent and ascorbic acid and determining the optical density at 880nm. Nitrate was reduced to nitrite by using cadmium column reduction method and estimated colourimetrically at 520nm by complexing with sulphanilamide and napthylethylene diamine (Grasshoff, 1983). The spectrum of rett liquor, its chloroform soluble fraction and water soluble fraction were scanned by using UV-Visible spectrophotometer (Hitachi Model 150-20) for a qualitative estimation of the major dissolved compounds.

The mortality tests were conducted in a temporary laboratory, set near the study area. *M.opima* of 22-24 mm size were collected and acclimised for 24 h. The sea water for experimentation was collected from the sampling site and filtered with glass wool filter. The rett liquor from the retting yards was collected immediately before the experiment each time. The bivalves were exposed in 10 l. of test medium which contained rett liquor in the concentration ranges 0, 5, 10, 15, 25%. The experiments were repeated thrice in duplicate series. The observations made and lasted for 96 hours an the toxicant solutions were changed after every 12 h interval. The animals were not fed during the period of experiments, and the water was not aerated. A set of control animals were also maintained in the same condition in water without the pollutant. The LC50 values computed employing approved methods.

Active clams extended the long siphons, while those inactive ones remained with tightly closed valves. Gaping of shells beyond 5 mm without extended siphons and inactivity to mechanical stimulations were the indices of death.

To study the effect of the liquor on the structure of the siphon, 10 numbers of *M. opima* of 20 to 24 mm size were exposed to 15% concentration of rett liquor. The siphon of 5 clams each were cut and fixed in Baker's formal calcium (Humason, 1972)at every 24 hour interval, for 96 hours. After 12 h fixation, the tissue was dehydrated in ethanol, cleared in xylol, and infiltrated with paraffin wax. Serial sections of 6 μm thickness were made, and stained with haematoxylene and counterstained with eosine. The stained sections, mounted in D.P.X. were observed under light microscope.

RESULTS

The polluted water in the coconut husk retting grounds had a greenish yellow colour with the foul smell of hydrogen sulphide. An yellowish scum was formed on the water surface. Temperature of the surface water was 31°C (at 11.30 AM). Dissolved oxygen was not present in detectable levels in the rett liquor and the pH recorded was 9. Salinity was found to be 28 ppt. Phosphates recorded 1.8 μg-at PO4-P/l and nitrates, 0.06 μg-at NO3-N/l.

From the absorption studies of the rett liquor, four absorption maxima in different wavelengths were observed. Chloroform extract gave three distinct absorption peaks apart

from the peak that was recorded close to UV-range which was repeated in the water extract also.

Mortality occurred in clams exposed to 15% of toxicant in 48h. 33 % of rett liquor was found to be lethal concentration at 48h exposure, 25 % at 72h and 16 % at 96h (Table VI.1). The clams exposed to 10 and 15 % of rett liquor were active with the shell valves open and siphons extended. All the clams exposed to 10% concentration survived 96h exposure while those in 50% recorded mortality. Clams dosed with 50% and above recorded mortality after 72 hour exposure.

Histo-pathological studies indicated cytological changes in the inhalent siphon of the clams exposed to rett liquor. Phagocytic cells with bluish violet stain can be observed in stained sections of inhalent siphons exposed to 15% rett liquor for 72 h (Fig. VI.1) while it is absent in the controls (Fig. VI.2). Size of phagocytes was found to increase with the time of exposure.

DISCUSSION

The unique conditions of coconut husk retting yards include the high organic load and large number of complex compounds released into the system by microbial action. The byproducts of the retting process remain largely responsible for the depletion in fishery in almost all estuarine systems of Kerala.

The oxygen deficient condition that prevailed in the retting grounds is because of the fact that this retting ground

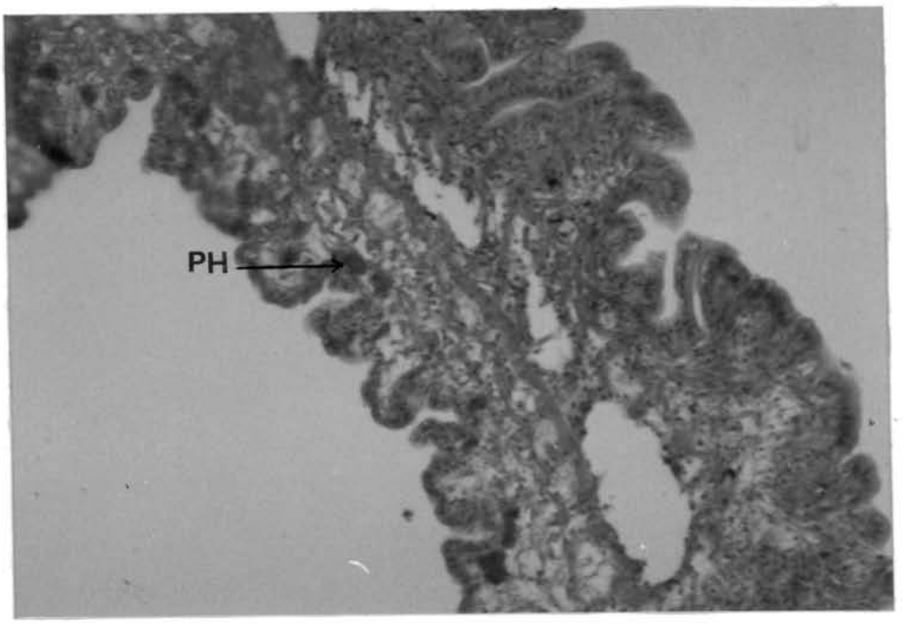

FIG. VI.1

SECTION OF INHALENT SYPHON EXPOSED TO 15% RETT LIQUOR

PH - Phagocytes.

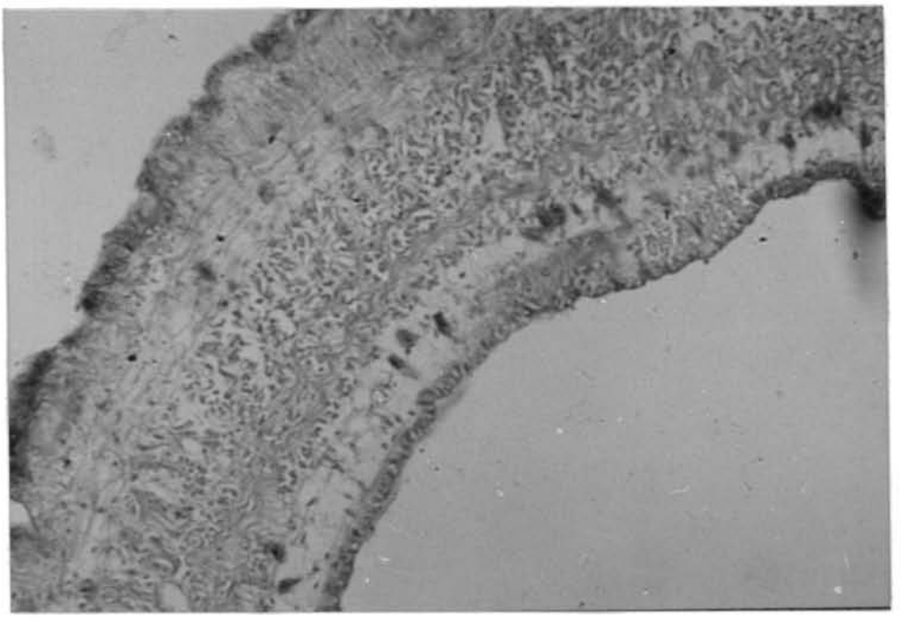

FIG. VI.2

SECTION OF INHALENT SYPHON (CONTROL)

TABLE VI .1

LETHAL CONCENTRATION

TIME (h)	LC-50 CONC. (%)	95% CONFIDENCE
48	33	20 - 54
72	25	17 - 35
96	16	12 - 21

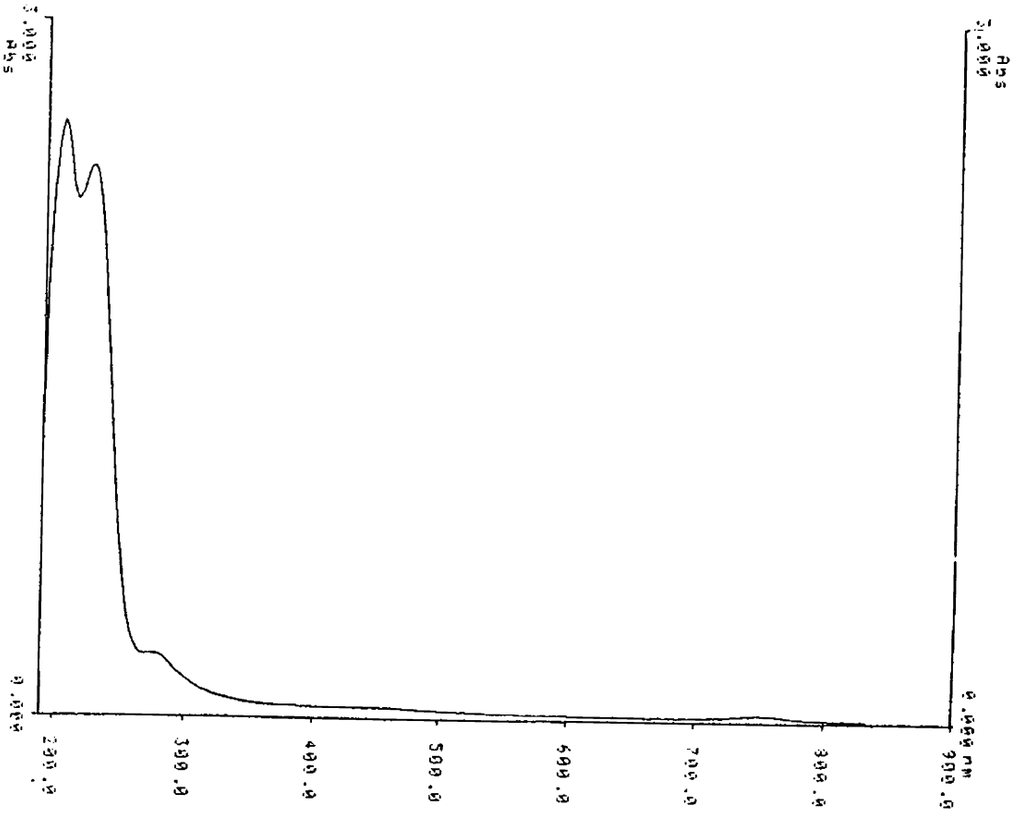

FIG. VI.3

SPECTRAL SCAN OF RETT LIQUOR

forms an isolated pocket of the lake. Eventhough there is tidal action, the degree of mixing was found to be weaker

Low oxygen concentration of 0.5 ml/l to 4.7 ml/l were recorded from the retting grounds, although a comparatively high concentration of 2.4 to 5.15 ml/l in a nearby clear zone was recorded by Remani *et al.* (1981). In the long term study on the ecological characteristics of retting ground, Azis and Nair (1986) also recorded lack of dissolved oxygen. The oxygen content ranged from 1.33 ml to 3.66 ml/l during August to October, while, rest of the months of the year recorded lack of dissolved oxygen. Thus an annual average of 0.83 ml/l was observed in the retting grounds while it was high throughout the year in a clean zone. The presence of oxygen during August to October months recorded in the retting grounds is due to the influx of freshwater in the monsoon season, which is evidenced by the decreased salinity recorded during the period.

Oxygen depletion is a particular characteristic feature of retting grounds and it is due to the high organic load of the retting yards. However, oxygen depletion is also reported from zones of high organic load due to sewage discharge (Unnithan *et al.*, 1975; Vijayan *et al.*, 1976).

Higher salinity of 28 ppt was recorded in the retting yard. Marine to backwater conditions were found to be best suited for the production of superior quality coir fiber with brighter colour and better strength. Unfortunately, this is the reason for the selection of marine and brackish water zones as sites for retting, which are the breeding and nursery

grounds of many valuable fish and shell fish. Kayamkulam lake is the retting ground for the topmost quality coir (Arattupuzha coir).

Surface water of retting grounds recorded a mean temperature level of 31°C which was higher than that of adjacent regions. Azis and Nair (1986), recorded lower temperature in the retting ground in comparison with clean zone, The reason for the lower temperature in the Nadayara was exclusively hydrological hence not pertinent here

High hydrogen ion concentration (pH=9) recorded may not be typical for retting environment since Azis and Nair (1986) recorded wide fluctuation in pH in the retting yard (4.2 to 8.9).

Average phosphate concentration is significantly high at the retting ground, whereas no significant difference was observed in the nitrate and nitrite concentrations. However, a predominant increase of nutrients was noticed during monsoon in the marginal clean zones.

The dissolved phosphate content in the retting zone recorded a low value of 1.8 µg PO4 - P at /l which is within the range of dissolved phosphate under normal conditions. Azis and Nair (1986) noticed an inverse relation between dissolved oxygen and phosphate in the retting zone. According to Stumm and Morrgan (1970) total phosphorus in unpolluted surface waters ranges from 00.3 and 1.3 µg PO4 - P at /l and levels upto 65 µg PO4 - P at/l is not uncommon in lakes which receive heavy domestic and agricultural drainage. Manikoth and Salih

(1974) recorded 2 to 5.3 μg PO4 - P at/l in Cochin estuary and that in various estuaries of Kerala ranged from 0.00 to 10.41 μg PO4 - P at/l (Saraladevi *et al.* (1983).

Dissolved nitrates estimated recorded an average value of 0.62 μg NO3 - N at/l. The value is low when compared to that in normal conditions. Manikoth and Salih (1974) recorded Nitrate nitrogen concentration ranging from 1.5 to 30 μg NO3 - N at/l from Cochin. Remani *et al.* (1981) recorded high value in the retting zone. Nitrate nitrogen of value 0 to 0.99 μg NO3 - N at/l was determined from the retting zone by Azis and Nair (1986).

High concentration of organic carbon observed in sediments of retting zone is due to the release of huge quantities of organic matter during the process of retting. Jayashanker (1966) recorded the organic carbon value ranging from 3.04 to 7.51 % when compared to the values encountered in the adjacent zones (0.32 to 0.51 %).

The spectral scanning of the filtered sample of the rett liquor (Fig. VI.3) in the range 200 - 900nm delineated predominant compounds of λ-max at 200, 235, 280, and 750 respectively. In order to classify the nature of these compounds into inorganic or organic, the rett liquor was subjected to chloroform extraction. The aqueous and organic phases were separated and their respective spectra identified. On comparison of the spectra of chloroform extract (Fig. VI.4) to that of water extract, (Fig. VI.5) it was noticed that the peak obtained at the range in the region with 200 — 300 nm is

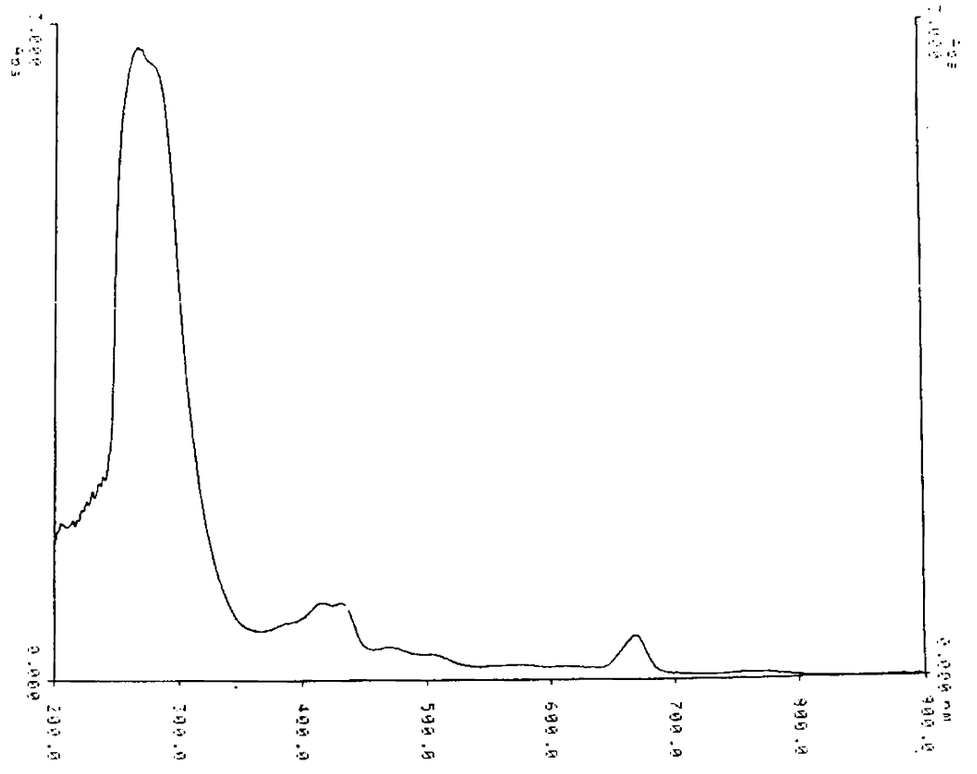

FIG. VI.4

SPECTRAL SCAN OF CHOLOROFORM EXTRACT OF RETT LIQUOR

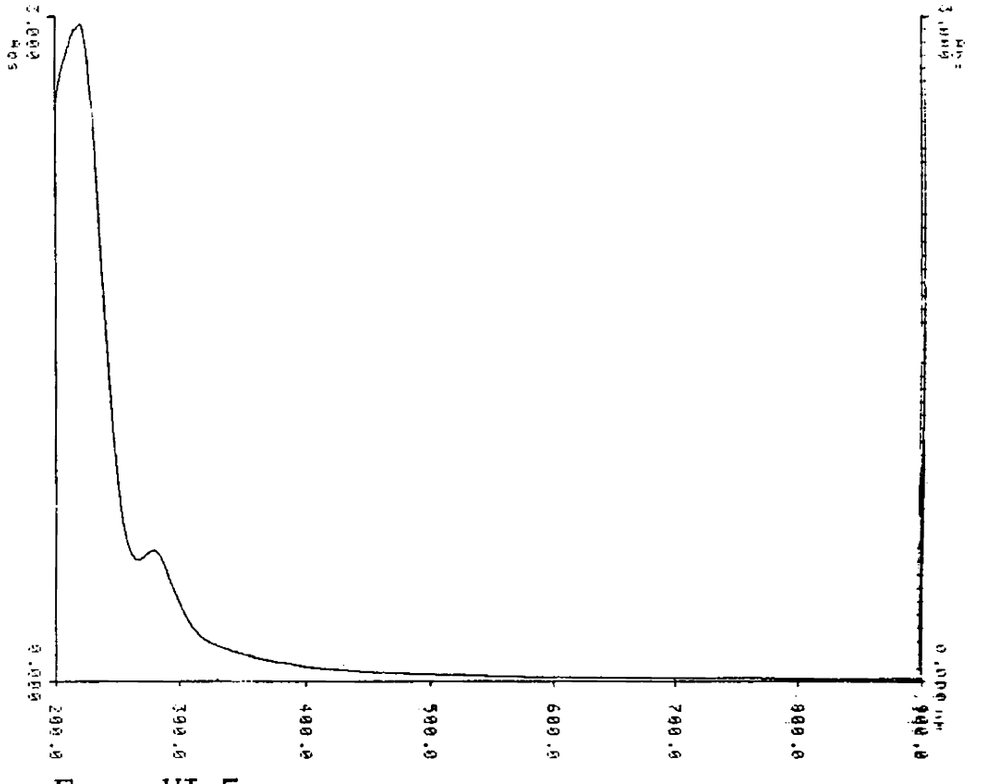

FIG. VI.5

SPECTRAL SCAN OF WATER EXTRACT OF RETT LIQUOR

common to both the fractions, indicating the presence of a coumpound which is soluble in both phases. Further it has been pointed out that a slight variability in the λmax value observed in this region is that which could be expected on the basis of "solvet effect" i.e., on changing the solvent (chloroform/water) a slight shift in λmax. is encountered. Obliviously, the organic phase has provided four well defined absorption peaks, which were absolutely absent in the aqueous extract. This indicates the presence of four characteristic organic compounds present in the ret liquor.

Aquatic organisms are highly vulnerable to the toxicity of pollutants. However, the animals tend to depend upon the defence mechanism, under stress as observed by the phagocytic cells in the exhalent siphons of th rett liquor exposed animals. No fish or shellfish population existed in the highly polluted and oxygen deficient retting yards studied from Kayamkulam. Decrease in meiofauna was recorded in the retting zone with only twelve species while the nearby clean zone represented 28 species (Azis and Nair, 1982; 1983).

The rett liquor, being a combination of many byproducts of the microbial process, exact evaluation of the pollutant concentration is difficult and hence different dilutions of the rett liquor was used to assess lethal toxicity. Mortality was noticed in the media which contained different concentrations of rett liquor. The clams were found to "clam up" in the media which contained more than 50% rett liquor. Exposure to such media beyond three days resulted in

death of all the experimental animals which indicates that death was owing to lack of oxygen due to prolonged anaerobic condition brought about by valve closure.

Natural retting of coconut husk is essentially a microbial process brought about by pectinolytic activities of bacteria, fungi, and yeasts liberating large quantities of organic substances like pectin, pectosan, fat and tannin into the medium. During the course of retting, polyphenols from the husks get constantly leached out into the ambient water. Polyphenols and pectin are major constituents representing as much as 75-76 and 16-17 g/kg respectively of the husk material (Jayashanker, 1966; Bhat, 1969). Marked features associated with retting are the depletion of oxygen, increase in suspended particle load in water and increase in the levels of hydrogen sulphide and biological oxygen demand.

Traditional method of retting consists of either keeping the husk in pits dug within the reach of the tidal action of backwater, or placing the husks in big coir nets known as Malis and burying in shallow brackish water for 6-12 months. The period of retting depends on the age of the husk and nature of ambient water. During the retting process, the husk becomes softened and the fibers get loosened resulting in leaching of the adhesives, tannin etc. It is known that husk retted in saline backwaters yield stronger and better coloured fiber because of the removal of the products of microbial action by constant tidal action. The periodical fleshing of

the ret water at the optimum level was found to be vital for an ideal retting (Jayashanker, 1968).

Prolonged periods of anoxic conditions associated with high concentration of sulphides and high BOD values had been most striking features of the retting zone.

The sediment of polluted zone composed of fine clay-sand rich in organic matter in contrast with clay sand with less organic matter in the clean zone. It was black in colour and with the malodour of H_2S. High concentration of organic carbon was observed in retting zones (3.04 -7.51 %) when compared to clean zone with low organic content (3.19 to 5.09%.) Jayashanker (1966).

The husk retting is being conducted at various location in the Kayamkulam lake. The heavy pollution caused by the pollutants from the retting yards is found to have detrimental effect on the clam population, by way of total elimination of various populations in the area.

The present chapter thus highlights the extent of pollution in this area which is well above the tolerance level of the estuarine flora and fauna as reflected by the complete absence of bivalve population in and within the vicinity of the retting zones.

Summary

SUMMARY

Marcia opima forms one of the most abundant bivalves collected by fishermen in Kerala. Though it formed a major share of the clam meat exported from Kerala, a scientific study is being done on the clam from the area for the first time.

The clam population is restricted to barmouth regions of Kayamkulam and Ashtamudi lakes. It forms the subsistence fishery of many local fishermen around Kayamkulam lake. A maximum density of 548 clams / sq.meter was recorded during the period of study. Wide fluctuations of salinity were recorded (2-33 ppt.). Tidal action results in the frequent variation in the salinity. The seasonal closure of the barmouth by sand also attributes to the fluctuations in the salinity conditions which are having profound influence in the population dynamics.

The study conducted on the reproductive biology of the clam, using histological preparations, clearly reveals the reproductive pattern of *M.opima*. The clam was found to be gonochoristic with no instance of hermaphroditism, recorded during the period of study. Clams were found to be sexually mature at a size of 18 to 20 mm size. Chi square test conducted on the male-female ratio of the clam showed that the deviation from the ratio is insignificant.

Gonadal follicles branched out occupying the complete interior of the lumen in the ripe clams. The stages of male

gonadal development is classified under five divisions for males and six for females. Each spawning cycles was followed by a short resting period of indeterminate sex. The clam underwent two complete spawning cycles each year. The first minor one commencing from April extending upto June, and the second major spawning commencing from October and continuing till February of next year. The drastic decline in the salinity during the early monsoon coinciding with the spawning and the early stages of developments have adverse effects on the productivity of the clam. A scientific monitoring of the hydrographic parameters and timely opening of the barmouth can protect the fishery to a great extent.

The monthly variations in biochemical composition (protein, glycogen and lipid) in the soft tissue indicate that it is more or less influenced by the reproductive status of the clam. The level of moisture was found to be primarily influenced by the salinity levels of the ambient media and secondarily to the reproductive stages of the clam. The maximum water content coincided with the low saline condition of the monsoon. Protein content in pooled samples recorded average values of 34.7 to 37.5 % of the total dry tissue. High values were recorded during gametogenesis and a decrease was noticed with the commencement of spawning. Protein analysis on separate sexes of the clam indicated almost uniform variation in male and female clams. A slight decrease in protein concentration was noticed in the indeterminate clams as they appeared in the post spawning stage.

The concentration of glycogen indicated wide fluctuations in pooled samples, with a range of 14.8 to 41.1% . Few levels coincided with gametogenic stages, while higher values were recorded during the spawning and post spawning stages. Males and females exhibited uniform pattern of glycogen concentration, while it was higher in indeterminate clams. Lipid concentrations in pooled samples as well as separate sexes of the clam indicated clear relationship with the reproductive cycles of the clam. The pooled samples recorded 3 to 6.1 % of lipid. The concentration of lipid increased with gametogenesis reachingmaximum during the ripe stage and decreased with spawning. Calorific values indicate that females are more nutritive than males. Females recorded an annual average of 3.06 Kcal/g whereas it was 2.81 Kcal/g in males.

The bioconcentration of metals viz. zinc, copper, manganese, and calcium provides a baseline data of the accumulated metals. Zinc recorded an annual average of 40 µg/g and the value showed irregular monthly variation. Male, female and indeterminate clams did not show variation in the rate of metal accumulation. Copper content in the soft tissue of *M.opima* recorded an annual average of 19 µg/g. The concentration of copper indicated a clear inverse relationship with the spawning cycle, with low copper content during spawning and high copper content during the stages of gametogenesis. Though the trend of copper accumulation in male, female and indeterminate clams followed the pattern

observed in pooled tissue, females showed a higher degree of accumulation when compared to males. Manganese ranked lowest of all the metals analysed. It was not present in detectable quantities in eight (sexes separated) samples analysed during the second year of the study. The concentration of manganese was found to vary with the salinity of the media. Calcium being an important major component, recorded high values with an annual average of 22.24 mg/g. Calcium concentration didn't show significant variation in relation to sex, however, it decreased during monsoon and postmonsoon period. The high concentration of calcium attributes to the nutritive value of the clam meat.

Coconut husk retting is being done in all estuarine systems of Kerala, causing serious pollution problem. The toxicological studies conducted with the complex pollutants from the coconut husk retting yards indicate the serious damage caused by the pollutant on the survival of the clam. UV-Visible scan of the rett liquor indicated four major peaks, each representing a compound in chloroform extract, and two peaks in water extract. Mortality tests show that 16 % concentration of the pollutant is lethal to the clam at 96 h. exposure. Histopathological studies indicated enlarged phagocytic cells in the siphon tissue of the clam exposed to 15 % of the pollutant for 72 h.

Studies on reproductive cycle and factors influencing reproduction provide valuable information for the scientific exploitation of the resource as well as to identify the factors

that adversely affect the reproductive potential. The investigation results bring to light the proximate composition for a better understanding of the nutritive status, similarly the the levels of metal bioconcentration too. Further, results of the toxicity studies focus attention on the coconut husk retting yards as a major source of aquatic pollution causing damage to fishery and indicate the need for efforts to protect the water quality.

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