REPRODUCTIVE PHYSIOLOGY OF INDIAN SPECIES OF THE GENUS *PERNA* (FAMILY MYTILIDAE)

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

DOCTOR OF PHILOSOPHY

OF THE

UNIVERSITY OF COCHIN

By

B. S. AJITHA KUMAR, M. Sc.

CENTRE OF ADVANCED STUDIES IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
COCHIN-682 018

MARCH 1984

Declaration

I hereby declare that this thesis entitled "REPRODUCTIVE PHYSIOLOGY OF INDIAN SPECIES OF THE GENUS *PERNA* (FAMILY MYTILIDAE)" has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Cochin-682 018,
March 1984.

(B. S. AJITHA KUMAR)

Certificate

This is to certify that the thesis entitled "REPRODUCTIVE PHYSIOLOGY OF INDIAN SPECIES OF THE GENUS *PERNA* (FAMILY MYTILIDAE)" is the bonafide record of the work carried out by Shri B. S. AJITHA KUMAR under my guidance and supervision and that no part thereof has been presented for the award of any other Degree.

Dr. K. Alagarswami,
Scientist S-3,
Central Marine Fisheries Research Institute,
Cochin-682 018.

Cochin-682 018,
March 1984.

P R E F A C E

The culture of marine organisms in the coastal waters and adjoining estuarine systems has developed on a large scale in different parts of the world. Recently India has made rapid strides in mariculture and technologies have been developed for production of several species of finfish, crustaceans, molluscs and seaweeds. In order to provide sufficient scientific insight into the basic and applied aspects of mariculture, the Central Marine Fisheries Research Institute (CMFRI) has taken up multidisciplinary researches. The Centre of Advanced Studies (CAS) in Mariculture of CMFRI which is an ICAR/UNDP/FAO Project forms the nucleus of this effort to carry out research of excellence and to provide advanced training in mariculture.

The candidate after obtaining an M.Sc. degree in Environmental Biology from the Tamil Nadu Agricultural University in March 1980, joined the CAS in Mariculture as a Senior Research Fellow in the first batch of the Ph.D. programme in July 1980. During the first semester, the candidate underwent course work in different aspects of mariculture. The curriculum of the course included detailed aspects of the biology, ecology and fishery of commercially important marine organisms, status of world mariculture, the resource potential of cultivable marine organisms, techniques

for the culture of the finfishes, shellfishes, sea weeds and mass culture of live-food organisms. The course work also involved practical training and study tours to different laboratories and mariculture field centres of CMFRI. During the second semester, the candidate made an in-depth study of the optional subject 'Reproduction in Molluscs' and passed the Ph.D. qualifying examination of the Univeristy of Cochin.

The candidate started his preliminary work on the research programme of 'Reproductive Physiology of Indian Species of the Genus Perna (Family Mytilidae)' by the end of the second semester. Subsequently full-time attention was given to the problem. The subject was chosen in view of the great importance given to mussel culture as a potential area for increasing seafood production in the country. The techniques of culture of green and brown mussel have been successfully developed at the CMFRI but seed production has remained one of the major constraints in expanding mussel culture. It was considered essential to acquire the basic knowledge on the physiology of reproduction of mussel to understand the problems of gametogenesis, maturation, spawning and seed production in the natural populations as well as under controlled conditions. The results would have practical applications both in collection of mussel seed in the sea by laying spat collectors and in management of reproduction in hatchery.

The natural mussel beds of Perna indica and P. viridis are widely distributed and, therefore, the planned programme

had to be carried out at different centres. Regular fortnightly samples of P. indica were collected from Vizhinjam, near Trivandrum and P. viridis from Elathur, near Calicut. All the laboratory works were carried out at Cochin. Experimental works relating to feed manipulation and ganglion ablation were carried out at Vizhinjam, Tuticorin and Madras. The electron microscopy examination was done at Madras. Thus, the candidate had to plan, organise and carry out the different aspects of the programme in great detail, show initiative in securing facilities for work at different centres, travel a lot and work very hard in tackling the comprehensive and composite problem taken up.

At the end, it was gratifying to note that all the efforts have been worthwhile. A great deal of additional information has been obtained on the reproductive physiology of P. indica and P. viridis. Some new knowledge has been acquired on the tropical Indian species of mussels. Some results have potential for practical applications in controlled reproduction of mussels.

ACKNOWLEDGEMENTS

With deep sense of gratitude I wish to express my sincere thanks to Dr. K. Alagarswami, M.A., Ph.D., Senior Scientist and my Supervising Teacher for his unstinted guidance, sustained interest, constructive criticism and affectionate treatment during the course of this investigation, but for which this piece of work would not have materialised. Dr. E.G. Silas, Director, C.M.F.R.I. and Sub-Project Co-ordinator, CAS in Mariculture, took a keen interest in my work programme and gave valuable advice, help and suggestions throughout my research work. I express my sincere gratitude to him for providing excellent facilities for the laboratory studies and field work.

I wish to recall with gratitude all the help and encouragement given by Dr. P.V. Ramachandran Nair, Senior Scientist, C.M.F.R.I., Cochin and Mr. V. Ramjukrishna Pillai, Scientist S-I, C.M.F.R.I., Cochin in providing all facilities of instrumentation and transportation during the course of the investigations.

My thanks are also due to the Officers-in-Charge and staff of Research Centres of CMFRI at Vizhinjam, Calicut, Madras and Tuticorin, especially to Shri. C. Mukundan, Dr. M. Kumaran and Shri. K. Nagappan Nayar, Officers-in-Charge

of Vizhinjam, Calicut and Tuticorin Research Centres, respectively, and to scientists Shri. K. Rangarajan and Shri. K.K. Appukuttan, for providing all the help during the collection of regular samples and conduct of experiments.

I am grateful to Dr. P. Vedavyasa Rao, Senior Scientist, C.M.F.R.I., for all the help received during the course work and for his kind guidance in administrative matters. Shri. M. Srinath, Scientist S-1, C.M.F.R.I helped me in the statistical analysis of data and I wish to record my sincere thanks to him.

It is my privilege to thank Miss. Asmini Joseph, my friend and fellow scholar, for her help during the study, especially in the estimation of chlorophyll a. My thanks are also due to the staff of CMFRI and staff and scholars at CAS in Mariculture at Cochin, for their timely help.

I express my gratitude to the Indian Council of Agricultural Research, New Delhi for offering me the Senior Research Fellowship to carry out this work at the ICAR/UNDP/FAO Centre of Advanced Studies in Mariculture, Central Marine Fisheries Research Institute, Cochin.

Finally, I owe much more than I can express to my parents, Smt. N. Bhamini Amma and Shri. C.R. Sivasankaran Nair, whose charisma and blessings enabled me to bring out this piece of work, humbly dedicating which at their feet, I feel honoured.

Cochin - 682 018,
March 1984.

(B.S. AJITHA KUMAR)

REPRODUCTIVE PHYSIOLOGY OF INDIAN SPECIES OF THE GENUS *PERNA* (FAMILY MYTILIDAE)

C O N T E N T S

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER I

GENERAL INTRODUCTION

The marine mussels (Family Mytilidae) enjoy a wide global distribution and occur from the high arctic waters of Spitsbergen (lat. 78.00°N; long. 15.00°E) in the north to Kerguelen Islands (lat. 48.3°S; long. 69.4°E) of the Indian Ocean in the south (Seed, 1976), spreading over the polar, temperate, subtropical and tropical climatic regimes. While the littoral and shallow sub-littoral waters are their natural habitat, occasionally they are found in deeper waters. Mussels have adapted to estuarine conditions as well. With such an ubiquitous distribution, combined with their economic significance as an important world food resource, mussels have been studied for their biology, physiology, ecology, farming and production (Bayne, 1976 *a*; Korringa, 1976; Lubet, 1976; Seed, 1976).

Six genera have been recognised (Soot Ryen, 1955) under Family Mytilidae of Class Pelecypoda, Phylum Mollusca, with several commercially important species, of which the European mussel or the blue mussel, *Mytilus edulis* has been investigated in great detail (Bayne, 1976 *a*). The Mediterranean mussel *M. galloprovincialis*, although treated as an independent species in the earlier literature (Soot-Ryen, 1955), is

considered as a variety of the larger M. edulis (Lubet, 1973). The genus Mytilus (Lamy, 1936) is distributed in Europe and North America. Aulacomya and Chloromya are known from restricted areas in Australia, New Zealand, Chile and Peru (Soot-Ryen, 1955; Seed, 1976).

The genus Perna Retzius, 1788 is distinguished from Mytilus by the anterior position of the pedal retractor muscle and also the absence of anterior adductor muscle (Lubet, 1973) besides differences in a few other characters (Kuriakose, 1980). It has a more restricted distribution in Brazil, Venezuela (Veles and Epifanio, 1981), New Zealand (Pike, 1971) and India (Kuriakose and Nair, 1976). The sea mussels of India were known under the genus Mytilus for a long time and two species, the green mussel Mytilus viridis (Linnaeus, 1758) and brown mussel Mytilus sp. were recognised by Hornell (1922). Kuriakose and Nair (1976) placed the Indian mussels under the genus Perna Retzius, 1788 and created a new species Perna indica for the brown mussel, while referring the green mussel to Perna viridis (Linnaeus, 1758).

The green mussel Perna viridis enjoys a wider distribution along the Indian coasts including Andamans (Jones and Alagarswami, 1973; Appukuttan, 1977). The species supports a subsistence fishery along the west coast of India, particularly the Malabar coast of Kerala, and the annual production is in the order of 2600 t (Silas et al., 1982). The brown mussel Perna indica has a restricted distribution, occupying the southern-most zone of the west coast from Varkala in Kerala to

Muttom in Tamil Nadu and contributes to a subsistence fishery with a production of 427 t annually (Silas et al., 1982). The mussel fishery has been described by Hornell (1922) and Jones (1950).

Mussel culture is an efficient method of converting marine phytoplankton into nutritious and palatable human food and the three-dimensional culture in the column waters of the coastal and bay areas enables achieving very high production rates. Among the molluscs, mussels with a world production of 328,517 t occupy the second place in culture next only to the oysters which yield 591,386 t (Pillai, 1979). Spain and the Netherlands lead in mussel production and France, Italy, Yugoslavia and the Philippines have commercial mussel farming operations. Korringa (1976) has given an extensive review of world mussel farming systems. Techniques of mussel culture have been recently developed in India, and it has been on an experimental scale (Qasim et al., 1977; Kuriakose, 1980; Appukuttan et al., 1980).

Since reproduction is the basis of production this aspect has been extensively studied in many parts of the world. Primarily, the annual reproductive cycle of mussels and the associated environmental factors exercising exogenous control on reproduction have received considerable attention and the works include - on Mytilus edulis: White (1937), Chipperfield (1953), Bayne (1964), and Wilson and Seed (1975) from England; Lubet and Le Gall (1967) from France; Moore and Reish (1969) from California; Sugiura (1959) and Hirai (1963) from Japan;

on M. galloprovincialis: Seed (1971) from S.W. England; Hrs-Brenko (1971) from Adriatic Sea; Coe (1932) and Young (1946) from California; on Perna viridis (= M. viridis) Paul (1942), Nagabhushanam and Mane (1975a) Rao et al. (1975), Narasimham (1980) and Parulekar and Dalal (1980) from India; and on P. indica: Kuriakose (1973) and Appukuttan and Nair (1980). Reproductive aspects of Perna perna have been studied by Lunetta (1969) from Brazil and by Carvajal (1969) from Venezuela.

Johnstone (1898) developed a scheme of classification of the reproductive stages of Mytilus. Later, Chipperfield (1953) improved upon it. Recently, Kelly et al. (1982) combined histology with autoradiography in describing the male reproductive cycle.

The peculiar nature of gamete proliferation in the mantle lobes of the mussel in the reproductive season and the function of the mantle as the storage organ during the non-reproductive season (Gabbott, 1975) were reasons for the biochemical studies on the animal in detail. Seasonal changes on the biochemical composition of the molluscs, especially bivalves, have been studied extensively and these works have been reviewed by Giese (1969), Gabbott (1976) and Sastry (1979). In M. edulis seasonal cycle of storage and utilisation of glycogen reserves is closely linked with annual reproductive cycle (De Zwaan and Zandee, 1972; Dare, 1973; Seed, 1975; Gabbott, 1976; Bayne, 1976 a; Zandee et al., 1980). Similar studies have been made on M. galloprovincialis (Costanzo, 1966)

and Perna perna (Lunetta, 1969). The participation of the adductor muscle (Gabbott, 1975) and hepatopancreas (Thompson, 1972; Thompson et al., 1974) in energy storage and gametogenesis in M. edulis has been reported.

In certain species of bivalves, a direct correlation has been found between phytoplankton abundance in the sea and the processes of reproduction. Gametogenesis, maturation and spawning take place at certain levels of phytoplankton productivity (Pieters et al., 1980; Zandee et al., 1980). Experimental work on this aspect has been limited (Velez and Epifanio, 1981).

Our knowledge on neurosecretion and its role in reproduction of bivalves is limited in spite of several good works carried out since 1955, including on M. edulis and M. galloprovincialis (Nagabhushanam, 1962 a, b, 1968; Gabe, 1965, 1966; Lubet, 1966; Martoja, 1972). A modern approach of graft and tissue culture has recently been adopted (Lubet and Mathieu, 1982).

Studies on neuroendocrine control of reproduction have been supported by experimental work on the ablation of cerebral ganglia, visceral ganglia and pedal ganglia at different stages of reproduction (Lubet, 1955, 1959, 1965; Anthoumisse, 1963; Nagabhushanam, 1964 g). Generally, the cerebral ganglia have been implicated in controlling reproduction and visceral ganglia in metabolic activities. It would be seen that most of the investigations cited above relate to the temperate European mussel Mytilus edulis and some have been

on the Mediterranean mussel M. galloprovincialis. Studies on tropical species of mussels have been very few, such as Carvajal (1969), Lunetta (1969) and Veles (1977). Some base-line biological studies have been made on the Indian species which also touch upon the reproductive aspects (Paul, 1942; Jones, 1950; Kuriakose, 1973; Rao et al., 1975). Nagabhushanam et al., (1975) and Mane and Nagabhushanam (1980), for the first time, paid attention to the neurosecretion aspect in Perna (= Mytilus) viridis. It is evident that a detailed and comprehensive study on reproduction of the Indian mussels has been lacking.

The present investigation on the "Reproductive physiology of Indian species of the genus Perna (Family Mytilidae)" was taken up to acquire such comprehensive knowledge on reproduction of the brown mussel P. indica and green mussel P. viridis.

Since physiology of reproduction has to be understood along with ecology and biology of reproduction, these aspects to the extent necessary were investigated. The knowledge and understanding gained on the reproductive physiology of the two species through this investigation will not only form a significant advancement over that of the past, but would also form the base-line information for management of reproduction of the species in controlled seed production.

CHAPTER II

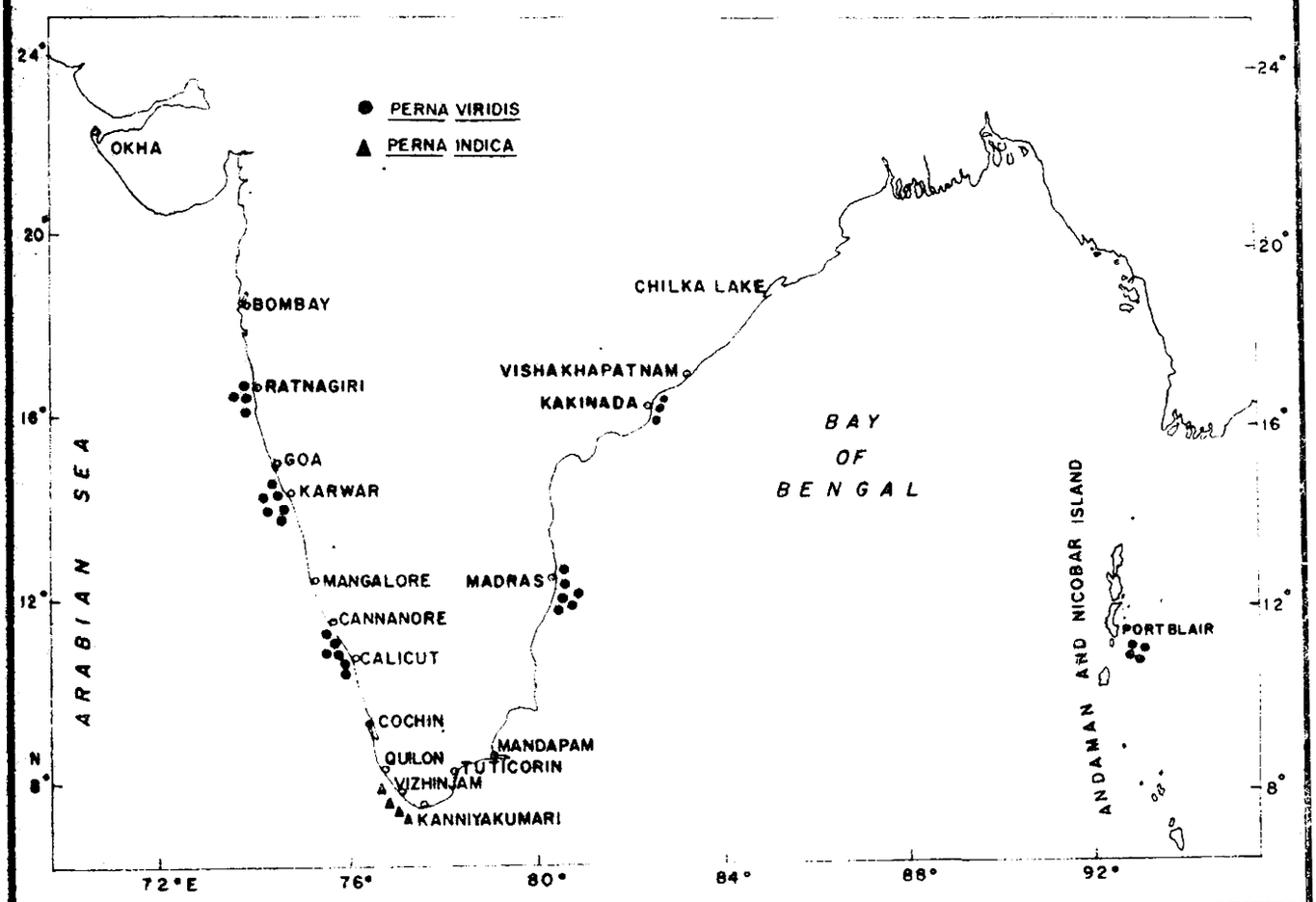
STUDY AREA AND MATERIALS

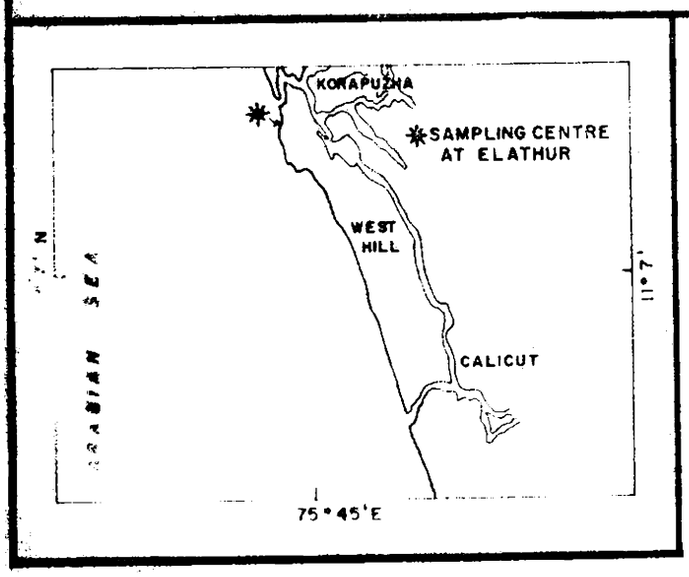
The centres of commercial exploitation of the mussels are along the west coast of India and intensive fishing at the subsistence level takes place along the Malabar coast from Cannanore to Koshikode for the green mussel Perna viridis (Linnaeus) (Pl. 1, A) and along the extreme south-west coast from Varkala to Muttom for the brown mussel Perna indica Kuriakose and Nair, (Pl. 1, B). Both the species live attached to the rocky substratum in the intertidal region and also on rocks in the subtidal region.

Perna viridis from Elathur

Elathur, a fishing village about 12 km north of Koshikode (lat. 11°15'N; long. 75°39'E) (Fig. 1), is one of the well-known centres for mussel fishery and was chosen for regular sampling for the study of reproductive physiology of Perna viridis from October 1981 to December 1982. The sampling site selected for the regular study was located in the subtidal granite rocks about 200 m away from the shore with a good settlement of mussel. All size groups were represented in the population. Regular sampling was done fortnightly from the selected site. Five random samples were drawn on each occasion

FIG. 1 MAP SHOWING THE DISTRIBUTION OF BROWN MUSSEL PERNA INDICA AND GREEN MUSSEL PERNA VIRIDIS ALONG THE INDIAN COAST AND THE SAMPLING CENTRES FOR THIS STUDY





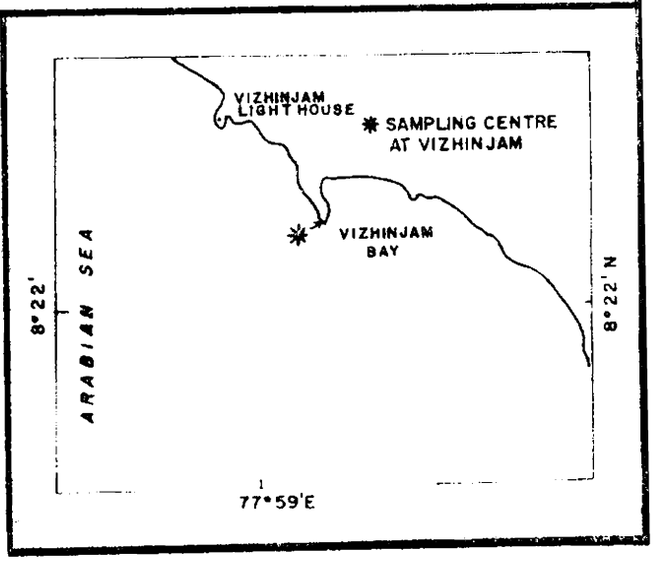


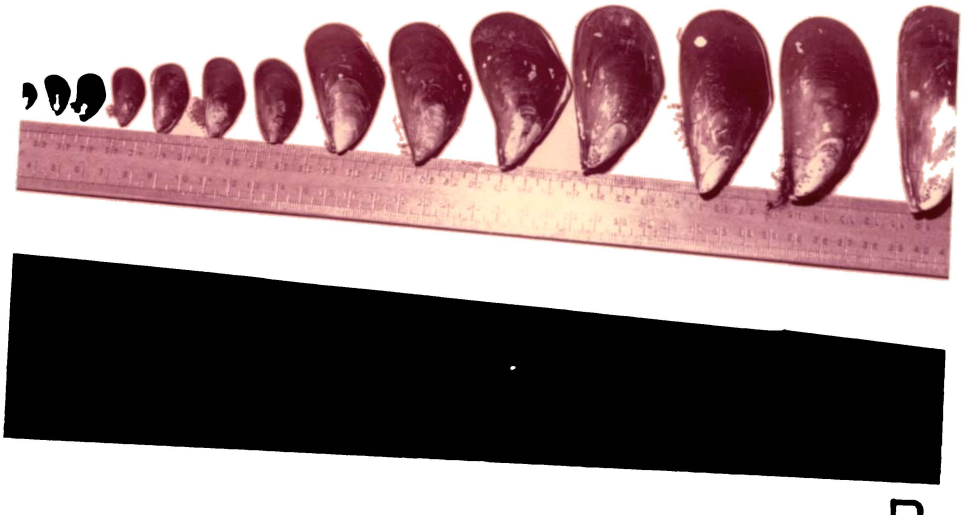
PLATE 1

A. The green mussel, Perna viridis (Linnaeus)

B. The brown mussel, Perna indica Kuriakose and Nair

PLATE 1

A



B

and pooled. The pooled sample was considered to represent the natural population in the mussel bed.

During the initial survey of the mussel beds at Elathur, it was observed that a site about 1.5 km south of the village was regularly used for retting of coconut husks. Pits of about 1-1.5 m depth were dug among the intertidal rocks and filled with husks. The sea water in the vicinity of such pits was discoloured due to pollution from this source. In order to study the effect of pollution on the reproduction of mussels found on the rocks of this area, regular fortnightly samples of sea water and mussel were collected throughout the period of study.

The biological samples and water samples collected from both the non-polluted and polluted areas at Elathur were treated first at the collection site and preserved appropriately for their intended purpose at the laboratory of the Calicut Research Centre of Central Marine Fisheries Research Institute after transporting the material by road over a distance of 12 km. The methods of treatment and preservation of samples are given in the relevant chapters.

Perna indica from Vizhinjam

For the studies on the brown mussel P. indica, Vizhinjam, a fishing village 15 km south of Trivandrum (lat. 8°22'30"N; long. 56°59'E) (Fig. 1), was chosen for collection of samples from October 1981 to December 1982. The sampling site was located in the intertidal area and the bed

was exposed during low tides. Fortnightly samples were collected randomly from 5 points and pooled. As in the case of green mussel, the biological and water samples were treated at the collection site and appropriately preserved at the Vizhinjam Research Centre of Central Marine Fisheries Research Institute which is about 1.5 km from the collection site.

Analysis of samples (biological and environmental) was carried out at the Central Marine Fisheries Research Institute, Cochin after further transport of the preserved materials from Calicut and Vizhinjam to Cochin by train.

Experiments on ganglion ablation

The effect of ganglion ablation on spawning was studied experimentally in both the species. The brown mussel P. indica was collected from the regular sampling site at Vizhinjam and the ganglion ablation studies were carried out at the Vizhinjam Research Centre of Central Marine Fisheries Research Institute from 15-30 July 1982. Similar studies on the green mussel P. viridis were carried out at the Kovalam Field Laboratory of Madras Research Centre of Central Marine Fisheries Research Institute during 22-31 May 1983. Samples for this study were collected from Ennoor, 30 km north of Madras and transported to Kovalam.

Experiments on induced maturation

The experimental work on induced maturation with ration in the brown mussel P. indica was carried out at the Tuticorin Research Centre of Central Marine Fisheries Research Institute. Samples were collected from Vizhinjam and transported by road to Tuticorin over a distance of about 220 km.

CHAPTER III

ECOLOGY OF MUSSEL BEDS

INTRODUCTION

Reproduction of the marine mussels has been correlated with factors such as food, water temperature, salinity and tides by many workers (Bayne, 1976 a; Sastry, 1979). Our knowledge on the ecology of mussel beds in India is based on the studies of Appukuttan et al. (1980) at Vizhinjam, Kuriakose (1980) at Calicut, Narasimham (1980) at Kakinada, Nagabhushanam and Mane (1975 a) at Ratnagiri and Parulekar and Dalal (1980) at Goa. But information on the hydrography of the Arabian Sea along the west coast of India has been well documented in the reports of Ramamirtham and Jayaraman (1960), Darbyshire (1967), Reddy and Sankaranarayanan (1968), Sharma (1968), Easterson and Mahadevan (1980) and others. The phytoplankton distribution and primary production of the region has been studied by Subrahmanyan (1959 a, b), Krishnamoorthy and Viswanathan (1968), Nair (1974) and others.

During the present study on the reproductive physiology of mussels (Perna indica and P. viridis), it was considered important to collect data on the environmental parameters of the mussel beds to understand the influence of

exogenous factors on the different processes of reproduction. The well-known parameters which have been established to play a role in marine bivalve reproduction such as temperature, salinity, turbidity, rainfall and phytoplankton production were selected for investigation.

MATERIALS AND METHODS

Fortnightly surface water samples were collected from the study area at Elathur and Vizhinjam during October 1981 - December 1982. All samples were collected between 0900 - 1000 hours on observation days. Atmospheric temperature and rainfall data were obtained from the Meteorological Department. Salinity was determined by the classical silver nitrate titration method. Dissolved oxygen content was obtained by Winkler method (Strickland and Parsons, 1968). Turbidity of the sea water in the mussel bed area was estimated by the visual method using Candle Jackson turbidometer and expressed as Jackson Turbidity Units (JTU) (APHA, 1980).

Phytoplankton productivity was estimated in terms of chlorophyll *a*. One litre of the water samples was filtered through 47 mm Whatman GF/C filter paper. Prior to filtration a few drops of 1 % magnesium carbonate suspension was added to the sample. After filtration the pigments were extracted with 90 % acetone at low temperature in darkness for about 20 hours. The extract was centrifuged and the colour intensity of the clear extract measured in EC Spectrophotometer (GS 865D) at 750 and 665 nm wavelength. The extract was acidified and the

absorbance was measured again. Acetone (90 %) was used as blank in all measurements. The amount of chlorophyll *a* was calculated, after correcting for pheopigments, in mg chlorophyll *a*/m^3 using the equation of Lorenzen (1967) as given in Strickland and Parsons (1968). The mean monthly values (average of the two fortnightly values) were found to indicate the fluctuations better and, hence, these values are presented and discussed. Where fortnightly values gave critical information (e.g. Chlorophyll *a*), they are dealt with as such in the text.

RESULTS

Green mussel bed at Vizhinjam

The hydrographic features and phytoplankton values of *Perna indica* bed at Vizhinjam, along with meteorological data, are presented in Table 1 and Fig. 2.

Temperature

The surface temperature of the sea water at Vizhinjam was 27.9°C in October 1981. It gradually increased with minor fluctuations and reached a maximum of 29.3°C in March 1982. In April 1982 the sea water temperature was 29.1°C when the atmospheric temperature was also high (33.1°C). After April there was gradual lowering of temperature reaching a minimum of 24.3°C in July 1982. Subsequently there was an upward trend, temperature reaching 29.1°C in October 1982. The surface

temperature was always above the minimum and below the maximum of atmospheric temperature (Table 1, Fig. 2). The lowest value of 24.3°C was recorded in July 1982 (south-west monsoon season) and the highest value of 29.3°C in March 1982 (Summer).

Salinity

The salinity of the sea water was 33.12 ppt in October 1981. It gradually increased to 35.69 ppt by April. After April there was a gradual reduction to 32.55 ppt in July. Subsequently an increasing trend was noticed upto September when the salinity was 35.85 ppt. With slight fluctuations the salinity again increased to 36.10 ppt in December 1982. The maximum salinity level of 36.68 ppt was observed during November 1982. The lowering of salinity coincided with the occurrence of heavy rainfall; the minimum 32.55 ppt was obtained in July 1982 (south-west monsoon) and 32.62 ppt in November 1981 (north-east monsoon). The relatively higher values during October-December 1982 were perhaps due to the delayed and subdued north-east monsoon.

Rainfall

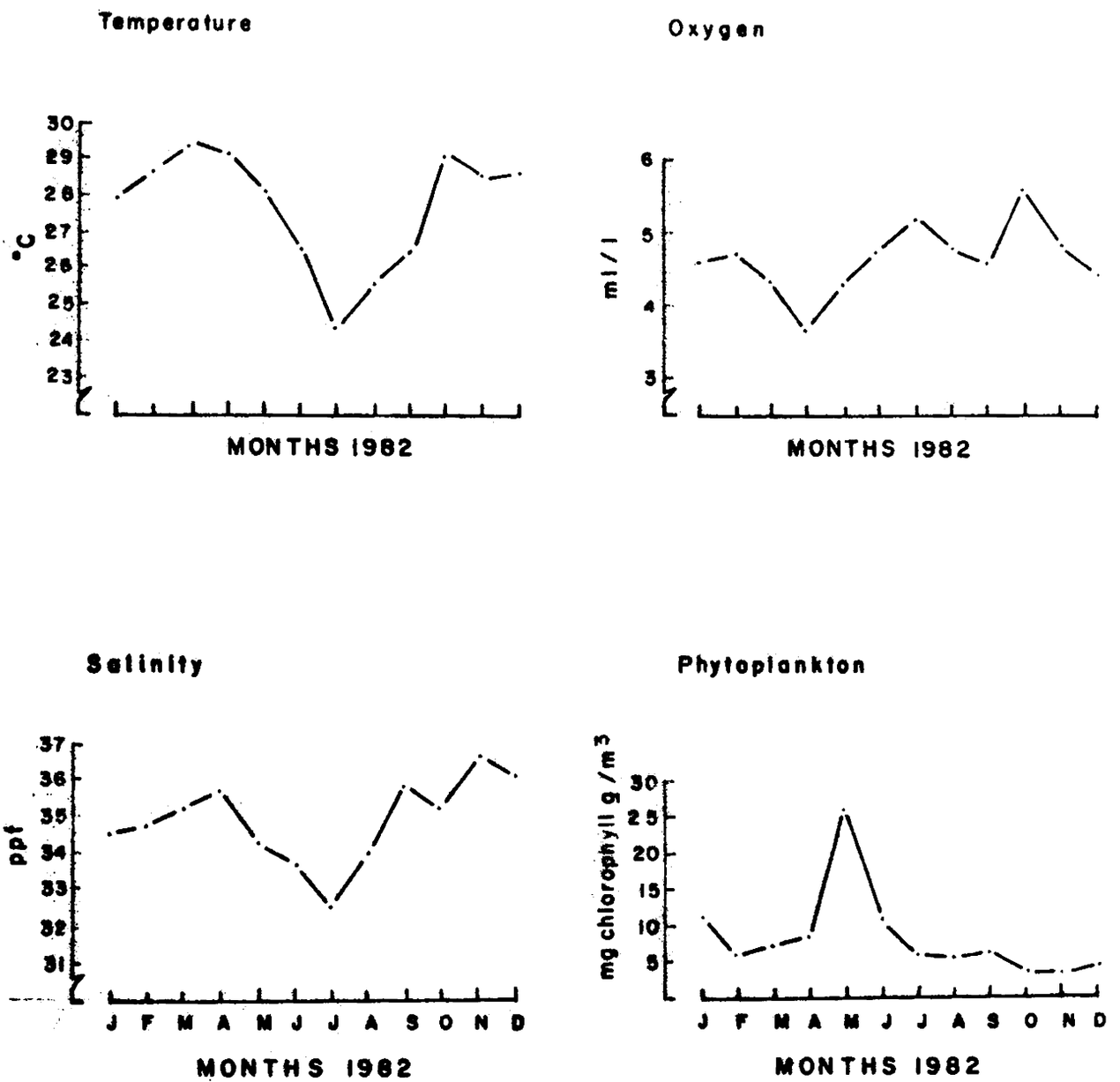
The Vizhinjam coast receives rainfall during the south-west monsoon as well as north-east monsoon. The north-east monsoon was active during October-December 1981 with the maximum number of rainy days (12) in October, the rainfall for the month being 291.8 mm. The south-west monsoon was active during May-July 1982. During this period the highest rainfall occurred in June (490.9 mm) with 23 rainy days. The south-west monsoon receded by the end of August. In October and November 1982, rain was felt again, at a lower intensity, due to north-east monsoon (Table 1).

Table 1 Seasonal variations in the environmental parameters of the mussel beds at Vizhinjam.

Month	Sea water temperature °C	Salinity ppt	Dissolved oxygen ml/litre	Turbidity JTU	Phytoplankton mg Chlorophyll g/m^3	Rainfall mm	Atmospheric temperature Minimum °C	Atmospheric temperature Maximum °C
Oct 81	27.9	33.12	4.67	NA	2.15	291.8	23.7	30.3
Nov 81	26.9	32.62	4.74	NA	2.19	156.0	23.5	30.1
Dec 81	28.4	33.54	4.10	NA	4.13	27.4	22.5	31.0
Jan 82	27.9	34.53	4.60	NA	11.02	0	21.1	30.9
Feb 82	28.6	34.70	4.70	NA	5.53	0	21.5	31.4
Mar 82	29.3	35.24	4.32	NA	7.38	0.5	24.7	32.6
Apr 82	29.1	35.69	3.64	245	8.46	49.6	25.8	33.1
May 82	28.1	34.32	4.33	470	28.57	294.5	25.2	32.3
Jun 82	26.5	33.75	4.77	435	10.52	480.9	24.0	29.9
Jul 82	24.3	32.55	5.19	540	5.92	147.8	23.6	29.5
Aug 82	25.6	33.95	4.78	365	5.54	117.7	23.7	29.7
Sep 82	26.5	35.85	4.60	180	6.37	17.2	24.0	30.5
Oct 82	29.1	35.22	4.56	200	3.94	120.3	23.9	31.2
Nov 82	28.5	36.68	4.85	263	3.12	176.6	24.0	31.1
Dec 82	28.6	36.10	4.45	200	4.03	23.8	22.1	31.1

NA - Data not available

Fig. 2 SEASONAL VARIATIONS IN THE ENVIRONMENTAL PARAMETERS OF THE MUSSEL BEDS AT VIZHINJAM



Dissolved oxygen

The dissolved oxygen content of the sea water showed fluctuations but no definite trend could be noticed. The minimum value obtained was 3.64 ml/l during April 1982 and the maximum 5.19 ml/l in July 1982 (Table 1, Fig. 2).

Turbidity

The sea water in the mussel bed area had low turbidity values during April-December 1982. Relatively higher turbidity values were coincident with the monsoons. However, the range was still low between 245 JTU in April and 540 JTU in July (Table 1).

Phytoplankton (Chlorophyll a)

Phytoplankton biomass was estimated as mg chlorophyll a per m^3. In October 1981, the chlorophyll a content was 2.15 mg. It was reduced to 1.64 mg in the first half of November following which there was gradual increase reaching a value of 14.85 mg in the first fortnight of January. Following a reduction to 5.53 mg in February, the chlorophyll content started increasing and reached its maximum of 38.49 mg/m^3 in the first fortnight of May corresponding to a phytoplankton bloom. The bloom was found to spread over the entire mussel bed area imparting a greenish brown colour to the water, which was dominated by diatoms Navicula sp., Asterionella japonica and Skeletonema costatum. Thereafter, chlorophyll a values gradually decreased to 3.12 mg in November. The minimum chlorophyll a value obtained was 1.64 mg in the first fortnight of November and the

maximum was 38.49 mg/m^3 in May 1982. The peak production was during May-June with a secondary peak in January (Table 1, Fig. 2).

Green mussel bed at Elathur

The hydrographic features of the water of the mussel beds of Elathur are presented in Table 2 and Fig. 3.

Temperature

The sea water temperature of the region fluctuated from 26.0°C (August 1982) to 29.9°C (June 1982). In October 1981, the temperature was 28.2°C and in November it increased to 28.9°C. After November, with slight fluctuations it reached 29.9°C in June 1982. Subsequently the temperature decreased to the minimum of 26°C in August and rose again to 28.2°C in December (Table 2, Fig. 3).

Salinity

The salinity variation during the period was from 35.40 ppt in October 1981 to 40.50 ppt in May 1982. From October to December 1981, the salinity remained low and steady (35.40-35.94 ppt) and increased abruptly to 39.85 ppt in January 1982. It reached the highest value of 40.50 ppt in May 1982. Later it decreased to 36.23 ppt in October 1982. During July to October 1982 salinity was low coinciding with heavy rainfall (Table 2, Fig. 3).

Rainfall

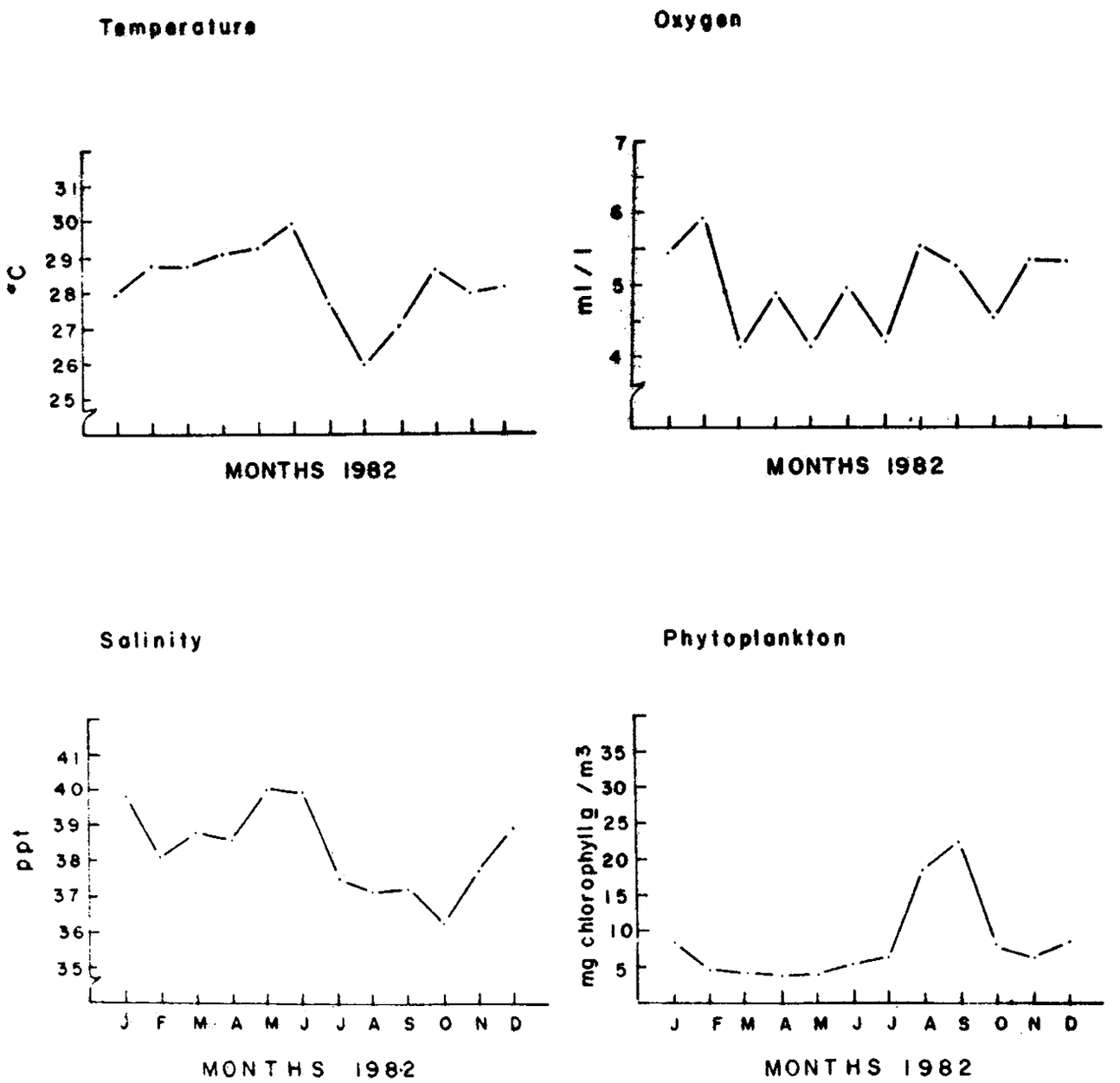
The south-west monsoon was very active during June-August 1982, with the monthly rainfall ranging 637.8 - 757.5 mm.

Table 2 Seasonal variations in the environmental parameters of the mussel beds at Elathur.

Month	Sea water temperature °C	Salinity ppt	Dissolved oxygen ml/litre	Turbidity JTU	Phyto-plankton mg Chlorophyll g/m^3	Rainfall mm	Atmospheric temperature Minimum °C	Atmospheric temperature Maximum °C
Oct 81	28.2	35.40	5.17	NA	3.31	263.3	24.1	30.2
Nov 81	28.9	35.94	5.17	NA	4.08	121.3	23.4	30.9
Dec 81	27.2	35.61	4.90	NA	4.28	24.2	22.2	31.6
Jan 82	27.9	39.85	5.44	NA	8.82	-	22.2	31.6
Feb 82	28.7	38.04	5.94	140	4.75	-	23.4	31.9
Mar 82	27.7	38.81	4.16	263	4.26	3.6	23.7	32.3
Apr 82	29.0	38.60	4.80	615	3.50	13.1	24.9	33.1
May 82	29.2	40.50	4.23	843	4.08	187.2	26.5	32.5
Jun 82	29.9	39.92	4.99	940	5.20	737.5	26.0	29.8
Jul 82	27.7	37.43	4.24	833	5.99	637.8	24.2	29.6
Aug 82	26.0	37.06	5.55	840	17.91	757.5	23.6	28.9
Sep 82	27.0	37.20	5.27	930	22.00	111.6	23.2	28.9
Oct 82	28.6	36.23	4.57	910	7.95	239.4	23.9	30.4
Nov 82	27.9	37.72	5.38	920	6.17	116.1	24.3	31.5
Dec 82	28.2	38.86	5.36	100	6.68	0	24.3	31.9

NA - Data not available

Fig. 3 SEASONAL VARIATIONS IN THE ENVIRONMENTAL PARAMETERS OF THE MUSSEL BEDS AT ELATHUR



The rains continued at reduced intensity from October to November 1982 bringing in the post-monsoon showers. During October-November 1981 also there was low precipitation (Table 2).

Dissolved oxygen

The dissolved oxygen of sea water ranged from 4.16 ml/l (March 1982) to 5.94 ml/l (February 1982). The dissolved oxygen content showed an irregular fluctuation during the period of observation (Table 2, Fig. 3).

Turbidity

The turbidity of the water at Kathur was high compared to that of the mussel beds at Vizhinjam. From February 1982 the turbidity began to increase and from May to November it was high (833 - 940 JTU) (Table 2).

Phytoplankton (Chlorophyll a)

The chlorophyll a values showed a marked seasonal trend during the period of study. In October 1981 the amount of chlorophyll a was 3.31 mg/m^3. It showed a gradual increase to 11.83 mg/m^3 in the first week of January 1982 (monthly average 8.82 mg). Subsequently chlorophyll a content decreased to 3.50 mg in April 1982. Later the chlorophyll level increased gradually and it touched a maximum of 37.25 mg/m^3 during first week of September corresponding to the occurrence of a bloom composed of a colonial blue-green alga. Thus, there were two peaks of phytoplankton production, a primary peak in the first week of September 1982 and a secondary peak in the first week

of January 1982 (Table 2, Fig. 3). The phytoplankton bloom could be judged by naked eye even from a distance during September 1982 as the colour of the water was greenish and the local people refer to this phenomenon as "kara".

DISCUSSION

The seasonal changes in the environmental factors of the mussel bed area at Vizhinjam and Elathur were found to be associated with the prevailing monsoon regime of the sea. The south-west monsoon was active at Vizhinjam during May-July with heavy rainfall and a moderate precipitation occurred due to north-east monsoon during October-December. The period of rainfall coincided with the period when salinity and temperature decreased and phytoplankton bloomed. These events occurred with a time lag of about one month at Elathur where the south-west monsoon was active during June-August. The role of monsoon in influencing the hydrographic features and primary and secondary production in the coastal waters of India has been clearly brought out by many workers (Rao and George, 1959; Subrahmanyan, 1959 a; b; Qasim et al., 1969 and Nair, 1974).

Both at Vizhinjam and Elathur the sea water temperature was comparatively high in the non-monsoon season (February, March and April 1982 at Vizhinjam and April to June 1982 at Elathur). The lowering of temperature in the monsoon period might have been caused by the precipitation. Ramamirtham and Jayaraman (1960) related the temperature fluctuations to climatic variation. The temperature fluctuations observed during the

present investigation at Vizhinjam agree with the findings of Appukuttan et al. (1980) for the period 1977-78. The salinity was found to decrease following the monsoon. The salinity trends at Vizhinjam closely agree with observations of Appukuttan et al. (1980).

The dissolved oxygen content of the sea water at Vizhinjam and Elathur did not show any regular pattern of variation except that the values were slightly high during the monsoon season. Rao and George (1959) also made similar observations and reported that, in the west coast, dissolved oxygen fluctuates from 3.54 to 5.13 ml/l with the maximum values in the rainy season. Nair (1974) also did not observe any seasonal trends in the dissolved oxygen content along the west coast of India.

The phytoplankton productivity of the mussel bed area at Elathur and Vizhinjam was influenced by a combination of factors such as rainfall, salinity and temperature. The phytoplankton bloom occurred in reduced salinity and temperature after the onset of monsoon. In Calicut, Subrahmanyan (1959 a, b) observed peak production of phytoplankton in June-July when the monsoon was very active with low salinity, temperature and high nutrients. During the south-west monsoon, the bottom mud laden with nutrients is released into the waters and brought to the surface layers by upwelling and turbulence caused by winds. Nair (1974) stated that in the coastal waters of India it is the nutrient replenishment that influence phytoplankton production directly and temperature also may play an indirect

influence in the regeneration of nutrient salts. Blooming of diatoms has been observed in the sea off Trivandrum during January-May (Menon, 1949). The occurrence of phytoplankton bloom, locally known as 'kara', was also reported by Jones (1950 at Vizhinjam during July-August, following the south-west monsoon.

CHAPTER IV

ANNUAL REPRODUCTIVE CYCLE

INTRODUCTION

The process of reproduction in molluscs involves germ cell differentiation, gonad development, maturation, spawning, fertilization, larval development and seed production. Reproduction may occur in a regular pattern resulting in annual or semi-annual cycle, though continuous spawning can also occur. These aspects have been studied with respect to marine invertebrates of polar, temperate and tropical regions (Giese, 1959). Environment plays an important role in the onset and progression of reproductive activity (Carriker, 1961; Sastry, 1963; Wilson and Hodgkin, 1967; Wilson 1969; Bayne, 1975 and Seed, 1976).

Reproduction in pelecypods has been reviewed by Franc (1960), Galstoff (1961), Fretter and Graham (1964), Bayne (1976 a) and Sastry (1979). The gametogenic processes of many marine bivalves have been studied, some of which are on Venus mercenaria (Loosanoff, 1937), Pinctada spp. (Tranter 1958 a, b, c, 1959), Argopecten (= Aequipecten) irradians (Sastry, 1963, 1966, 1968) and Placopecten magellanicus (Naidu, 1970). Among the Indian species, the reproductive

aspects have been investigated on several species and the list includes Katelysia opima by Mane (1974, 1981) and Nagabhushanam and Mane (1975 b), Donax faba by Alagarswami (1966), Donax cuneatus by Nagappan Nayar (1955), Meretrix casta by Durve (1964), Cresmostrea madrasensis by Rao (1951, 1953, 1956), and C. cucullaeoides by Durve (1965).

The reproductive activity of the animals in a population is described in terms of the gonad index which is a measure of the gonad development. The gonad index of Mytilus edulis has been studied by Seed (1975) and Seed and Brown (1975). The changes in the gonad index and digestive gland index were studied in Argopecten (= Aequipecten) irradians by Sastry (1979). Histological technique has been used to ascertain the reproductive activity of the mussels and several reproductive stages have been identified. Johnstone (1898) outlined a method of staging which was later developed by Chipperfield (1953). Many authors have subsequently revised the scheme (Lubet, 1957, 1959; Seed, 1969 a, 1975; Lunetta, 1969; Bayne 1976 a). These schemes provide a simple, yet precise, method for examining the onset, progress and duration of the reproductive cycle. More recently, using histology and autoradiography, Kelly et al. (1982) detailed a scheme for the classification of the male gonad.

Among the earlier publications on the reproduction of mussels, the most comprehensive one is that of Field (1922) on Mytilus edulis from the Atlantic coast. Data on the reproduction of M. edulis of the European coast were published

by several authors (Chipperfield, 1953; Seed and Brown, 1975 and Bayne, 1976 a). For M. californianus a prolonged reproductive season was reported by Whedon (1936) and Young (1942). Reproduction of the Mediterranean mussel M. galloprovincialis was investigated by Lubet (1959), Costanzo (1966), Bourcart and Lubet (1965), Rensoni and Giusti (1972) and Lubet et al. (1981). The reproductive cycle of M. galloprovincialis from Northern Adriatic sea and M. edulis from Long Island Sound was studied by Hrs-Brenko (1971).

The early work on reproduction of the Indian mussel is that of Paul (1942). The reproductive processes of Perna viridis at Ratnagiri were investigated by Nagabhushanam and Mane (1975 a) and at Kakinada by Narasimham (1980). The annual reproductive cycle of P. indica was studied by Kuriakose (1973) and Appukuttan et al. (1980). The reproductive activity of the cultured mussel (P. viridis) in Goa has been studied by Parulekar and Dalal (1980).

A sound knowledge of the biology of reproduction has been considered essential for the study of reproductive physiology. Therefore, during the present investigation, observations were made on the different reproductive phases and their seasonality in the mussels P. indica and P. viridis.

MATERIALS AND METHODS

Samples of *Perna indica* and *P. viridis* were collected respectively from Vizhinjam and Elathur at the sites referred to in Chapter II. These sites came under exploitation by the fishermen and, therefore, in order to get a representative sampling of the population, mussels were collected from five spots randomly selected on the fort-nightly sampling days. The collections were pooled. Mussels were divided into five size-groups, namely those below length of 15 mm, 15-30 mm, 30-45 mm, 45-60 mm and those above 60 mm. From the sample, 20 animals were drawn from each of the above five size-groups for detailed studies. Seasonal differences were observed in the availability of size-groups due to recruitment and exploitation and there were occasions when only one size-group (45-60 mm) was present at the sites. Thus the sample size ranged from 20 to 100 animals depending on seasonal variations in the exploited population. For histological examination, the size-group 45-60 mm, which was present throughout the year, was selected.

The mussels were fixed in 5 - 7 % buffered neutral formalin at the site. In the laboratory the colour, texture and fullness of gonad were noted visually and a smear examination was done on all the animals in the selected sample. Ten animals were treated further for histological studies. Based on the results of smear test and histological study, the reproductive stage was assigned to each animal and the data

were used in calculating Mean Gonad Index of the sample and sex ratio. The fortnightly data did not generally indicate much variations within any calender month and, therefore, the data were pooled for each month and presented here. Wherever differences were noticed, these are discussed in the text.

Histological studies were carried out on mantle tissues fixed in Zenker's fluid. The fixed tissues were washed in running tap water overnight and with iodine in 70 % alcohol (Lugol's solution) to ensure complete removal of mercury. Dehydration of the tissues was affected in alcohol grades from 10 % to absolute alcohol. The tissues were then treated with methyl salicylate and cleared progressively in alcohol-xylene mixture and finally in xylene. After paraffin infiltration at 60°C in hot air oven, the tissues were embedded in paraffin wax of congealing point 56 - 58°C. Sections were cut in 6 - 8 µm thickness on a rotary microtome and fixed on glass slides. The sections were deparaffinised in xylene, hydrated through the descending series of alcohol and distilled water and stained with Delafield's haematoxyline and counter stained with eosin (Humason, 1962). Stained sections were dehydrated, cleared in xylene, mounted on DeX and stored briefly for histological examination.

The Mean Gonad Index (MGI) of the population was calculated by the method of Seed and Brown (1975) used on M. edulis. The number of animals in each stage of gonad development was multiplied by the numerical ranking of the stage and the sum total of these products was divided by the

number of animals in the sample to obtain the index. However, the numerical ranking for the different reproductive stages assigned by Seed and Brown (1975) could not be followed in the present study on P. indica and P. viridis. These authors treated the developing stages of the European mussel as I to IV in the order of progression and the spawning stages in the reverse order of IV to I, denoting spent and undifferentiated gonads as stage 0 and fully ripe gonad as stage V. In the Indian species the progress of reproductive activity takes place in quick succession and, therefore, a spread-out classification appeared unrealistic. The classification adopted by Velez and Epifanio (1981) for the tropical Venezuelan mussel Perna perna was found suitable to describe the reproductive stages of the tropical Indian mussels. The numerical ranking given for the different stages in the present study is as follows :

Stage	Gonad condition	Numerical ranking for MGI
0	indeterminate	0
1	incipient	1
2	advanced	2
3	ripe	3
4	spawning, spent	0

RESULTS

Reproductive system of mussel

The gonad in *Perna indica* and *P. viridis* is a diffuse organ and spreads into the mantle and labial palps. The gonad proper, which is present behind the foot and below the byssal retractor muscle, remains dormant as a small, thin, slightly elongated tissue during the resting phase. It grows in size with the onset of gametogenesis and attains its maximum bulk in fully mature animals. The mantle, except the posterior margin, is occupied by reproductive tissue during the active phase of reproduction. The reproductive follicles in the mantle are packed tightly in mature condition and are connected by anastomosing minor canals which join and lead to major genital canals in different regions. These major canals combine on either side of the body and lead to a paired gonoduct which discharges the gametes at spawning. The growth and development of reproductive tissue is concurrent and uniform in all the organs stated above. Therefore, in the present investigation, the mantle tissue was taken to represent the reproductive state of the animal, as has been done in many earlier studies, and a portion of the central region of the left mantle was examined.

Reproductive stages

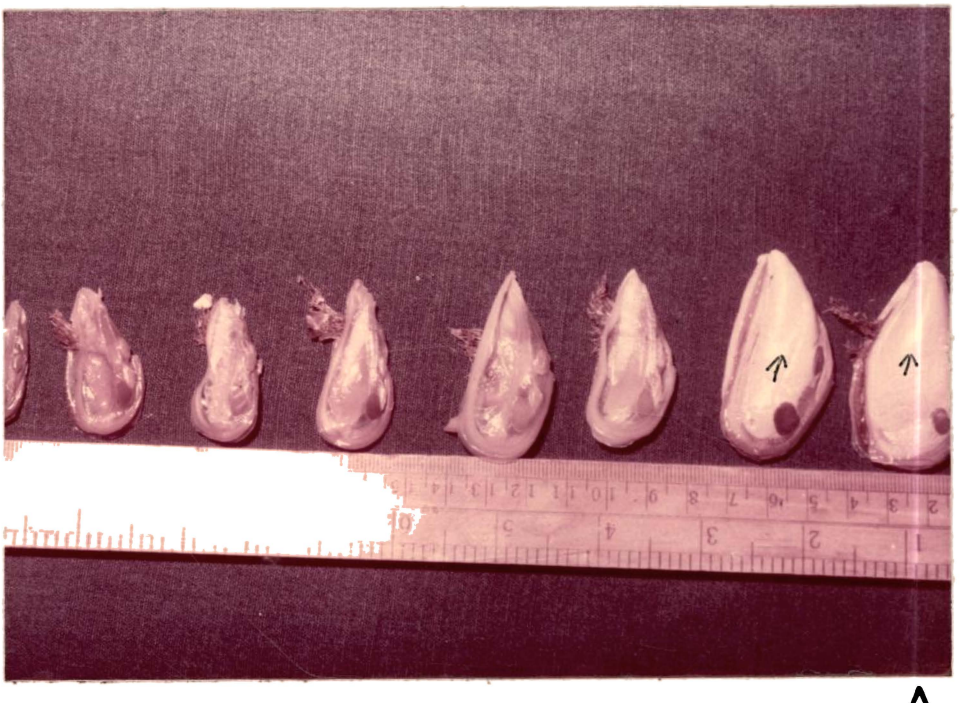
Indeterminate (stage-0): During the non-reproductive phase the mantle is generally thin and transparent (Pl. 2, A). With accumulation of glycogen, the mantle tissue becomes

PLATE 2

A. Progressive stages (from left to right) in glycogen accumulation in the mantle tissue of Perna indica, during the non-reproductive phase. Arrow points to the whitish mantle with high glycogen content.

B. Progressive changes in the colour and size of the digestive gland of Perna indica associated with reproductive cycle. Arrow indicates digestive gland. Clock-wise from bottom right: mussels in reproductive stages 0, 1, 1, 2, 2, 3, 3, 4 and 4

PLATE 2



A

B

thicker and turns milky white in colour. There is no trace of reproductive activity in the gonad, but elaboration of connective tissue takes place. The connective tissue is both adipogranular (AG) and vesicular (VCT) in nature during later stages, due to glycogen accimulation. The number of AG cells is higher than VCT (Pl. 3, A, B). The digestive gland is large, soft and dark green in colour (Pl. 2, B). The adductor muscle is thick and muscular in nature with reddish brown colour.

Female

Stage-1: Sex differentiation occurs with the formation and differentiation of the gonial cells in the connective tissue (Pl. 3, B, C). With the formation of follicles the gametogenic process is initiated and the follicles begin to proliferate occupying more of the space of connective tissue (Pl. 3, F). The mantle is thick and milky white due to glycogen accumulation. The size and the colour of the digestive gland and adductor muscle is reduced by the onset of gametogenesis. With the progress of oogenesis the digestive gland slightly shrinks in size and the dark green colour undergoes a faint change to a paler shade (Pl. 2, B). The reddish brown colour of adductor muscle also gets slightly reduced.

Stage-2: In the stage-2 animals the colour change of the gonad is quite evident. The mantle is orange-red in colour with a few patchy white areas due to the presence of

PLATE 3

A & B Histological sections of mantle tissue of *Perna indica* showing the presence of both adipogranular (AG) and vesicular connective tissue (VCT) cells.

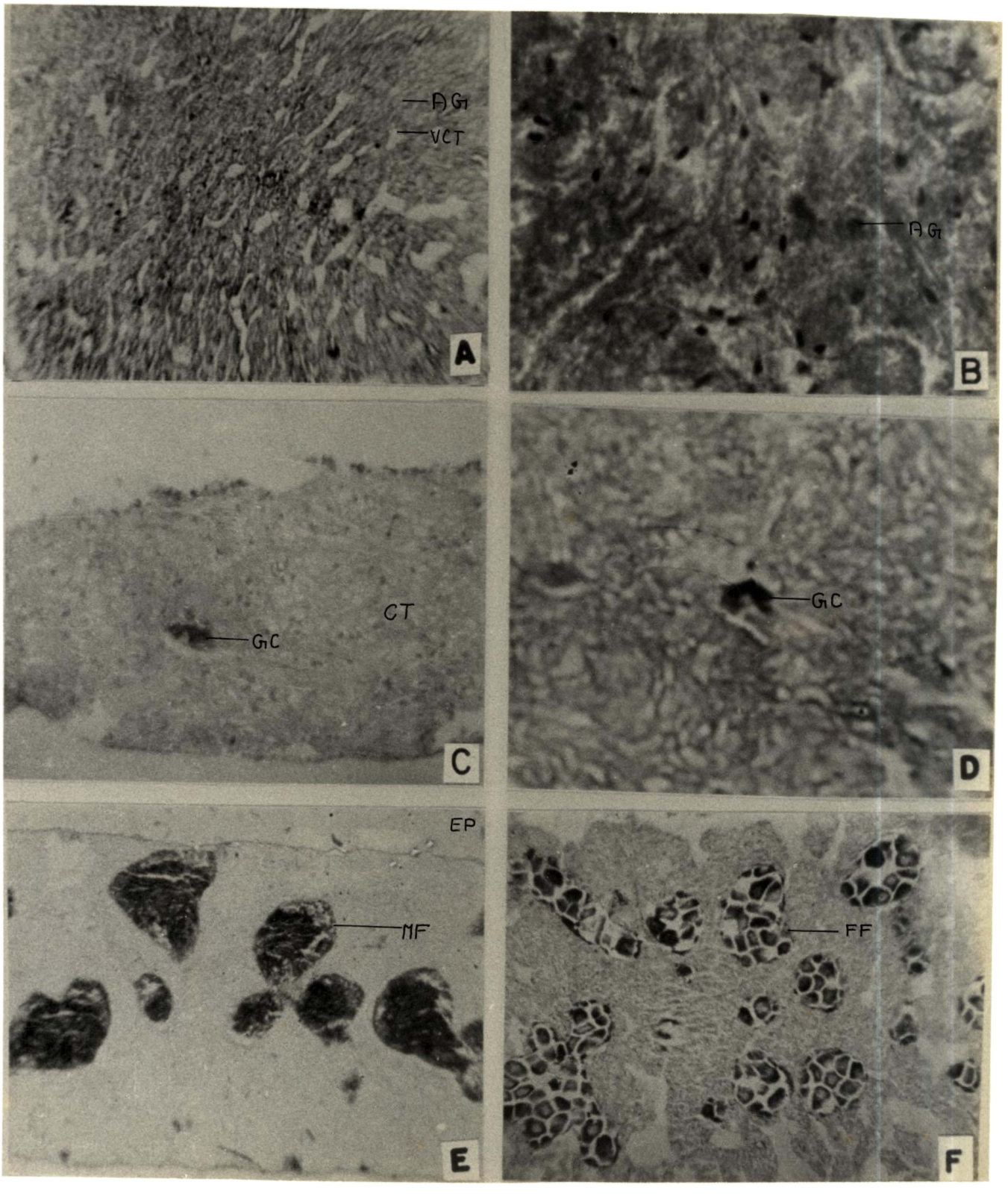
C & D Histological sections of mantle tissue of *P. indica* showing the differentiation of gonial cells in the connective tissue.

E Proliferation of male reproductive follicles in the connective tissue of *P. indica*.

F Proliferation of female reproductive follicles in the connective tissue of *P. indica*.

AG - Adipogranular cells; CT - Connective tissue;
EP - Epithelial layer;
FF - Female follicle; GC - Gonial cells; MF - Male follicle;
VCT - Vesicular connective tissue.

PLATE 3



glycogen. The mantle is packed with the developing follicles containing oogonia as also oocytes. The oogonia lie close to the follicular wall and, by mitotic divisions, give rise to primary oocytes. The oocytes are attached to the follicular wall with a stalk (Pl. 4, B, D, F and Pl. 5, B). The stalked oocytes enter into the vitellogenic phase and yolk material gets accumulated in the ooplasm. The mantle increases in thickness due to the active gametogenic process and becomes flabby. The digestive gland is pale green in colour and gets further reduced in size. The adductor muscle turns brown with reduction in size and texture.

Stage-3: The mantle is thick, flabby, slimy and packed with reproductive follicles. The colour of the mantle is deep red or orange-red. The surface of the ripe mantle shows a granular texture. Histological examination shows that the follicles occupy the entire mantle tissue except the posterior margin with apparently no connective tissue and contain stalked oocytes as well as free spherical ova (Pl. 5, B, D). The germinal vesicle is distinctly seen in the free ova. The ooplasm surrounding the germinal vesicle is rich in yolk materials. The digestive gland turns yellowish green in colour and is further reduced in volume. The adductor muscle becomes flabby and pale brown in colour.

Stage-4: The animals are in the spawning phase (Pl. 5, E). The breakdown of the germinal vesicle of the ova is observed only after spawning and upon the ova coming in contact with seawater. After the discharge of eggs, the

PLATE 4

A, C & E Stages of development of male follicle in Perna indica as revealed in histological sections;

A. An enlarged male follicle on the left side of the section;

E. Growth of male follicles during active gametogenic phase;

E. Fully mature male follicles.

B, D & F Stages of development of female follicle in Perna indica;

B. A female reproductive follicle in the early gametogenic phase;

D. Female follicles in active phase;

F. Enlarged view of oocytes in active gametogenic phase showing stalked and free oocytes in the follicle.

AG - Adipogranular cells; CT - Connective tissue;

FF - Female follicle;

MF - Male follicle; SO - Stalked oocytes.

PLATE 4

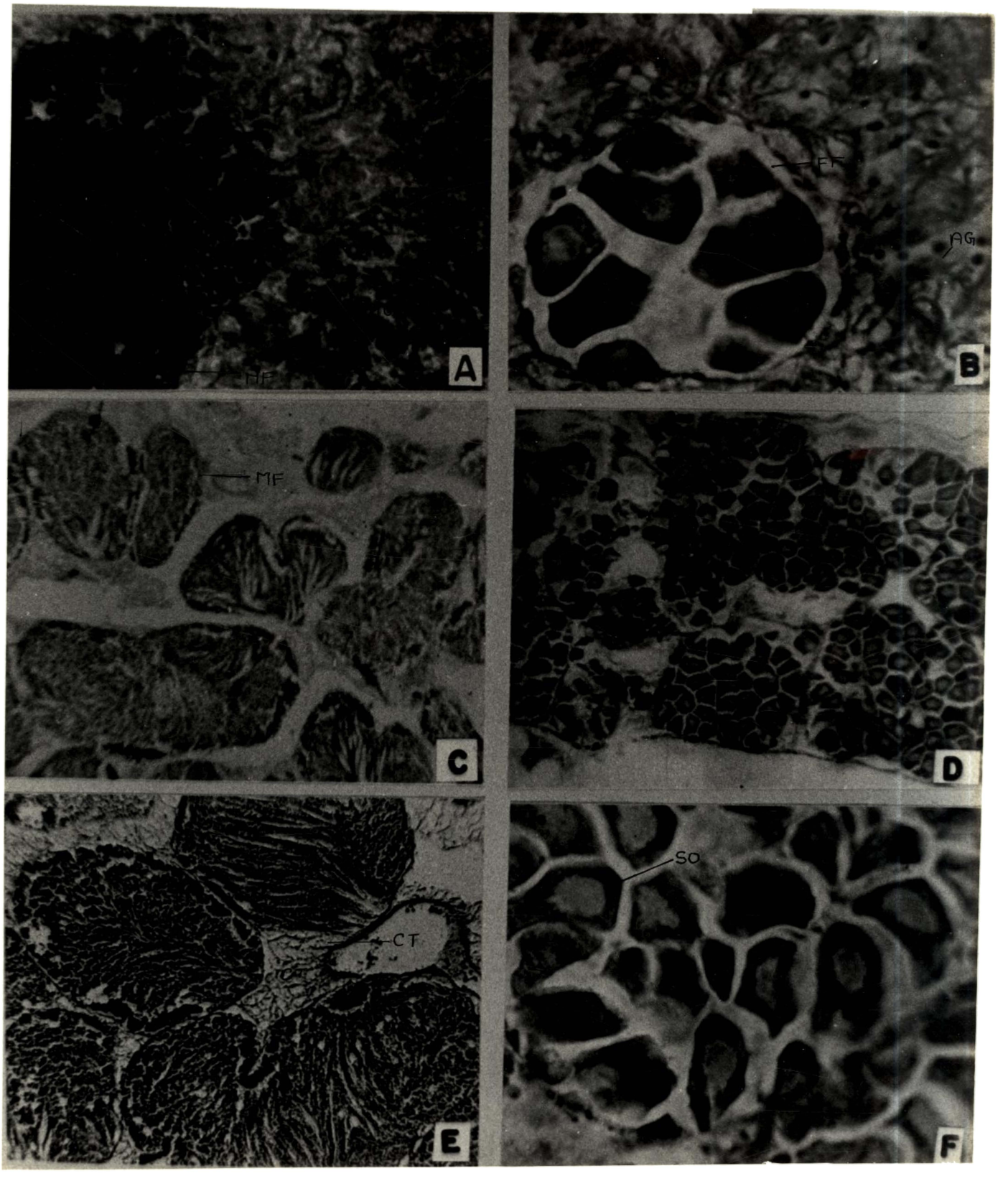


PLATE 5

A. An enlarged section of fully mature male follicles of *P. indica* showing the arrangement of sperm bundles which lose follicular identity before spawning.

B. Female follicles in stage 3 animals.

C. The peripheral area in the section of a mature male follicle showing the presence of spermatogonia and spermatids indicating simultaneous gametogenic process along with maturation.

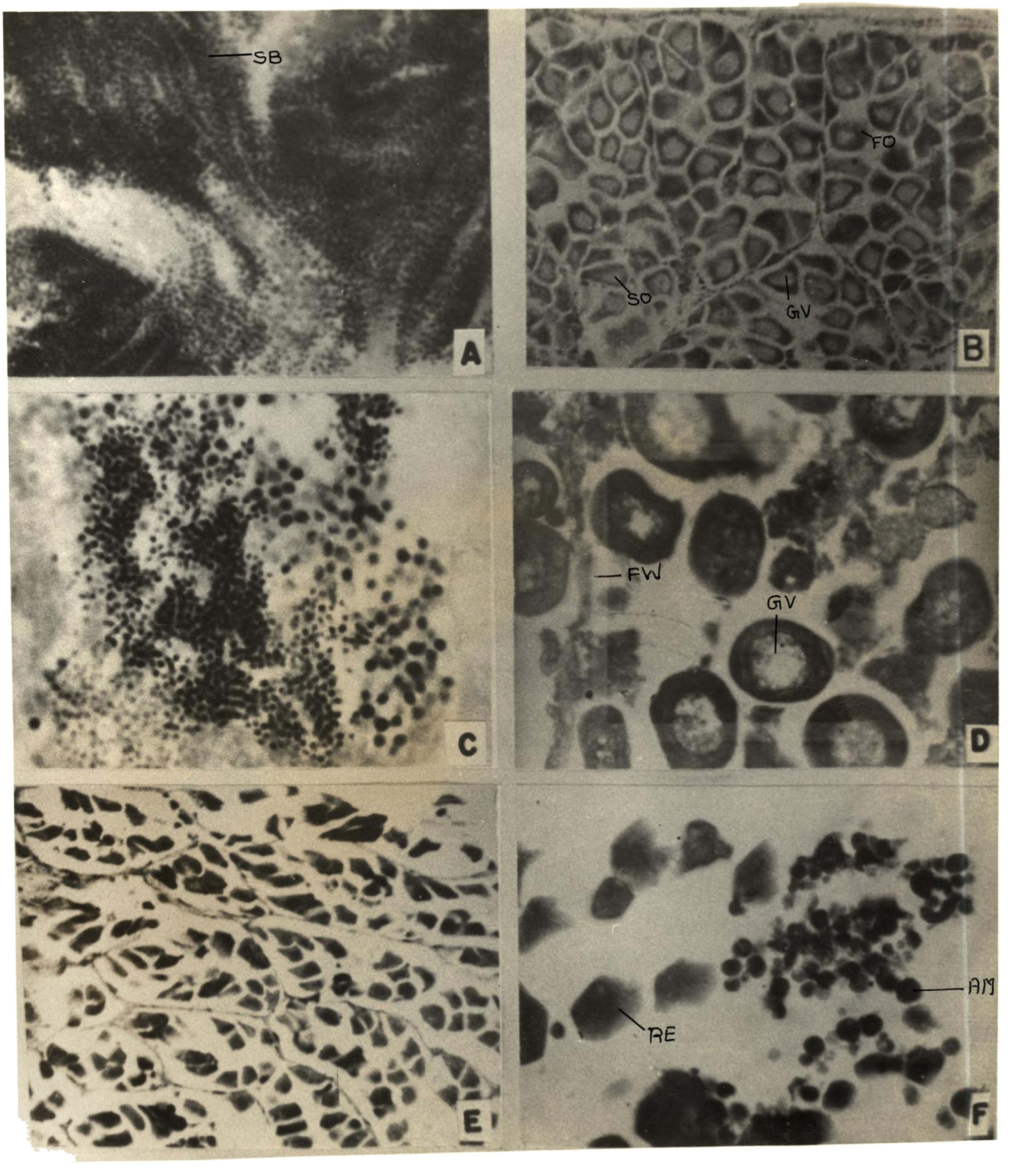
D. An enlarged section of a mature female follicle showing the disrupting follicular wall and mature ova with prominent germinal vesicle.

E. Section through the mantle of a spawning mussel indicating the absence of free ova (which have been spawned) and presence of stalked oocytes.

F. An enlarged view of a section through mantle of a spent animal showing the presence of residual eggs and amoebocytes.

AM - Amoebocytes; FO - Free oocytes; FW - Follicular wall;
GV - Germinal vesicle; RE - Residual eggs; S - Spermatids;
SB - Sperms in bundles; SG - Spermatogonia; SO - Staled oocy

PLATE 5



colour of the mantle gets progressively reduced and finally becomes membranous and transparent. Presence of amoebocytes is observed in the spent animals for the resorption of the residual eggs by autolysis (Pl. 5, F). Soon after spawning the digestive gland and adductor muscle begin to regain their size and colour. After the resorption of the residual eggs the gonad enters the resting phase by elaboration of connective tissue and the sex becomes indeterminate.

Male

Stage-1: In the stage-1 animals, sex differentiation is just initiated with early gametogenesis (Pl. 3, E). Gonial cells begin to make appearance amidst the connective tissue by the disintegration of the latter (Pl. 4, A). The spermatogonial cells appear in the periphery of the follicle and the spermatocytes are formed inside the follicular area. The occurrence of primary and secondary spermatocytes is also evidenced during the initial stages of development (Pl. 5, C). The initiation of the gametogenic activity results in reduction of the size of digestive gland (Pl. 2, B) and adductor muscle.

Stage-2: The mantle is pale yellow or dirty white in colour, loose and flabby. The reproductive follicles are found to spread in the mantle occupying more space in the connective tissue (Pl. 4, C). The follicles contain secondary spermatocytes and spermatids. The interfollicular connective tissue disintegrates with the progress of spermatogenesis.

The digestive gland is further reduced in size and is pale green in colour. The adductor muscle turns brown with reduction in size and texture.

Stage-3: In this stage the gonad is fully ripe. It is loose, flabby and pale yellow in colour. Histological examination of the mantle shows that the follicles are densely packed and contain spermatozoa (Pl. 4, E). The follicular boundaries even disappear or merge with each other and the spermatozoa are seen as bands in the mantle (Pl. 5, A). The sperms are found to ooze out of the mantle on slight pressure. The digestive gland is very thin and yellowish in colour. Similarly the adductor muscle is thin and flabby and pale brown in colour.

Stage-4: This is the active spawning stage of the animals. After the expulsion of sperms the mantle becomes membranous and transparent. Residual sperms and amoebocytes are observed. By the resorption of residual sperms and elaboration of connective tissue, the animal enters the spent-recovery and indeterminate stage. After spawning the digestive gland and adductor muscle regain their colour, size and texture.

Annual reproductive cycle

The distribution of different reproductive stages in Perna indica and Perna viridis during the period from October 1981 to December 1982 is presented in Tables 3 and 4 respectively.

Perna indica

The *Perna indica* population at Vizhinjam was observed to have only a single reproductive season in a year. The reproductive activity extended over a period of eight months, from February to September 1982. The animals were in the indeterminate stage (non-reproductive phase) from October 1981 to February 1982 and also during October-December 1982 (Table 3). Gonial cell differentiation commenced in February end and early stages of gametogenesis were observed till the middle of April 1982, when 20 % of the animals were still in the germinal cell differentiation stage. In April, 75 % of the animals were in the active gametogenic phase (stage-2) and a small percentage has advanced to the ripe phase. The percentage of ripe animals (stage-3) showed a rapid increase from 5 % in April to 55 % in May and 85 % in June and a maximum 92 % of this stage was observed in July. Mussels in the spawning/spent stage appeared from the second half of May till the first week of September, but the spawning activity in the entire population was the highest in August, with 80 % of the animals in stage-4. Spawning was mild in the previous months with 15 % of the population in stage-4 during May and June and 8 % in July. By the end of September all the animals entered the indeterminate stage. Elaboration of the connective tissue was observed in the animals collected during October-December.

The data in Table 3 show that in *P. indica* there is one clear and well-defined reproductive cycle which is annual in nature. Growth of gonad and gametogenesis commence in

Table 3 Distribution of reproductive stages and sex ratio in the population of Perna indica at Vizhinjam

Month	Non-reproductive phase %	Reproductive phase %	Sex ratio M : F	Percentage distribution of reproductive stages (male and female combined)			
				1	2	3	4
Oct 1981	100	-	-	-	-	-	-
Nov 1981	100	-	-	-	-	-	-
Dec 1981	100	-	-	-	-	-	-
Jan 1982	100	-	-	-	-	-	-
Feb 1982	61	39	80:20	100	-	-	-
Mar 1982	13	87	65:35	67	33	-	-
Apr 1982	-	100	58:42	20	75	5	-
May 1982	-	100	57:43	-	30	55	15
Jun 1982	-	100	52:48	-	-	85	15
Jul 1982	-	100	49:51	-	-	92	8
Aug 1982	-	100	50:50	-	-	20	80
Sep 1982	88	12	52:48	-	-	-	100
Oct 1982	100	-	-	-	-	-	-
Nov 1982	100	-	-	-	-	-	-
Dec 1982	100	-	-	-	-	-	-

February; maximum gamete proliferation takes place in April; maturation and vitellogenic activity is the highest in June-July; and the highest synchronised spawning activity takes place in August. The animals remain in the vegetative or non-reproductive phase during October-January.

Perna viridis

On the other hand, the green mussel Perna viridis at Elathur was observed to have two reproductive seasons, one major and another minor. The data are presented in Table 4. The major reproductive season extended for five months from July to November 1982. The reproductive phase commenced by the beginning of July with germ cell differentiation and early gametogenesis in 40 % of the total population. During the second fortnight of July and early August 1982 active gametogenesis was observed and 50 % of the animals became ripe by the second half of August. Spawning commenced in early September and active spawning was noticed in September (60 %) and October (80 %). Spawning continued in November with 93 % of the animals in spawning/spent-recovery stage. Maximum spawning was complete by end of October when most of the animals were in the spent condition and a small percentage had entered the resorption stage. The animals entered the non-reproductive phase by November-December 1982.

The secondary reproductive season was observed from January to March 1982. An interesting observation was that only animals of size above 60 mm participated in the

Table 4 Distribution of reproductive stages and sex ratio in the population of Perna viridis at Elathur

Month	Non-reproductive phase %	Reproductive phase %	Sex ratio M : F	Percentage distribution of reproductive stages (male and female combined)			
				1	2	3	4
Oct 1981	-	100	48:52	-	-	80	20
Nov 1981	76	24	52:48	-	-	61	39
Dec 1981	32	68	52:48	-	-	-	100
Jan 1982	*100	-	-	-	-	-	-
	**84	16	47:53	12	18	52	18
Feb 1982	*100	-	-	-	-	-	-
	**85	15	52:48	6	12	58	24
Mar 1982	*100	-	-	-	-	-	-
	**84	16	50:50	-	6	18	76
Apr 1982	100	-	-	-	-	-	-
May 1982	100	-	-	-	-	-	-
Jun 1982	100	-	-	-	-	-	-
Jul 1982	60	40	54:46	28	72	-	-
Aug 1982	-	100	48:52	-	50	50	-
Sep 1982	-	100	53:47	-	-	40	60
Oct 1982	-	100	49:51	-	-	20	80
Nov 1982	10	90	50:50	-	-	7	93
Dec 1982	100	-	-	-	-	-	-

EXPLANATORY NOTE

* Figures in this row are for mussels 60 mm and below in size (range 28 - 60 mm)

** Figures in this row are for mussels above 60 mm size (range 61 - 81 mm)

reproductive activity (Table 4). Those 60 mm and below continued to be in the resting phase. During the second fortnight of January 1982, while 84 % of the population was in the resting stage, 16 % showed sex differentiation. Of these, 12 % were in the initial gametogenic phase, 18 % in the active gametogenic phase, 52 % in the fully ripe condition and 18 % showed spent condition. In February 1982, the majority of the animals (58 %) was in the ripe stage and 24 % in the spent condition. During March, while 18 % of the animals were in the fully ripe condition, 76 % were in the spent condition. From April to the middle of July 1982 the animals again entered the non-reproductive phase.

Examining the data for the period October-December 1981 in Table 4, it is inferred that the major reproductive season of 1981 would have been slightly delayed by a time factor of about one month. While majority of the population was in stage-4 in September-October 1982, most of the animals were still in stage-3 during the same months in 1981. Similarly, whereas 93 % of the animals were in stage-4 in November 1982, such a condition was observed during 1981 only in December.

Considering the secondary minor reproductive activity during January-March 1982 in the above light, it is seen that the *P. viridis* population, upon remaining in the resting phase at the end of the major spawning for a very short spell, again enters the reproductive phase in January 1982. It is to be inferred that animals of size below 60 mm could not activate

themselves for the secondary reproductive phase so quickly perhaps due to depletion of energy reserves at the previous spawning; those above 60 mm are likely to have had a higher energy reserve even at the end of spawning and were able to enter the secondary reproductive phase during January-March 1982.

Sex ratio

Sex ratio in the population of P. indica showed a male dominance in the initial phase of reproductive activity (Table 3). Starting with a male-female ratio of 80:20 in February 1982 at the onset of gametogenic activity, the ratio shifted in favour of females with a progressive increase in the female population, reaching near equality in ratio (49:51) in July when 92 % of the population was in fully mature condition and equality (50:50) in August, the month of peak spawning activity. It would appear that sex differentiation to female is a slow process and catches up shortly before peak spawning.

In the case of P. viridis population the male-female ratio was about equal (Table 4) during both the major and secondary reproductive seasons, ranging from 47:53 to 54:46.

Mean Gonad Index (MGI)

Data on mean gonad index of the mussels are presented in Table 5 and Fig. 4.

In P. indica the MGI was 0 from October 1981 to January 1982 and from September to December 1982. The MGI was

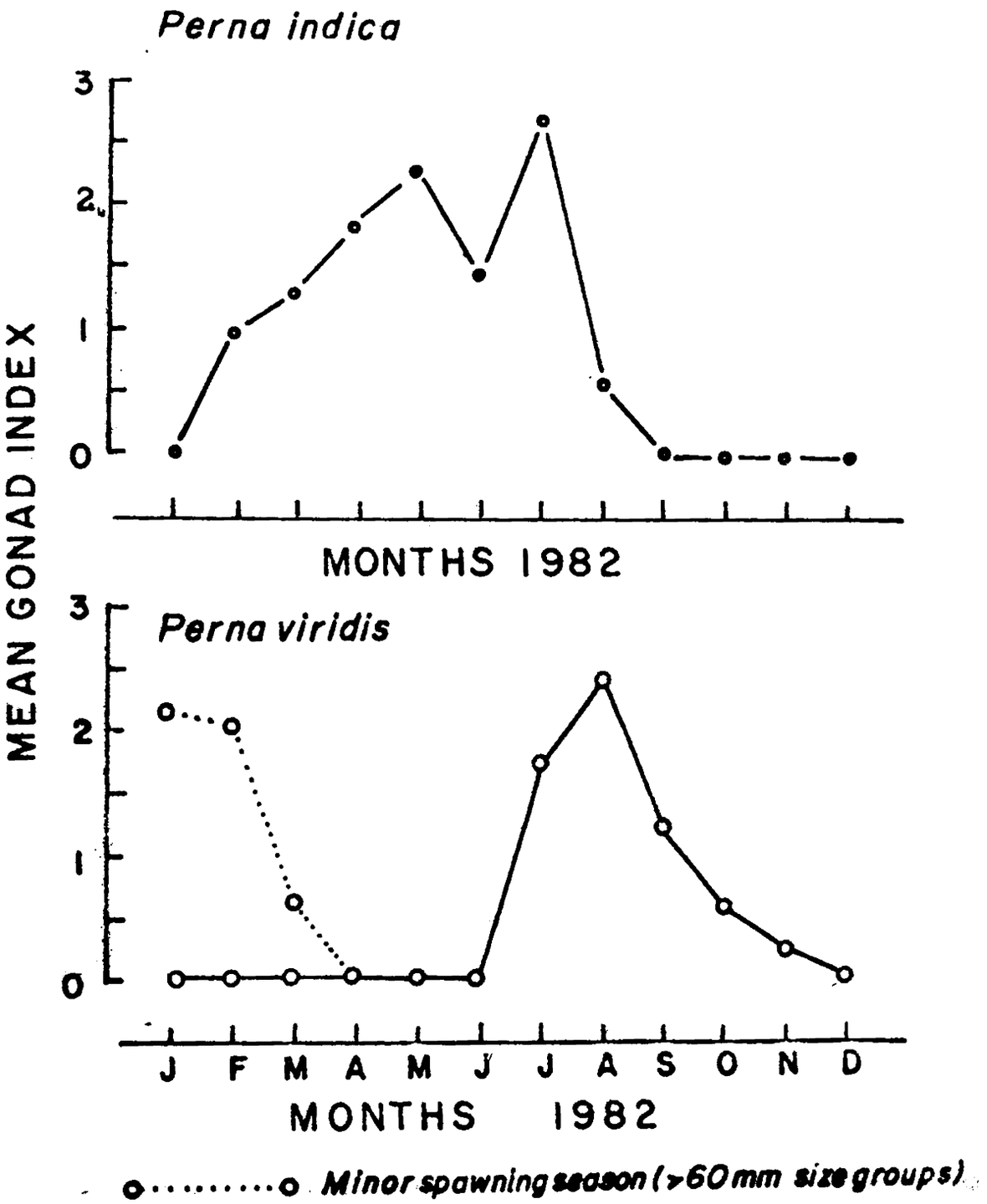
Table 5 The seasonal variation in mean gonad index of *Perna indica* and *P. viridis*

Month	Mean Gonad index	
	P. indica	*P. viridis*
Oct 1981	0	2.40
Nov 1981	0	1.88
Dec 1981	0	0
Jan 1982	0	0* 2.16**
Feb 1982	1.0	0* 2.06**
Mar 1982	1.33	0* 0.64**
Apr 1982	1.85	0
May 1982	2.28	0
Jun 1982	1.43	0
Jul 1982	2.77	1.72
Aug 1982	0.60	2.40
Sep 1982	0	1.20
Oct 1982	0	0.59
Nov 1982	0	0.22
Dec 1982	0	0

* Animals below 60 mm size group

** Animals above 60 mm size group

Fig. 4 SEASONAL VARIATION IN THE MEAN GONAD INDEX OF *PERNA INDICA* AND *PERNA VIRIDIS*



1.00 in February and 1.33 in March. From April 1982 the MGI increased gradually and attained 2.28 in May and in July it was 2.77. In August the MGI sharply reduced to 0.60 coinciding with spawning.

In P. viridis the mean gonad index of the animal was 0 from January to June 1982 in the animals below 60 mm in size. In July the MGI was 1.72 and by August it increased to 2.40. By September, the MGI began to fall and reached 0.22 in November. In December the animals were in the non-reproductive phase. During the minor spawning season, only the animals above 60 mm size participated in the reproductive activity. In January the MGI was 2.16 and it got reduced to 2.06 in February and 0.64 in March.

DISCUSSION

The reproductive system of Perna indica and P. viridis closely resembles that of Mytilus edulis as described by White (1937) and Lubet (1959). The extension of reproductive follicles into the mantle during the reproductive phase, which otherwise functions as the storage organ in the non-reproductive season, was well elucidated by Bayne (1976 a, b) and Bayne et al. (1982). The changes in colour and size of digestive gland are clearly associated with the progress of reproductive activity and are indications of energy transfer from digestive gland to reproductive tissues. The changes in adductor muscle would also suggest a similar role.

The occurrence of adipogranular (AG) and vesicular cells (VCT) in the mantle connective tissue has been reported by Bayne et al. (1982), Lowe et al. (1982) and Kelly et al. (1982). Lunetta (1969) had observed only vesicular cells in the connective tissue of Perna perna. In P. indica and P. viridis of the present study, adipogranular cells were more numerous in the connective tissue than the vesicular cells. There is reduction in the abundance of ADG with germ cell proliferation which has been attributed to lysosomal activity (Moutteville, 1974; Mathieu, 1979), the energy being utilised in gametogenesis.

The mean gonad index was high during the period of active gametogenesis and maturation, and decreased with spawning. Seed and Brown (1975) and Bayne (1976 a) observed similar variations in the gonad index of Mytilus edulis.

The populations of P. indica and P. viridis studied were observed to have a single major spawning season, though in P. viridis a minor spawning season also occurred. In the population of P. indica, the period of reproductive activity extended from February to August with peak spawning in July-August. Jones (1950) reported an extended breeding season for the brown mussel at Vizhinjam with peak breeding activity during June-August. Appukuttan and Nair (1980) reported spawning in this species by the end of May lasting till September with peak spawning in July-August. In P. viridis from Elathur, spawning season was from July to November with

intensive spawning in September and October. Nagabhushanam and Mane (1975 a) reported that M. viridis at Ratnagiri spawns from June to early September. These authors also reported a minor spawning during February-March as has been observed during the present study.

The minor spawning of P. viridis at Elathut during January to March in which only the higher size group (above 60 mm) participated was partial and incomplete. During these months the primary productivity of the area was also found to be low (Chapter III). Such partial spawning by the larger size groups during the low productivity periods has been reported to occur in the Danish waters (Jensen and Spark, 1934). The second spawning in P. viridis may be related to the energy reserve and requirements of the animal.

According to Dr. B.L. Bayne (personal discussion)* the reproductive effort of bivalves can be expressed by the term Pr/P, where Pr = energy available for gamete production and P is the sum of energy for somatic growth (Pg) and for gamete production (Pr). He considers reproductive effort as a function of size besides other factors. The larger animals, having attained a higher somatic growth, may be able to convert the food energy derived from the minor peak of phytoplankton bloom during December-February to reproductive effort. On the other hand, the smaller animals (below 60 mm), may utilize this

---

* The valuable suggestions given by Dr. B.L. Bayne, Director, NERC Institute for Marine Environmental Research, Plymouth, U.K., during his visit to Cochin in December 1983 are sincerely acknowledged.

energy source for somatic growth and may not be able to convert this energy to gamete production. This appears to be a plausible explanation of the situation observed during the secondary minor reproductive phase of P. viridis at Elathur.

The observation on the dominance of males in P. indica in the early stages of reproduction and the gradual shift to an almost equal distribution of the sexes agrees with that of Nagabhushanam and Mane (1975 a) on P. viridis. According to Velez and Epifanio (1981), the availability of food plays a role in sex determination of experimental animals (P. perna). They found that low nutrition and high temperature lead to dominance of males. While this aspect is dealt with in Chapter IX of this study, it is indicated here that if this observation holds true for the natural populations also, the dominant male ratio observed in P. indica during February-March might be due to low levels of chlorophyll a (5.53 - 7.38 mg/m^3) and higher temperatures (28.6° - 29.3°C) prevailing in the area during the period.

CHAPTER V

ECOPHYSIOLOGY OF REPRODUCTION

INTRODUCTION

A number of investigations on reproduction of marine invertebrates have been directed to elucidate the exogenous factors which control reproduction and these have been reviewed by Giese (1959), Giese and Pearse (1974) and Sastry (1979). Giese (1959) suggested that the precise pattern of a reproductive cycle depends upon the external factors which entrain or time the endogenous drive. Pearse and Barksdale (1984) emphasised on investigations on the temporal patterns of reproduction by shallow water marine invertebrates in the Indian Ocean and on "the environmental factors used by animals as cues to determine when they 'should' spawn, and the selective factors ultimately determining optimal spawning times".

Orton (1920) proposed that temperature was the most important exogenous factor regulating reproduction of marine invertebrates of temperate waters. This was confirmed by Nelson (1928), Wilson and Hodgkin (1967), Pearse (1968) and Mileikovsky (1970). Thorson (1946) studied the reproduction and larval development of Danish marine bottom invertebrates and related temperature with their reproductive activity.

Loosanoff and Davis (1950, 1963) made use of temperature in controlling reproduction of many marine bivalves. Effect of temperature on the reproductive activity of mussels has been worked out by Young (1945), Allen (1955) and Moore and Reish (1969). Bayne (1965) used a combination of temperature and chemical stimulation to induce spawning in mussels and described a relationship between gametogenesis and rate of change of temperature. Some data on the tropical species have also been obtained (Carvajal, 1969; Lunetta, 1969).

Along the Indian coasts, sea water temperature is relatively high throughout the year without drastic fluctuations and generally does not fall below the optimum requirements of many molluscs (Rao, 1951). Durve (1965) suggested that temperature might not influence the spawning of marine bivalves but considered salinity as an important factor in initiation of spawning. It was confirmed by the studies of Alagarswami (1966) on Donax faba, Nagabhushanam and Mane (1975 a) on Mytilus viridis (= Perna viridis) and Nagabhushanam and Mane (1975 b) on Katelysia opima. The lowering of salinity was considered the stimulus to induce spawning in marine invertebrates (Panikkar and Aiyer, 1939; Paul, 1942). Parulekar and Dalal (1980) found the annual cycle of reproduction in P. viridis closely associated with the temporal variation in temperature and salinity.

The role of food (phytoplankton) in regulating gametogenesis has recently been stressed (Giese and Pearse, 1974). Bayne (1975), Zandee et al. (1980) and Pieters et al. (1980) related food availability with production of ripe gametes

and spawning in Mytilus edulis. In the temperate zones bivalves elaborate large amounts of gametes during spring coinciding with the spring bloom of phytoplankton and spawning starts by a rise in temperature in summer (Broom and Mason, 1978). According to Lubet (1973) gonad development in Mytilus is controlled by neuroendocrine factors but temperature and food act to synchronise or induce the different stages of gametogenesis. Parulekar and Dalal (1980) correlated gonad growth in Perna viridis with phytoplankton abundance.

Since the reproductive physiology of marine invertebrates is closely linked with the biotic and abiotic parameters of the environment, the present study on Perna indica and P. viridis included investigations on the ecophysiological aspects. The annual reproductive cycle of the species presented in Chapter IV is discussed here in conjunction with the cyclical changes in the environmental parameters, data on which have been presented in Chapter III. In addition, data on pea-crab infestation and pollution due to coconut husk retting have been presented and discussed, in as much as they interfere with the reproductive activity of the mussel.

RESULTS AND DISCUSSION

Influence of temperature

The environmental data for Perna indica from Vizhinjam and P. viridis from Elathur have been presented in Tables 1 and 2 (Chapter III) and reproductive stages in different months in Tables 3 and 4 (Chapter IV).

In the case of P. indica, the annual reproductive cycle can broadly be divided into three phases, namely

a) Non-reproductive phase (October to January)

b) Gametogenic phase (February to April) and

c) Maturation and spawning phase (May to September).

During the non-reproductive phase (October-January) the temperature range is 27.9° - 29.1°C, with a seasonal mean of 28.5°C. In the gametogenic phase (February-April), the range is 28.6° - 29.3°C and the seasonal mean is 29.0°C. In the maturation and spawning phase, the range is 24.3° - 28.1°C, the seasonal mean being 26.2°C. It is significant to note that within the narrow annual range of 24.3° - 29.3°C, the mean temperature is moderately high (28.5°C) during the non-reproductive phase, marginally elevated (29.0°C) during the gametogenic phase and is the lowest (26.2°C) during the maturation and spawning phase. It could be inferred that gametogenesis commences with a slight elevation of temperature (from 28.5° to 29.0°C), maturation of gametes is accelerated with a fall in temperature (to the mean 26.3°C during May-July) and spawning reaches its peak in the lowest temperature mean of 26.05°C (August-September).

On the above pattern, the reproductive cycle of P. viridis at Elathur can be broadly divided into four phases, namely

a) Non-reproductive phase (April-June)

b) Gametogenic phase (July-August)

c) Spawning phase (September-November) and

d) Non-reproductive/secondary spawning phase (December-March).

The range and seasonal mean temperatures for the above phases are as follows: (a) 29.0° - 29.9°C, 29.37°C; (b) 26.0° - 27.7°C, 26.85°C; (c) 27.0° - 28.6°C, 27.83°C; (d) 27.7° - 28.7°C, 28.12°C. The correlation that emerges in this species is different from that of P. indica. Gametogenesis commences on the decrease of seasonal mean temperature from 29.37°C to 26.85°C and the spawning season is marked by a slight rise to 27.83°C. The non-reproductive/secondary spawning period (mussels above 60 mm alone participates in the partial and subdued spawning activity) is marked by a further elevation in temperature to 28.12°C. It is seen that in both species a higher temperature (relative to the narrow annual range) prevails during non-reproductive phase than during the active reproductive phase.

Influence of salinity

Treating the salinity data at Vizhinjam from Table 1 (Chapter III) for P. indica in the same sequence of reproductive cycle as for temperature, it is seen that the seasonal mean for the non-reproductive phase is the highest at 35.63 ppt, gametogenic phase is at 35.21 ppt and maturation and spawning phase is at 34.08 ppt. Within the narrow annual variations (32.55 ppt in July to 36.68 ppt in November), the non-reproductive phase has higher values of salinity as compared to the active reproductive phase. A slight reduction

from 35.63 ppt to 35.21 ppt appears to activate the gametogenic phase and further reduction to 34.08 ppt leads the animals to maturation and spawning. Maturation appears to be accelerated at the lowest seasonal mean salinity of 33.54 ppt during May-July and spawning reaches its peak at a slightly higher mean of 34.90 ppt during August-September.

In the case of P. viridis at Elathur (Table 2, Chapter III), the non-reproductive phase has the highest seasonal mean salinity value of 39.67 ppt, the gametogenic phase 37.25 ppt, spawning phase 37.05 ppt and non-reproductive/ secondary spawning phase 38.89 ppt. Again it is seen that the non-reproductive phase is marked by higher salinity values and the active reproductive phase by lower values. Gametogenesis appears to be triggered by a decrease in salinity from the seasonal mean of 39.67 ppt to 37.25 ppt and spawning occurs around this salinity (37.05 ppt). Further elevation of salinity to 38.89 ppt results only in partial and incomplete spawning among the older groups of mussels.

Influence of rainfall

Under a monsoon regime, almost all the environmental parameters of the sea are influenced by its duration and force. In this study, rainfall is taken as the indicator of the duration and force of monsoon (Tables 1 and 2, Chapter III). At Vizhinjam 74.1 % of the annual rainfall of 1431.9 mm is during the maturation and spawning phase of P. indica, 22.4 % during the non-reproductive phase and 3.5 % during the gametogenic phase. Gametogenesis starts in February with 0

rainfall but proceeds with precipitation. Maturation becomes accelerated and spawning commences with heavy rainfall in May (294.5 mm). The heaviest rainfall of 480.9 mm in June takes the process of maturation to a peak (85 % in stage-3) and accelerates spawning. Spawning continues vigorously during the subsequent months (July-September) under decreasing rainfall.

At Elathur, gametogenic process in *P. viridis* commences with heavy rainfall (637.8 mm) in July and accelerates in August under a similar spell of heavy rainfall (757.5 mm). Fifty percent of the annual rainfall of 2793.8 mm during the above two months coincides with gametogenic phase. Spawning commences early September with reduced rainfall and is completed during September-November, the period accounting only for 16.4 % of rainfall. There is no rain (except 3.6 mm in March) during December-March when the animals are in the non-reproductive/ secondary spawning phase. The non-reproductive phase coincides with 33.6 % of annual rainfall during April-June, June alone having a high rainfall of 737.5 mm.

Influence of nutrition

In the present study, a major chlorophyll *a* peak was observed at Vizhinjam in May with a value of 25.57 mg/m^3 and a minor secondary peak in January with a value of 11.02 mg/m^3 (Table 1, Chapter III). The minor peak in January leads to gametogenic activity in *P. indica* from February and the major peak in May leads to acceleration of maturation and spawning.

In the case of P. viridis at Elathur, gametogenesis commences in July with a subtle increase in chlorophyll a content to 5.99 mg/m^3 from 5.20 mg/m^3 in June (Table 2, Chapter III). Maturation accelerates with increase in chlorophyll a values and spawning commences in September when phytoplankton production is at its peak (22.00 mg/m^3). A minor secondary peak of 8.82 mg/m^3 in January appears to support the secondary, partial and incomplete spawning observed during January-March.

Effects of pollution

The pollution caused by coconut husk retting activity at Elathur, about one km away from the regular sampling site, was found to have an effect on the reproductive activity of the green mussel population of that area. The environmental parameters which showed differences between the regular sampling site and coconut husk retting site are given in Table 6. The range of temperature at the retting site was from 27.3°C to 33.0°C and the maximum temperature was observed during April 1982. The salinity ranged from 24.9 ppt to 40.72 ppt and the maximum salinity was observed during 1982. The dissolved oxygen was comparatively less in the retting area. The lowest oxygen content of 3.09 ml/l was observed in March 1982 and the highest 4.78 ml/l in October 1981. In the non-polluted area the oxygen level was 4.16 ml/l and 4.57 ml/l during March and October respectively. The water in the polluted area was highly turbid. The chlorophyll a values were consistently low in all months. The water that stagnated in the pits where coconut husk

Table 6 Some of the environmental parameters at the coconut husk retting (polluted) area compared with those of the regular sampling site (non-polluted) at Kathur

Month	Dissolved oxygen ml/litre		Turbidity JTU		Phytoplankton biomass mg chl. a/m^3	
	Polluted area	Non-polluted area	Polluted are	Non-polluted area	Polluted area	Non-polluted area
Oct 81	4.78	5.17	200	NA	2.81	3.31
Nov 81	4.40	5.17	200	NA	2.89	4.08
Dec 81	4.06	4.90	725	NA	2.82	4.28
Jan 82	4.05	5.44	600	NA	5.43	8.82
Feb 82	4.54	5.94	500	140	2.72	4.75
Mar 82	3.09	4.16	900	263	2.34	4.26
Apr 82	3.14	4.80	1000	615	2.12	3.50
May 82	3.11	4.23	1000	843	2.93	4.08
Jun 82	4.17	4.99	1000	940	3.74	5.20
Jul 82	4.15	4.24	1000	833	2.95	5.99
Aug 82	4.36	5.55	960	840	7.50	17.91
Sep 82	4.67	5.27	1000	930	7.36	22.00
Oct 82	4.37	4.57	1000	910	7.05	7.95
Nov 82	4.50	5.38	1000	920	4.45	6.17
Dec 82	4.16	5.36	1000	100	2.88	6.68

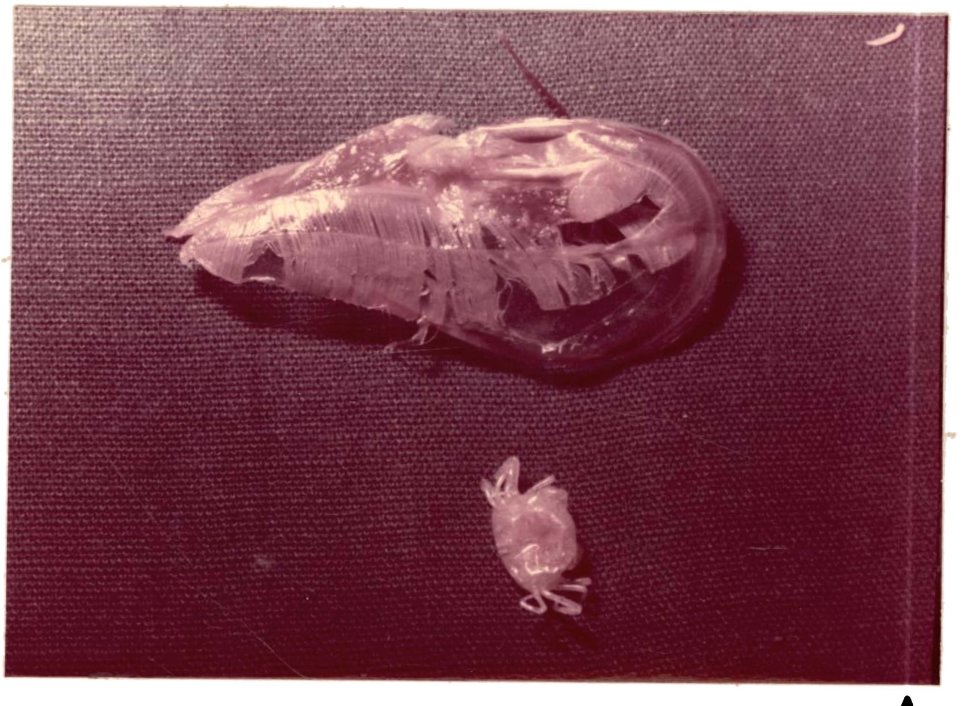
NA - Data not available

PLATE 6

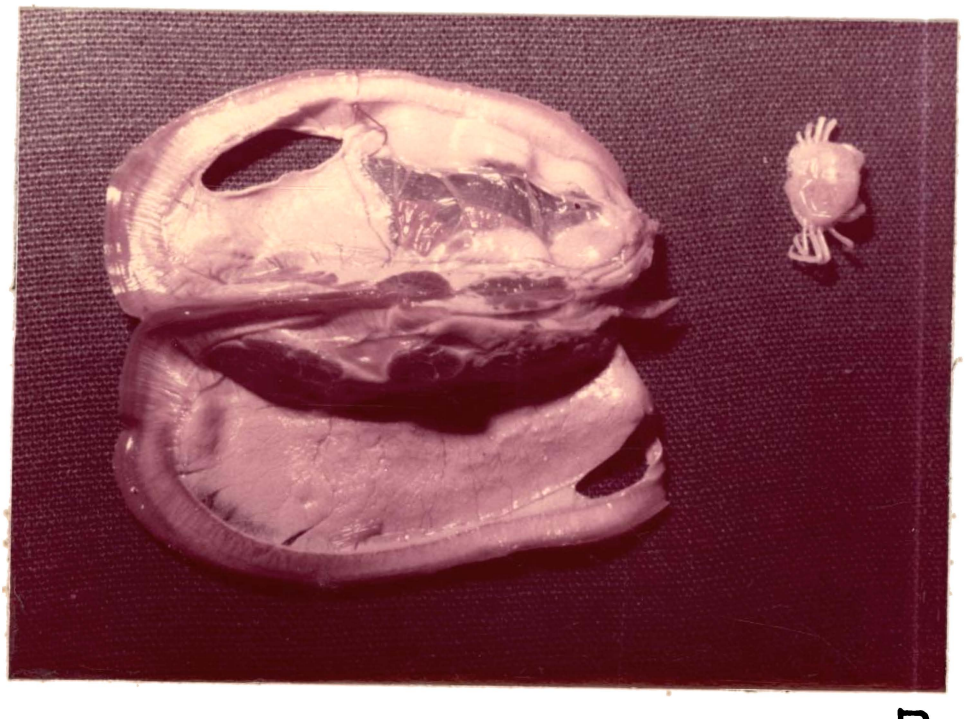
A. Perna indica in sexually indeterminate stage infested with the pea-crab (Pinnotheres sp.) showing the fibrous nature of the mantle and emaciated body as a whole.

B. Showing damage caused by the pea-crab to the mantle of a mature male P. indica at the site of 'lodging'. The rest of the mantle shows normal mantle tissue.

PLATE 6



A



B

was dumped for retting was darker, emitting foul smell of hydrogen sulphide and phenolic compounds.

The reproductive processes of the mussel collected from the polluted area were found to be similar to those of the animals collected from the non-polluted area. A striking difference was that the entire P. viridis population in the polluted area was constituted by males and not a single female was recorded throughout the period of observation. The spawning activity of the males showed an incomplete expulsion of the gametes. The animals in the population did not exceed 50 mm size whereas in the other parts of the bed, animals of 60 to 80 mm size were common.

Effects of pea-crab infestation

In the regular sampling of P. indica at Vizhinjam, instances of pea-crab infestation were noticed in certain times of the year. The percentage incidence is presented in Table 7. Both sexes of mussels were affected and the percentage of infestation was higher in males than in females. In all cases only a single pea-crab (carapace width 7-10 mm) was noticed per mussel. The pea-crabs were found to occupy the mantle cavity of mussels above 30 mm in size. The crabs seemed to cause damage to the gills and soft tissues (Pl. 6 A and B). The digestive gland of the infested mussels appeared pale and thin indicating nutritional stress. The whole body was transparent and the tissues were fibrous. In cases where the pea-crabs had infested fully mature mussels, the mantle at the site was transparent without reproductive tissues. When infestation occurs in the

Table 7 Percentage incidence of pea-crab (*Pinnotheres* sp.) in *P. indica*

Month	Total no. of animals	No. of infested mussels	% incidence of infestation
Oct 81	20	-	-
Nov 81	26	2	7.6
Dec 81	18	-	-
Jan 82	16	2	12.5
Feb 82	20	-	-
Mar 82	20	-	-
Apr 82	22	1	4.5
May 82	31	3	9.67
Jun 82	20	1	5.00
Jul 82	17	4	23.53
Aug 82	20	2	10.00
Sep 82	17	-	-
Oct 82	20	-	-
Nov 82	18	2	11.11
Dec 82	27	-	-

early stages of reproductive activity, further development was totally inhibited, resulting in a transparent tissue mass with fibrous texture. Probably the decreased food availability to the mussel due to the consumption of the food strings by the pea-crab affected the mussels and the stored energy in the digestive gland and the adductor muscle could have been mobilised for the survival of the animals.

DISCUSSION

"Ecophysiological studies on the effect of external factors on the reproduction of Lamellibranchs have emphasized the action of temperature, the somewhat fragmentary ecological data collected in the field serving as framework for research in the laboratory" (Lubet, 1980-81). Based on temperature requirements for reproduction, Loosanoff and Nomejko (1951) identified physiologically different races of Crassostrea virginica along the Atlantic coast of U.S.A. The M. edulis populations in the British waters are observed to have different seasonal reproductive cycles in different localities (Bayne, 1976 a) and even in the same locality the reproductive activity varied with the habitat. It has been established in the case of Mytilus edulis (Bayne, 1976 a) and Pinctada fucata (Wada, 1976) that gametogenesis and maturation is a function of temperature and time which is expressed as degree-days.

A review of the Indian literature on the annual reproductive cycle of the marine bivalves in relation to environmental conditions shows the truly sub-continental nature of the problem, and the ecophysiological aspects involved in

gametogenesis and spawning appear specific for each species and each location. Alagarswami (1966) stated that the marine and estuarine molluscs fall into different categories ranging from the ones having a restricted spawning season (Katelysia opima) to those spawning more or less continuously throughout the year (Crassostrea madrasensis). The related temperature and salinity data (largely these two parameters have been emphasised) would show the dominant role of salinity on reproduction than temperature though the associated pattern of fluctuations of the two is clear (Rao, 1951; Nayar, 1955; Durve, 1965).

The present data on Perna indica and P. viridis show that a relatively higher temperature prevails during the non-reproductive season than during the active reproductive season. Supporting data for this observation come from the works of Rao (1951) on Crassostrea madrasensis from Adyar backwaters, Nayar (1955) on Donax cuneatus from Palk Bay and Alagarswami (1966) on D. faba from Gulf of Mannar. From the Venezuelan and Brazilian coasts, Perna perna has been found to spawn when the temperature has declined to 22°C after a maximum of 28°C (Carvajal, 1969; Lunetta, 1969). In this particular respect, the tropical species appear to be opposed to their temperate counterparts which spawn in higher temperatures. However, there are also examples where spawning takes place in rising or high temperatures as in Perna viridis from Kakinada during January-May (Narasimham, 1980), the secondary spawning of C. madrasensis during March-April (Rao, 1951), the second peak spawning of D. faba during May-June (Alagarswami, 1966), as also the

secondary spawning of P. viridis of the present study during January-March. With the exception of P. viridis from Kakinada, all the other cases involve only a secondary spawning and, in many cases, these are partial. Thus the primary or major spawning of these species appear generally related with lowering of temperature.

Evidence to relate marine bivalve spawning with low salinities is indeed overwhelming in the Indian literature (Rao, 1951; Abraham, 1953; Nayar, 1955; Durve, 1965; Alagarswami, 1966 and others). Nagabhushanam and Mane (1975 a) observed in Mytilus viridis (= P. viridis) from Bhatia creek in Ratnagiri a major spawning during July-August coincident with salinity decrease and moderate temperature. The salinity decrease was very marked, from 25.6 ppt in June to 3 - 9 ppt in July as the samples came from the creek which is subject to heavy fresh-water drainage during monsoon. The authors observed a minor spawning during February-March which coincided with a rise in salinity (to 32.5 ppt in February) and low temperature. Nagabhushanam and Mane (1975 a) considered salinity as a determining factor in controlling the reproductive cycle of M. viridis.

Further south, from Goa, Rao et al. (1975) observed that the older M. viridis (length 60 mm and above) have a prolonged spawning period from about July to December with peak spawning in September-November and the younger groups (below 60 mm length) breeding from January to April, with a minor peak during February-March.

The present results on P. viridis at Kathur, which is further south from Goa, have shown a major spawning season for the whole adult population during September-November and a secondary spawning season only for the older mussels during January-March. There appears to be a difference in timing of spawning of the P. viridis populations along the west coast which may be dependent on the location of the beds. Those which are located in the creeks as in the case of Bhatia creek population (Nagabhushanam and Mane, 1975 a) spawns in July-August under the influence of sudden lowering of salinity (3 - 9 ppt in July) due to freshwater influx, and those in the intertidal coastal waters (present study) spawn later during September-November at a marginally lower normal salinity (37.0% ppt in September-November). The rate of decrease in salinity is so vast in the two ecosystems, being 88.3 % in the Bhatia creek and 6.2 % at Kathur, between June and July, and yet the decrease leads to spawning of the population in Bhatia creek and gametogenesis and spawning at Kathur.

Secondary spawning of the mussel at all the three centres has been observed and in all cases, the spawning is partial and incomplete, not leading to any notable spatfall. While Rao et al. (1975) noticed that only the younger ones below 60 mm in size, arising from previous monsoon spawning, participate in the reproductive activity during January-April, the present study shows an opposite situation where only the older ones (above 60 mm) go through the secondary spawning activity during January-March. The environmental factors responsible for the secondary spawning are not well defined.

P. viridis along the east coast has shown year-to-year variations in the seasonal cycle of reproductive activity, as observed during 1974-1976 at Kakinada by Narasimham (1980). In general, the spawning is prolonged extending from December to July, with a peak during January-May. Contrary to the west coast situation, the peak spawning in the Kakinada mussel population coincides with rising temperature and high salinity and the animals are mostly in resting condition during the period when the temperature shows a decreasing trend and the salinity is low.

Under raft culture, the transplanted mussel shows a different type of reproductive cycle, as investigated by Parulekar and Dalal (1980). Gametogenesis was observed in young mussels of 7-8 mm size under raft culture (Parulekar and Dalal, 1980) as against 15.5 mm in the natural beds (Rao et al., 1975). Spawning commences in February and lasts till May, with a peak in May. During June-July, with the monsoon active, they are in the resting phase. From August gametogenesis recommences and the mussels have a second spawning season from September to November. This phase of the spawning agrees well with the observations made in the present study at Ennur. According to Parulekar and Dalal (1980), the peak spawning coincides with favourable conditions of temperature, salinity and abundance of food material and the resting phase coincides with the lowering of temperature, salinity and scarcity of food.

The green mussel (P. viridis) has a wide distribution along the Indian coasts. The data on reproductive cycle

discussed above would show that the different populations in different areas (the differences being coastal, latitudinal and locational) have specifically adopted to the environmental conditions prevailing in their situations. The influence of salinity on gametogenesis and spawning also appears to differ temporally in these populations.

Kuriakose (1973) observed spawning in *P. indica* at Vizhinjam from July to December with peak during August-November. Redevelopment of gonad after the above spawning is rare and therefore there is no secondary spawning in the species. According to Kuriakose (1973) the spawning period coincides with decrease in salinity (March-June range 34.14 - 35.68 ppt; July-December range 33.33 - 34.30 ppt) and the spawning peak goes with an increase in temperature. The same species, under raft culture, spawns from end of May till September with peak spawning during July-August (Appukuttan *et al.*, 1980). These authors also observed year-to-year variations in the seasonal cycles.

Rainfall would appear to act through the changes brought about in temperature and salinity and also through its influence on nutrient enrichment leading to phytoplankton blooms. Earlier workers on marine bivalve reproduction in India had not paid any attention to the role of nutrition in gametogenesis and spawning. Parulekar and Dalal (1980) associated this factor with growth and reproduction of *P. viridis*. In the present study on *P. viridis* from Kalthur and *P. indica* from Vizhinjam, the influence of phytoplankton

blooms on gametogenesis and spawning has been clearly shown. This correlation is more striking in the cold water and temperate species where phytoplankton production and blooms are limited to a few months in a year as has been seen in Acrocalanus irradians (Sastry, 1968) and Placocalanus magellanicus (Thompson, 1977).

The retting of coconut husk is brought about by the pectinolytic activity of micro-organisms, especially bacteria and fungi. During the process of retting large quantities of organic substances like pectin, pentosans, fat and tannins are liberated into the medium (Jayasankar, 1966). The noticeable features associated with retting are the offensive odour resembling that of hydrogen sulphide during decomposition of pectin, a rise in turbidity and temperature, gas formation, depletion of oxygen, reduction in productivity and colour change of the medium (Asis and Nair, 1976). The 'all-male' population observed in the coconut husk retting area may be due directly to the above pollution effects or indirectly to nutritional deficiency or a combination of both.

It has been seen that pea-crab infestation affects the normal nutritional balance of P. indica and also interferes with reproduction. Similar observations have been made earlier on M. edulis by Berner (1952) and Young (1960). The former observed partial or complete cessation of production of gametes due to larger pea-crab infestation. Silas and Alagarswami (1967) noticed conspicuous depressions caused by pea-crab on the gonad of Meretrix casta.

CHAPTER VI

GAMETE MORPHOLOGY AND PHYSIOLOGY

INTRODUCTION

The morphology of spermatids and sperms of Mytilus edulis was described by Franzen (1955) using light microscopy. Studies on the ultrastructure of sperm made progress with the work of Niijima and Dan (1965) on M. edulis and Boureart et al. (1965) on Perna perna. Idelman (1967) reviewed the work on the ultrastructure of the sperm of mussels. According to Boureart et al. (1965), the spermatozoa of Mytilus perna consists of an acrosome, a middle piece and a flagellum. The role of acrosome in fertilisation of M. edulis ova was discussed by Hauschka (1963), Niijima (1963) and Dan et al. (1972).

The structure of the egg of Mytilus was described by Humphreys (1962) and Dan (1962) and has been reviewed by Reverberi (1971). Longo and Anderson (1969) and Durfoot (1973) studied the heterogeneous aggregation of yolk bodies, lipid droplets and mitochondria in the ooplasm of mature egg. During the present investigation the gross morphology of the gametes of P. indica and P. viridis was studied. Preliminary observations were made on the ultrastructure of the sperm of P. indica using scanning electron microscopy.

Gamete viability is a vital aspect in the management of reproduction of the candidate species in aquaculture. The recent trend in commercial molluscan aquaculture is the hatchery production of seed where seed availability is the major constraint (Davis, 1969; Alagarswami, 1980; Silas, 1980).

One of the major problems in seed production is induced maturation and spawning (Loosanoff and Davis, 1963; Alagarswami *et al.*, 1981). Induced maturation, spawning and fertilisation efficiency have been dealt with by many workers (Loosanoff and Davis, 1963; Ino, 1972; Morse *et al.*, 1976; Alagarswami, 1980).

Literature on bivalve sperm and egg viability is limited. Some knowledge has been obtained through the works of Longo and Anderson (1969) on *M. edulis*, Wilson (1969) on *Xenostrobus securis* and Lough and Gonor (1971) on *Adula californiensis*. However, there have been several works on the effect of salinity and temperature on the metamorphosis and development of mussel larvae (Bayne, 1976 *a*; Hrs-Brenko, 1971).

In relation to the hatchery production of seed, the importance of cryopreservation of teleost sperms and eggs has been realised in recent times (Billard, 1981; Stoss and Donaldson, 1982). Literature on the cryopreservation of gametes of bivalves are very limited (Longwell and Stiles, 1968; Lannan, 1971 and Hughes, 1973). Realising the importance of such studies in aquaculture, experimental work was carried out during the present investigation, on gamete survival in different salinities and short-term preservation of sperms of the mussel species.

MATERIALS AND METHODS

Fully ripe mussels Perna indica and P. viridis were collected from the natural habitat and maintained in tanks containing normal sea water at ambient temperature. The eggs and sperms were collected in the natural spawnings in the laboratory and examined under phase contrast and dark field microscope. For SEM (Scanning Electron Microscopy) examination, the sperms of P. indica were fixed in buffered formaldehyde and post-fixed in 2 % osmium tetroxide. Fixation was done at low temperature (5°C) in darkness and the excess osmium tetroxide was removed by repeated washings in deionised water. A drop of this sperm suspension was transferred to a glass substratum, coated with gold and scanned in the Scanning Electron Microscope (PSEM 501).

For the experiments on gamete viability, clear and unpolluted sea water (sal. 32.7 ppt) collected from the open sea was filtered and used in preparing the experimental salinity levels. Lower salinities (20.6 and 27.1 ppt) were made up by diluting sea water with distilled water. Higher salinities (35.2, 38.7, 43.8 and 48.1 ppt) were prepared by concentrating the sea water in a deep-freeze for freezing out the fresh water. Although the experiments were carried out in ambient temperature, the range was controlled within 26-27°C by keeping the beakers in a water bath and frequently changing this water with stored tap water. Motility of the sperm was taken as the criterion in determining sperm viability. Males, during the experiment, naturally spawned in the laboratory and

the milt initially settled down. Using a pipette, 0.1 ml of the milt was drawn and introduced into each of the beakers containing 100 ml of experimental medium (different salinities). The sperms were dispersed uniformly by stirring. At 15 minutes intervals, 0.1 ml of sperm dilution was drawn from each of the beakers, placed on a glass slide and gently topped by a cover-glass. Under a magnification of 50, the sperms which showed motility were counted under 10 fields of view of the phase contrast microscope and the number of sperms per field of view was averaged. The sperms on slide were then fixed by adding formalin through a needle tip at the margin of cover-glass. Counting of the total sperms was done again under 10 fields and averaged. The percentage motility at different intervals was calculated as follows :

$$\frac{\text{Aver. No. of motile sperms per field of view}}{\text{Total No. of sperms per field of view}} \times 100$$

The eggs collected after spawning were suspended in sea water of different salinities in 100 ml beakers. Samples of eggs were withdrawn at 15 - 30 minute intervals from the suspension and allowed to fertilise with freshly spawned sperms. The viability of the eggs was calculated in terms of the percentage of eggs fertilized as indicated by the cleavage and further development at each salinity level.

Short-term preservation of sperms was experimented at 7°C in a refrigerator and 0°C in a deep-freeze. Sperms collected from fresh spawnings of mussel were transferred to sterilised test-tubes containing sterilised sea water

(sal. 32.7 ppt) and were immediately stored at the above temperatures. Mantle tissue in fully mature condition was cut and stored similarly. The preserved material was taken out at intervals (6 hours to 10 days), thawed under room temperature and examined for sperm motility under microscope.

RESULTS

Structure of Spermatozoa

Under light microscope, the sperm of both Perna indica and P. viridis appears as an oval body tapering to a narrow pointed anterior end (Pl. 7, B). Posteriorly, a delicate flagellum-like tail gives motility to the sperm. The sperm of P. indica, when scanned under the electron microscope, revealed four distinct parts namely, acrosome, nucleus, middle piece and tail (Pl. 7, C, D, E, F). The acrosome, which is anterior in position is funnel shaped with the 'cup' portion turned towards the nucleus and the 'stem' portion free anteriorly. The acrosome measures 9 µm in length. The nuclear mass is cylindrical with a slight bulge in the middle portion where it is the broadest. The nucleus is followed posteriorly by the mid-piece, at the base of which emerges the flagellum-like tail. The tail of the spermatozoa measures about 56 µm in length and 0.8 µm in diameter which is uniform throughout its length except at the base near the mid-piece where it is slightly broader (Pl. 7, E, F). The total length of sperm including the tail portion is about 70 µm.

PLATE 7

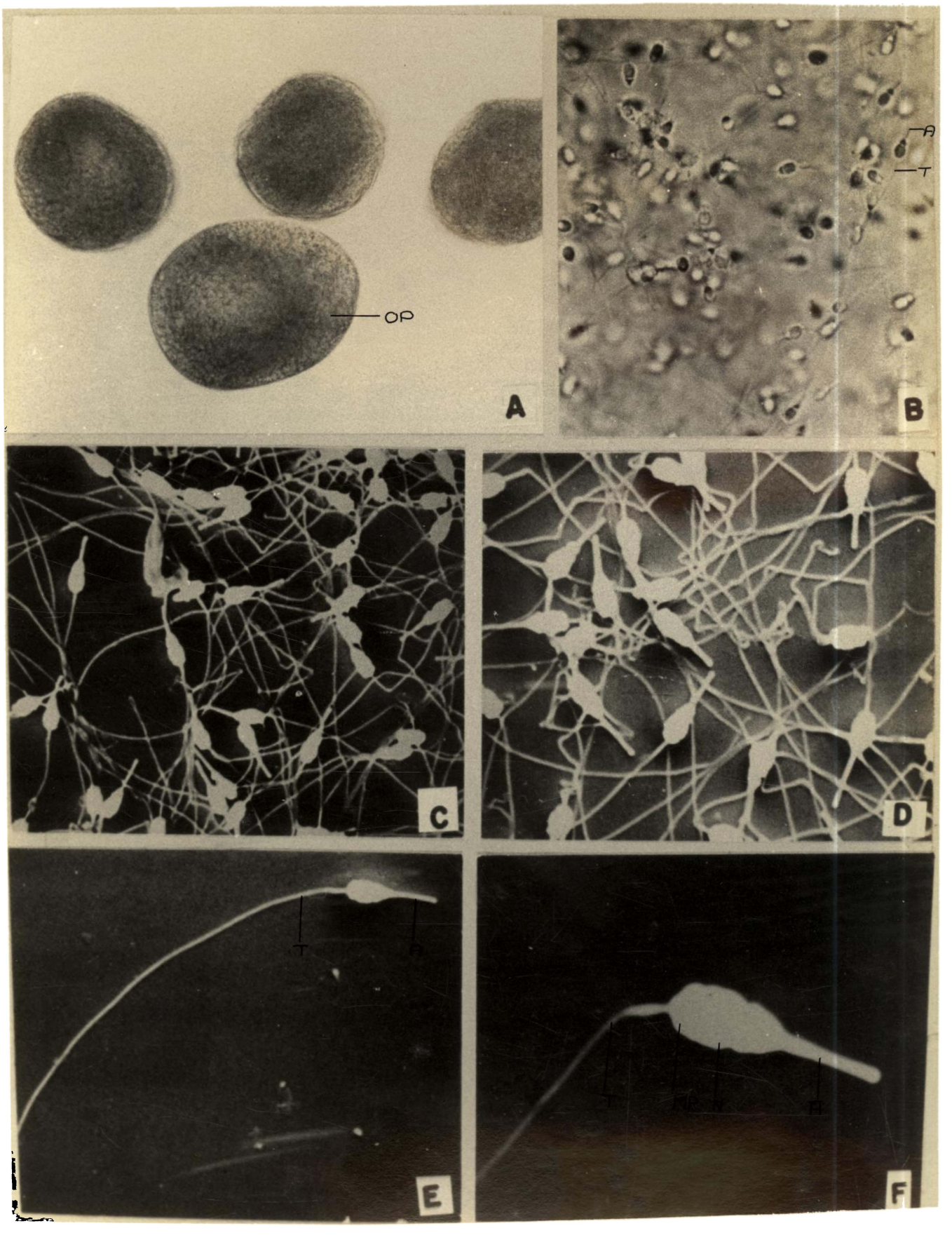
A. Spawned eggs of *Perna indica* (x 625).

B. Spermatozoa of *P. indica* viewed under light microscope (x 1500).

C-F Scanning Electron micrographs of *Perna indica* spermatozoa. (C x 2500; D x 3750; E x 3750; F x 5000).

A - Acrosome; MP - Middle piece; N - Nucleus; CP - Cytoplasm; T - Tail.

PLATE 7



Structure of egg

The fully mature follicular egg of both species is irregular in shape with a prominent germinal vesicle that occupies 1/3 of the egg mass (Pl. 5, D). Peripherally, the egg has a coat of vitelline membrane which is separated from the rest of the egg by a perivitelline space. The spawned eggs measure 50 - 60 µm in diameter. They are spherical in shape with the granulated ooplasm distributed throughout the egg mass except for a small transparent area in the middle of the egg (Pl. 7, A). The vitelline coat and perivitelline space are conspicuous and the eggs appear orange red in colour. The ooplasm contains lipid dropletz, yolk granules and other cytoplasmic organelles. Small vesicles have also been observed to occur in the periphery of the ooplasm.

Sperm viability in different salinities and durations

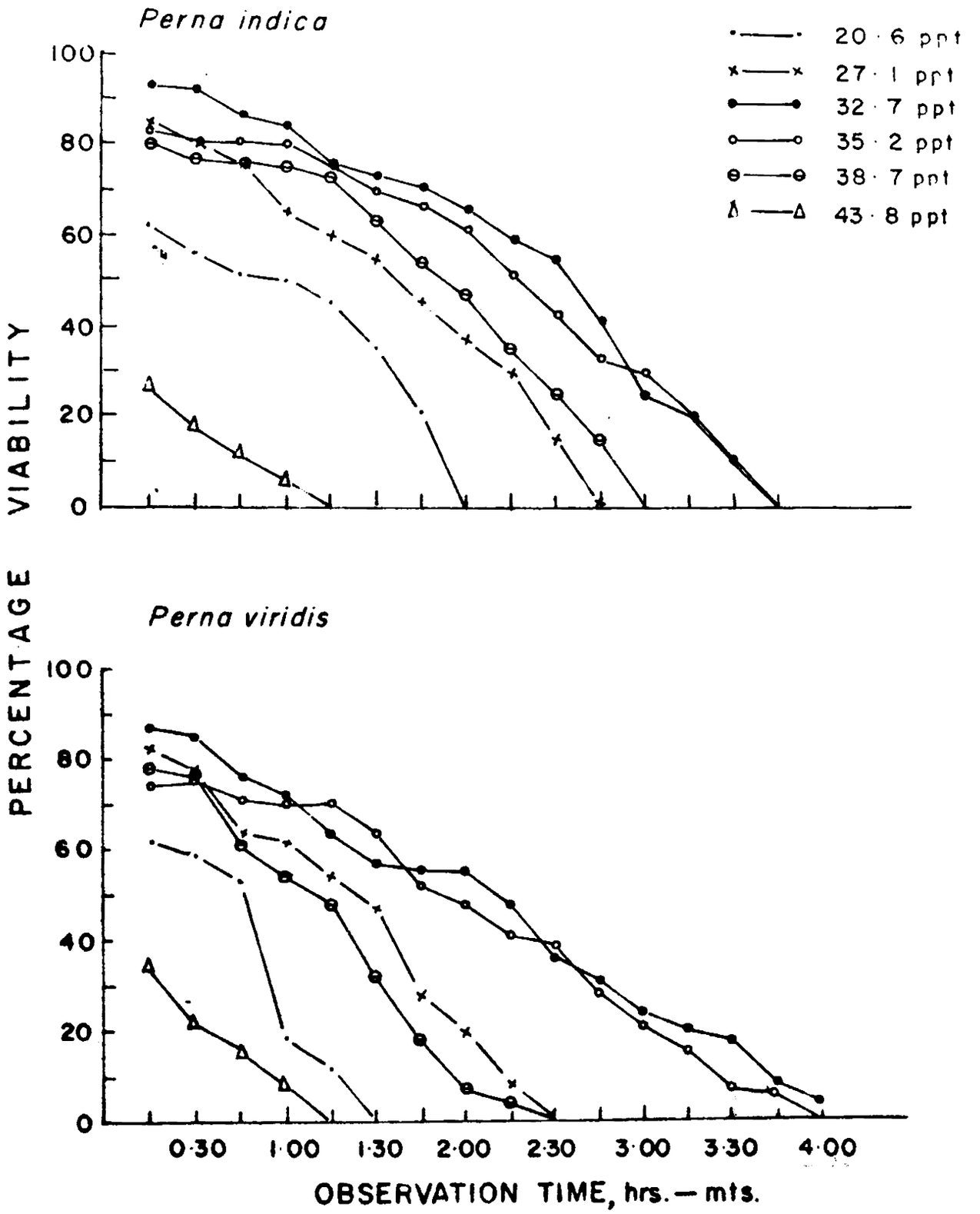
Perna indica

The experimental results on sperm motility in P. indica are presented in Table 8, Fig. 5. At the start of experiment majority of the sperms (62 - 93 %) were active in the salinity range 20.6 - 38.7 ppt; but later the percentage of motile sperms in all treatments decreased. The salinity of 32.7 and 35.2 ppt seemed to be the most suitable for the survival of sperms. At 20.6 ppt all the sperms became non-motile within 2 hours (h) and at 27.1 and 38.7 ppt total mortality was observed by the third hour. In the salinity of 43.8 ppt 26 % of the sperms were viable at 15 minutes (min) whereas at 48.1 ppt viability was nil. The 50 % sperm viability level was

Table 8 Sperm motility percentage in *Perna indica* at different salinities

Salinities ppt	20.6	27.1	32.7	35.2	38.7	43.8
Duration of experiment	Sperm motility %					
15 min	62	85	93	84	80	26
30 min	56	80	92	80	77	18
45 min	51	76	86	80	76	12
60 min	50	65	84	80	75	6
1 h 15 min	45	60	76	75	73	0
1 h 30 min	35	55	73	70	63	-
1 h 45 min	21	45	71	67	54	-
2 h	-	37	66	62	47	-
2 h 15 min	-	30	59	51	35	-
2 h 30 min	-	15	55	43	25	-
2 h 45 min	-	1	41	33	15	-
3 h	-	-	25	29	-	-
3 h 15 min	-	-	20	20	-	-
3 h 30 min	-	-	11	10	-	-
3 h 45 min	-	-	2	-	-	-

Fig 5 EFFECT OF SALINITY ON SPERM VIABILITY OF *PERNA INDICA* AND *PERNA VIRIDIS*



obtained approximately at 60 min in 20.6 ppt, 1 h 45 min in 27.1 ppt, 2 h 30 min in 32.7 ppt, 2 h 15 min in 35.2 ppt and 1 h 45 min in 38.7 ppt.

Perna viridis

The results for P. viridis are presented in Table 9, Fig. 5. At 15 min after collection the majority of the sperms (62 - 87 %) were motile in the salinity range 20.6 - 38.7 ppt. The percentage motility got reduced with lapse of time, the rate of decrease being dependant on the salinity of the medium (Fig. 5). At 20.6 ppt the percentage motility reduced to 43 % by 45th minute and further reduced to zero in 1 h 30 min. The reduction was comparatively gradual in the salinity level 27.1 ppt, though all the sperms became non-motile in 2 h 30 min. Compared to the above salinity levels, those at 32.7 and 35.2 ppt showed higher survival rate. About 5 % of the sperms were motile even after 4 h of exposure. At 38.7 ppt the sperm motility decreased rapidly; the number of motile sperms was nil at 2 h 30 min. At 43.8 ppt 34 % of the sperms were viable and total mortality occurred within one hour and no sperms survived at 48.1 ppt. The 50 % sperm viability level was obtained approximately at 30 min in 20.6 ppt, 1 h 15 min in 27.1 ppt, 2 h 15 min in 32.7 ppt, 2 h in 35.2 ppt and 60 min in 38.7 ppt.

Egg viability in different salinities and durations

P. indica

Fertilisation of eggs occurred at salinities from 20.6 to 43.8 ppt. But the rate of fertilisation differed

Table 9 Sperm motility percentage in *Perna viridis* at different salinities

Salinities ppt	20.6	27.1	32.7	35.2	38.7	43.8
Duration of experiment	Sperm motility %					
15 min	62	82	87	74	78	34
30 min	59	78	85	75	76	21
45 min	43	64	76	71	61	16
60 min	18	62	72	70	54	8
1 h 15 min	12	54	64	70	48	0
1 h 30 min	-	47	57	64	32	-
1 h 45 min	-	28	55	52	18	-
2 h	-	20	85	48	7	-
2 h 15 min	-	8	48	41	4	-
2 h 30 min	-	-	36	39	-	-
2 h 45 min	-	-	31	28	-	-
3 h	-	-	24	21	-	-
3 h 15 min	-	-	20	15	-	-
3 h 30 min	-	-	18	7	-	-
3 h 45 min	-	-	8	6	-	-
4 h	-	-	5	-	-	-

(Table 10, Fig. 6). At 20.6 ppt, the eggs were fertilised but further development was affected. At 27.1 ppt, 78 % of the eggs were fertilised when examined after 15 minutes of exposure. The percentage of viable eggs decreased gradually. In the salinity levels of 32.7, 35.2, 38.7 and 43.8 ppt the egg viability was 82 %, 87 %, 76 % and 49 % respectively. Reduction in the rate of fertilisation was noticed in all the cases but it was gradual in 35.2 ppt compared to the other three treatments. In 43.8 ppt the percentage viability of the eggs was 49 % to start with; but by the 4th hour all the eggs became non-viable. In 48.1 ppt fertilisation was nil. In none of the treatments, the eggs were viable after 7 hrs. The 50 % egg viability level was obtained approximately at 15 min in 20.6 ppt, 2 h 30 min in 27.1 ppt, 3 h 30 min in 32.7 ppt and 35.2 ppt and 1 h 30 min in 38.7 ppt.

Perna viridis

In the salinity 27.1, 32.7 and 35.2 ppt the eggs were viable upto 7 h and the percentage viability of the eggs was high in 32.7 ppt (15 %) (Table 11, Fig. 6). In 38.7 ppt and 43.8 ppt salinity level, eggs were viable upto 6 h and 5 h respectively. In the lower (20.6 ppt) and higher (48.1 ppt) salinity, fertilisation and further development of the eggs were poor. The percentage egg survival and fertilisation were high in the initial stages of observation (15 min) and then gradually reduced. The reduction was gradual at 30 - 38 ppt. The 50 % egg viability level was obtained approximately at 15 min in 20.6 ppt, 4 h in 27.1 ppt, 4 h 30 min in 32.7 ppt, 8 h in 35.2 ppt and 2 h 30 min in 38.7 ppt.

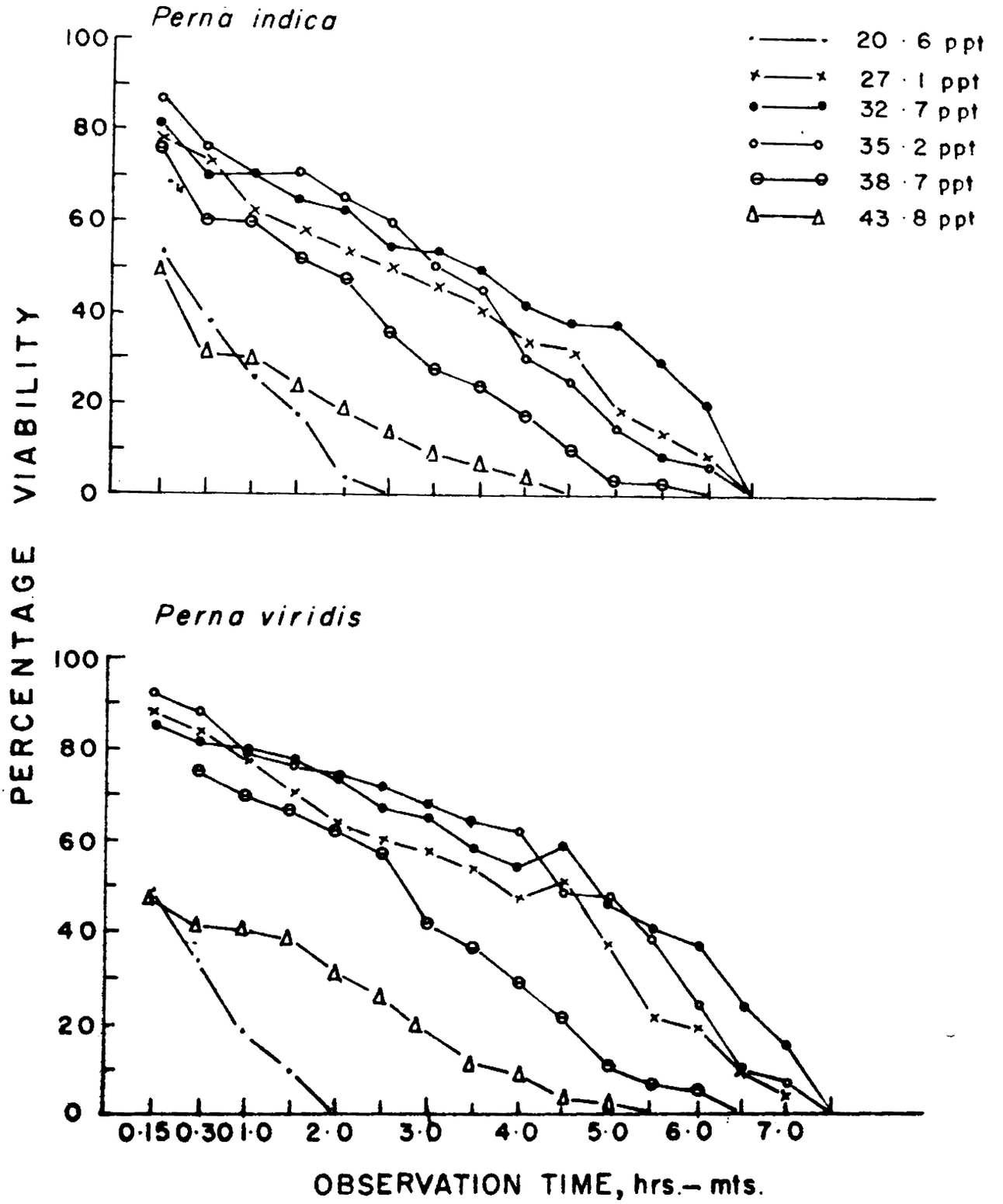
Table 10 Egg viability percentage in *Penna indica* at different salinities

Salinities ppt	20.6	27.1	32.7	35.2	38.7	43.8
Duration of experiment	Egg viability %					
15 min	54	78	82	87	76	49
30 min	38	73	70	76	60	31
1 h	26	62	70	76	60	30
1 h 30 min	18	58	65	71	52	24
2 h	4	54	63	71	48	19
2 h 30 min	0	50	55	65	36	14
3 h	-	46	54	60	28	9
3 h 30 min	-	41	50	50	24	7
4 h	-	34	42	45	18	4
4 h 30 min	-	32	38	30	10	-
5 h	-	19	38	25	4	-
5 h 30 min	-	14	29	15	3	-
6 h	-	9	20	9	-	-
6 h 30 min	-	-	-	7	-	-

Table 11 Egg viability percentage in *Perna viridis* at different salinities

Salinities ppt	20.6	27.1	32.7	35.2	38.7	43.8
Duration of Experiment	Egg viability %					
15 min	46	88	86	92	95	47
30 min	34	84	82	88	75	42
1 h	18	78	80	79	70	40
1 h 30 min	10	71	78	76	67	38
2 h	0	64	74	74	62	31
2 h 30 min	-	60	67	72	58	26
3 h	-	58	65	68	42	18
3 h 30 min	-	54	58	64	37	12
4 h	-	47	54	62	29	9
4 h 30 min	-	42	51	58	23	3
5 h	-	37	46	47	11	2
5 h 30 min	-	21	41	38	8	-
6 h	-	19	37	24	5	-
6 h 30 min	-	9	24	10	-	-
7 h	-	4	15	7	-	-

Fig 6 FFFECT OF SALINITY ON EGG VIABILITY OF *PERNA INDICA* AND *PERNA VIRIDIS*



Gamete preservation

Sperms preserved at 0°C and 7°C did not show any difference due to temperature. Upto 72 h, the preserved sperms showed normal motility on thawing. On day 5, the motility was very much reduced and, on day 10, none of the sperms were motile. These results are only indicative of sperm viability on preservation as they could not be confirmed by fertilization. While sperms preserved in the form of milt gave the above results, the mantle tissue preserved as such gave completely negative results.

Spawned eggs of both species of mussel were preserved in the same way as for the sperms. When removed after 6 h and thawed, they were not fertilised with addition of fresh sperms.

DISCUSSION

Kuriakose (1973) observed that the sperm of P. indica is small, pin shaped with an oval head measuring 5 - 7 µm and a vibratile tail 18 - 20 µm in length. The SEM examination shows a larger oval shaped sperm. The results of the present investigation agree with the observations made by Sourcart et al. (1965) on Mytilus perna with regard to structural details of nucleus, middle piece and tail. The light microscopic observations on the egg of P. indica and P. viridis, which are similar, agree with the findings of Kuriakose (1973). In the light microscope itself, the acrosome and midpiece were quite visible; the long tail could be made out on close examination. In the dark field microscopy the details could be observed

even more clearly. The detailed morphology was revealed in the SEM examination.

The gamete viability at 50 % level gives similar results at salinities 32.7 and 35.2 ppt but the viability gets reduced on either side of this range. The 50 % viability level appears better at 27.1 ppt than at 38.7 ppt. Bayne (1965) observed on M. edulis that fertilisation occurs successfully in the salinity range 15 - 40 ppt. In the lowest experimental salinity of 20.6 ppt, sperm viability appears relatively better than egg viability and in the high salinity of 43.8 ppt egg viability appears greater. However, these extremes are not met with in the coastal areas. The annual salinity range is 32.55 - 36.68 ppt at Vizhinjam and 35.40 - 40.50 at Elathur. During spawning season of P. indica the salinity range in the natural beds is 32.55 - 35.85 ppt and of P. viridis the range is 36.23 - 37.72 ppt. The experimental data have shown the high viability of gametes in the above ranges.

Perna viridis also occurs in estuarine conditions as in Bhatia creek in Ratnagiri and, as reported by Nagabhushanam and Mane (1975 a), the population spawns during July - August when the salinity is 3.0 - 9.0 ppt in July and 18.0 ppt in August. It would appear that this population has its own adaptation to have high gamete viability at these lower salinities. The salinity in August (18.0 ppt), when most of the spawning is completed by this population, is slightly above the minimum (15 ppt) of the fertilisation salinity range given by Bayne (1976 a).

In the present study the sperms were observed to survive to a maximum of 4 h and eggs upto 7 h. Longo and Anderson (1969) reported that in M. californianus the eggs remained viable for 4 - 6 h after spawning, while the sperms were found to lose motility in 1 - 2 hrs at 18°C.

Longwell and Stiles (1968), Lannan (1971) and Hughes (1973) worked with cryopreserved sperms of oyster species Crassostrea virginica and C. gigas in experimental fertilisation. Hughes (1973) used dimethylsulfoxide (DMSO) of 5 % and 10 % concentration as cryoprotectant and froze and stored the ampoules containing sperm suspension in liquid nitrogen. The short-term preservation experiments carried out during the present study are of a preliminary nature and need to be pursued further.

CHAPTER VII

BIOCHEMISTRY OF REPRODUCTION

INTRODUCTION

In marine mussels, as also in other bivalves, a discrete biochemical storage site as the adipose tissue is lacking, and the synthesised nutrients are stored in the somatic or reproductive organs (Giese, 1959). The process of gametogenesis demands high energy. The relationship between food availability (energy source) and gonad development has been studied in detail (Sastry, 1968; Bayne, 1975; Gabbott, 1975, 1976). When food is in abundance, the nutrients are assimilated and stored in different organs. This may happen during the non-reproductive period. While protein and lipid reserves are reported to accumulate in the non-mantle tissues of M. edulis, glycogen is observed to be stored in the mantle when abundant food is available (Gabbott and Bayne, 1973; Zandee et al., 1980). Obviously this leads to a 'nutrient storage cycle' related to the reproductive activity of the organisms (Gabbott, 1975, 1976; Bayne, 1976 a; Zandee et al., 1980 and Pieters et al., 1980).

The literature on seasonal biochemical variations in Mytilus edulis and M. galloprovincialis has been reviewed by Giese (1969), Bayne (1976 a) and Sastry (1979). The role of

lipids in the economy of the marine invertebrates was reviewed by Giese (1966). Goddard and Martin (1966) reviewed the carbohydrate metabolism and Florkin (1966) the nitrogen metabolism of molluscs. The general observations have been that the amounts of carbohydrates, proteins and lipids increase as gonad development proceeds and then declines following spawning (Gabbott, 1975, 1976; Sastry, 1979; Zandee et al., 1980).

Seasonal changes in the body weight in relation to reproductive activity have been reported in Tellina tenuis (Ansell and Trevallion, 1967); M. edulis (Zandee et al., 1980) and Pecten maximus (Comely, 1974). Dry weight variations are mainly due to variations in the carbohydrate or glycogen content, which are the important energy reserves, as observed in M. edulis (Gabbot, 1976) and M. viridis (Mane and Nagabhushanam, 1975).

Gabbott and Bayne (1973), Gabbott (1975), Bayne (1976 a) and Bayne et al. (1982) reported the utilisation of glycogen reserves in adult mussels during gametogenesis. Synchrony between glycogen breakdown and vitellogenesis has been observed in M. edulis by Lubet (1959). The initiation of gametogenesis is followed by a period of vitellogenesis and a storage loss of carbohydrate in M. edulis (Sastry, 1979; Zandee et al., 1980; Pieters et al., 1980). Ansell et al. (1964) and Ansell and Lander (1967) have observed reciprocal relationship between carbohydrates and proteins throughout the year following reproductive activity in Mercenaria mercenaria.

While studying the effect of starvation on the digestive gland of M. edulis, Thompson et al. (1974) found distinct changes in the weight and biochemical composition of the tissue. The increase of lipid level in the stage III animals is reported to be due to the conversion of the pre-stored glycogen into lipid material in the egg in the case of female (Pieters et al., 1980; Zandee et al., 1980). In Placopecten magellanicus, however, Thompson (1977) did not observe any mobilisation of energy reserves from the somatic to the reproductive tissue during growth and maturation.

Zandee et al. (1980) observed in M. edulis that while the lipid content in the body remained constant until spawning, protein and glycogen declined during maturation. Pieters et al. (1980) observed fluctuations in the protein, glycogen and lipid content in M. edulis with repeated shedding of gametes.

The biochemical composition of the egg of M. edulis was reported by Bayne et al. (1975) and Pieters et al. (1980). The effect of different stress conditions, in the egg of M. edulis was studied by Bayne, Gabbott and Widdows (1975). Striking change in the biochemical content of the eggs under stress was found to be in their lipid contents.

Most of the above studies on reproduction - related biochemical changes in mussel are in temperate species of Mytilus and very little work has been done in tropical species. Mane and Nagabhushanam (1975) and Nagabhushanam and Mane (1978) described the annual reproductive cycle in relation to biochemical variations in M. viridis. Wafer et al. (1976)

estimated the biochemical changes with respect to nutrient value of green mussel P. viridis. The proximate composition of mussel meat has been studied with reference to processing (Muraleedharan et al., 1982). A comprehensive investigation on the seasonal biochemical variations in the mantle, adductor muscle and digestive gland as related to the reproductive cycle in the two species of Indian sea mussel Perna indica and P. viridis has been made and the results are reported here.

MATERIALS AND METHODS

The fortnightly samples of P. indica and P. viridis from the mussel beds of Vizhinjam and Elathur, respectively, were deep-frozen and transported to the laboratory at Cochin in hotlined flasks (Chapter II). Mussels 45 - 60 mm in length and in the same reproductive stage were selected for biochemical investigation. The two fortnightly samples in a month were pooled and 10 - 30 animals were drawn from the pooled sample for biochemical estimations. Monthly mean values were derived from the data.

For the dry weight and water content estimation of whole body, 40 % of the animals in the selected sample were used. The soft body was removed entirely from the shell. The tissues were blotted and weighed immediately to obtain wet weight. The tissues were dried to constant weight at 90 ± 5°C (for 24 to 36 h) in a hot air oven and weighed (dry weight) after cooling in a desiccator over P_2O_5. The percent water content was calculated from the above data. For the biochemical

estimation on different organs the remaining 50 % of the animals in the selected sample were utilised. The mantle, digestive gland and adductor muscle were excised, cleaned with double distilled water to remove any adhering salts, and the wet weight, dry weight and water content determined as described above.

The glycogen content of the mantle, digestive gland and adductor muscle was determined by the method of Kemp et al. (1954). The tissue was powdered to obtain 15 - 20 mg of each and the amount of glycogen was determined as percentage of dry weight.

The protein content of the mantle, digestive gland and adductor muscle was determined by Lowry's method (Lowry et al., 1951). The dry tissues were treated with 10 % TCA to precipitate the protein. The precipitate was dissolved in 1 N sodium hydroxide and the amount of protein determined using folin-ciocalteu reagent. The optical density measurements were taken on Spectronic - 20. The protein content of the samples was determined from the optical density using a standard graph prepared using Bovine serum albumin. The protein content was calculated as percentage of dry weight of the organ.

The procedure recommended by Folch, Lees and Sloane Stanley (1957) was used to estimate the amount of lipids. The dry powdered samples (30 - 50 mg) were homogenised with 25 ml of 2:1 (v/v) chloroform:methanol of analar grade. The total lipids were subsequently isolated from the

chloroform-methanol after evaporating the solvent and drying the residue over P_2O_5 overnight. The lipid content was determined as percentage of dry weight.

The digestive gland index (DGI) was calculated on dry weight basis of the digestive gland to that of the mantle-free body dry weight (Thompson et al., 1974).

RESULTS

The monthly mean values of the biochemical constituents of the body tissues of Perna indica and P. viridis are given in Tables 12 to 20 and Figs. 7 to 15. For April-August 1982 in P. indica and October-December 1981 and July-October 1982 in P. viridis, the values for male and female are given separately. For the other months, the values stand for the sexually indeterminate animals.

Perna indica

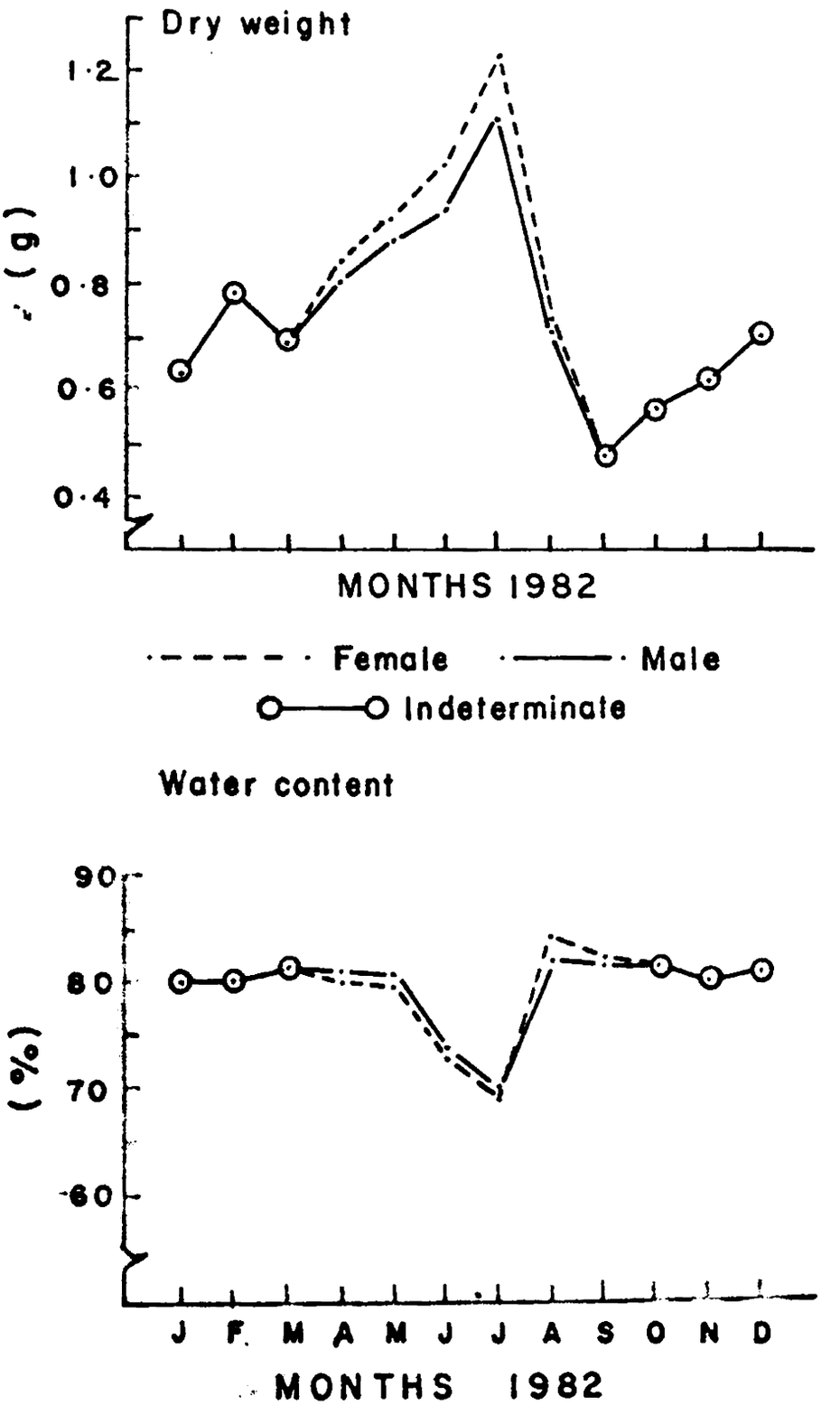
Whole body

Dry weight: The seasonal variations in the dry weight of the whole body of P. indica are presented in Table 12 and Fig. 7. Differences in dry weight were distinct between the non-reproductive and reproductive phase. In the former phase, the values ranged 0.584 - 0.788 g during October 1981-March 1982 and 0.486 - 0.716 g during September-December 1982. It is also observed that there was consistent and gradual increase in dry weight from post-spawning to

Table 12 Stages of maturity, body dry weight and percentage water content in *Perna indica*

Month	Dominant maturity stage	Sex	Body dry wt (g)	Water content %
1981				
Oct	0	-	0.584 ± 0.117	81.76 ± 3.71
Nov	0	-	0.592 ± 0.093	82.18 ± 2.74
Dec	0	-	0.598 ± 0.203	79.48 ± 3.16
1982				
Jan	0	-	0.632 ± 0.116	80.26 ± 2.24
Feb	0	-	0.788 ± 0.136	80.32 ± 2.74
Mar	0	-	0.684 ± 0.125	81.48 ± 3.16
Apr	1	M	0.796 ± 0.102	80.69 ± 3.86
		F	0.842 ± 0.097	80.18 ± 3.72
May	2	M	0.886 ± 0.094	80.53 ± 2.86
		F	0.902 ± 0.214	79.64 ± 4.32
Jun	3	M	0.934 ± 0.089	73.78 ± 2.14
		F	1.021 ± 0.148	72.93 ± 2.76
Jul	3	M	1.112 ± 0.080	70.16 ± 2.30
		F	1.230 ± 0.117	69.28 ± 1.13
Aug	4	M	0.719 ± 0.168	82.17 ± 1.45
		F	0.731 ± 0.182	84.23 ± 2.74
Sep	0	-	0.486 ± 0.209	81.49 ± 3.06
Oct	0	-	0.571 ± 0.307	81.53 ± 3.21
Nov	0	-	0.623 ± 0.184	80.24 ± 3.78
Dec	0	-	0.716 ± 0.093	81.26 ± 3.12

Fig. 7 SEASONAL VARIATION IN THE BODY DRY WEIGHT AND PERCENT WATER CONTENT OF *PERNA INDICA*



pre-gametogenic phase. In the reproductive phase, the dry weight increased steadily from 0.796 g in April 1982 to 1.112 g in July 1982 for males, and from 0.842 g to 1.230 g in July 1982 for females, corresponding with increasing reproductive activity from stage-1 to stage-3. In August 1982, the values decreased with peak spawning activity.

Water content: Data on percentage of water content of the animal are presented in Table 12 and Fig. 7. The water content was high in the non-reproductive phase as compared to the reproductive phase. It ranged 79.48 - 82.18 % during October 1981-March 1982 and 80.24 - 81.53 % during September-December 1982, all data relating to non-reproductive phase. During April-July 1982, corresponding to initiation of gametogenesis to maturation, the values were low, and in the decreasing order, from 80.69 % to 70.16 % for males and from 80.18 % to 69.28 % for females. During stage-4 (August 1982), the values started increasing with 84.23 % for female, being the highest value.

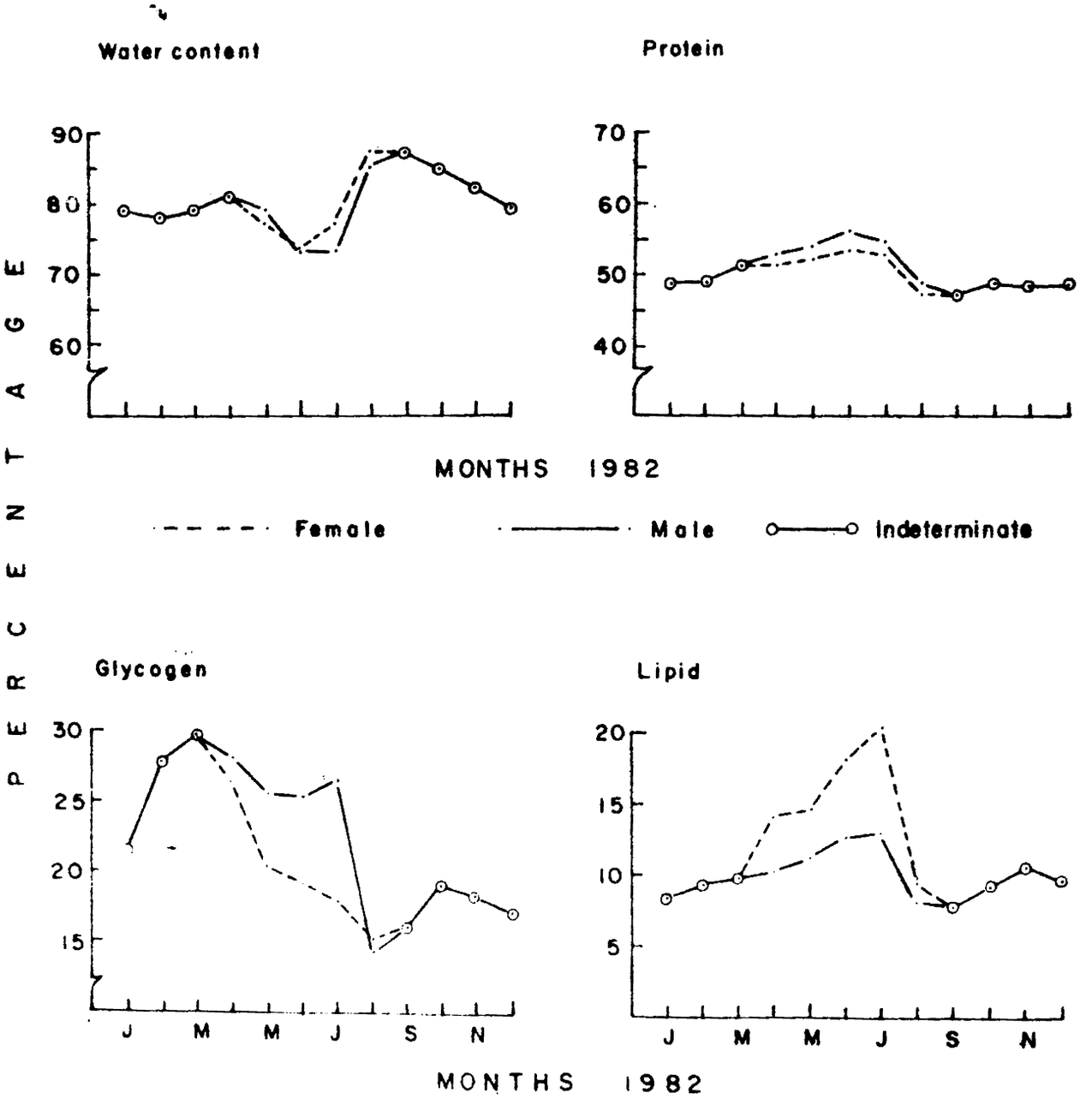
Mantle tissue

Water content: The monthly mean values of water content of the mantle are given in Table 13. The amount of water in the mantle of the males varied from 72.81 % to 85.16 % and that of females from 73.62 % to 87.24 % during the 15 months of observations. As presented in Fig. 8, the water content decreased May-July when the animals were in stages-2 and 3. Following spawning in August the water content increased further.

Table 13 Seasonal variations in the percentage water content, protein, glycogen and lipids in the mantle tissue of the mussel *Perna indica*

Month	Dominant maturity stage	Sex	Water content	Protein	Glycogen	Lipid
1981						
Oct	0	-	80.84 ± 1.81	48.06 ± 1.72	14.24 ± 1.49	9.83 ± 1.50
Nov	0	-	80.11 ± 2.16	47.32 ± 1.36	15.16 ± 1.07	8.54 ± 0.61
Dec	0	-	78.63 ± 1.74	49.71 ± 2.72	17.30 ± 1.59	8.72 ± 0.59
1982						
Jan	0	-	79.42 ± 2.84	48.20 ± 3.15	21.60 ± 1.83	8.34 ± 0.94
Feb	0	-	78.24 ± 3.47	46.28 ± 3.42	27.50 ± 0.95	9.12 ± 1.94
Mar	0	-	78.86 ± 2.81	51.02 ± 1.87	29.86 ± 1.58	9.60 ± 1.45
Apr	1	M	80.91 ± 1.74	52.50 ± 1.34	28.40 ± 1.47	10.10 ± 0.59
		F	79.12 ± 2.68	51.18 ± 1.23	26.50 ± 1.58	14.25 ± 1.62
May	2	M	79.24 ± 3.14	53.90 ± 1.12	25.50 ± 1.12	11.06 ± 0.94
		F	77.16 ± 2.68	62.34 ± 1.04	20.18 ± 1.26	14.80 ± 1.53
Jun	3	M	72.81 ± 2.42	55.51 ± 2.39	25.13 ± 1.57	12.33 ± 1.73
		F	73.62 ± 3.17	53.22 ± 1.67	18.64 ± 1.49	18.24 ± 1.07
Jul	3	M	73.28 ± 3.26	54.25 ± 1.89	26.94 ± 1.39	12.67 ± 2.28
		F	77.18 ± 1.43	52.88 ± 2.58	17.62 ± 1.78	20.72 ± 2.17
Aug	4	M	85.16 ± 3.38	48.64 ± 2.15	14.20 ± 1.62	8.16 ± 0.95
		F	87.24 ± 1.97	47.09 ± 3.15	15.18 ± 1.83	9.24 ± 1.57
Sep	0	-	86.98 ± 4.39	46.92 ± 3.79	16.12 ± 1.62	8.42 ± 1.37
Oct	0	-	84.70 ± 3.52	48.54 ± 2.68	18.78 ± 0.50	9.34 ± 1.32
Nov	0	-	82.37 ± 4.23	48.24 ± 3.88	18.37 ± 1.62	10.56 ± 0.95
Dec	0	-	79.48 ± 1.29	46.55 ± 2.73	17.38 ± 1.35	9.62 ± 1.93

Fig. 8 SEASONAL VARIATIONS IN WATER CONTENT AND BIOCHEMICAL COMPOSITION OF MANTLE TISSUE OF *PERNA INDICA*



Protein: The protein level in the mantle changed remarkably in relation to the reproductive activity of the animal (Table 13). It varied from 47.09 % to 53.22 % in the females and from 48.64 % to 55.51 % in the males. An increase in the protein content was evident with sex differentiation and maturation (Fig. 8). Between July and September 1982 the protein content decreased drastically and reached 46.92 % during September. During the non-reproductive period the relative proportion of protein was less than 50 %.

Glycogen: The glycogen level started accumulating in the gonad tissue considerably from January to March when the animals were immature, but as gametogenesis started the glycogen level declined drastically and further reduced as spawning commenced (Table 13, Fig. 8). The glycogen was minimum in August-September during which the majority of the mussels were in the spent condition. Similar reduction in the glycogen content was observed in the previous year also (October-November 1981). The total range of glycogen content was from 14.24 % in October 1981 to 29.86 % in March. The glycogen content of the mantle in females was comparatively lower than that in the males. In July the glycogen content of the males was 26.94 % while that of females was 17.62 %.

Lipid: The lipid content of the mantle was also related to the maturity stages of the animals (Table 13, Fig. 8). The lipid level increased from April to July and reached a peak in mature mussels by June-July with 12.67 % in males and 20.72 % in females. The lipid level decreased to

8.16 % in males and 9.24 % in females following spawning and began to build up after September.

Adductor muscle

Water content: The percentage water content of the adductor muscle showed only minor variations throughout the period of observation (Fig. 9). The range of water content was 71.24 - 78.18 % in males, 73.68 - 77.09 % in the females and 69.86 - 76.42 % in indeterminates (Table 14). The percentage of water was high during the period of spawning.

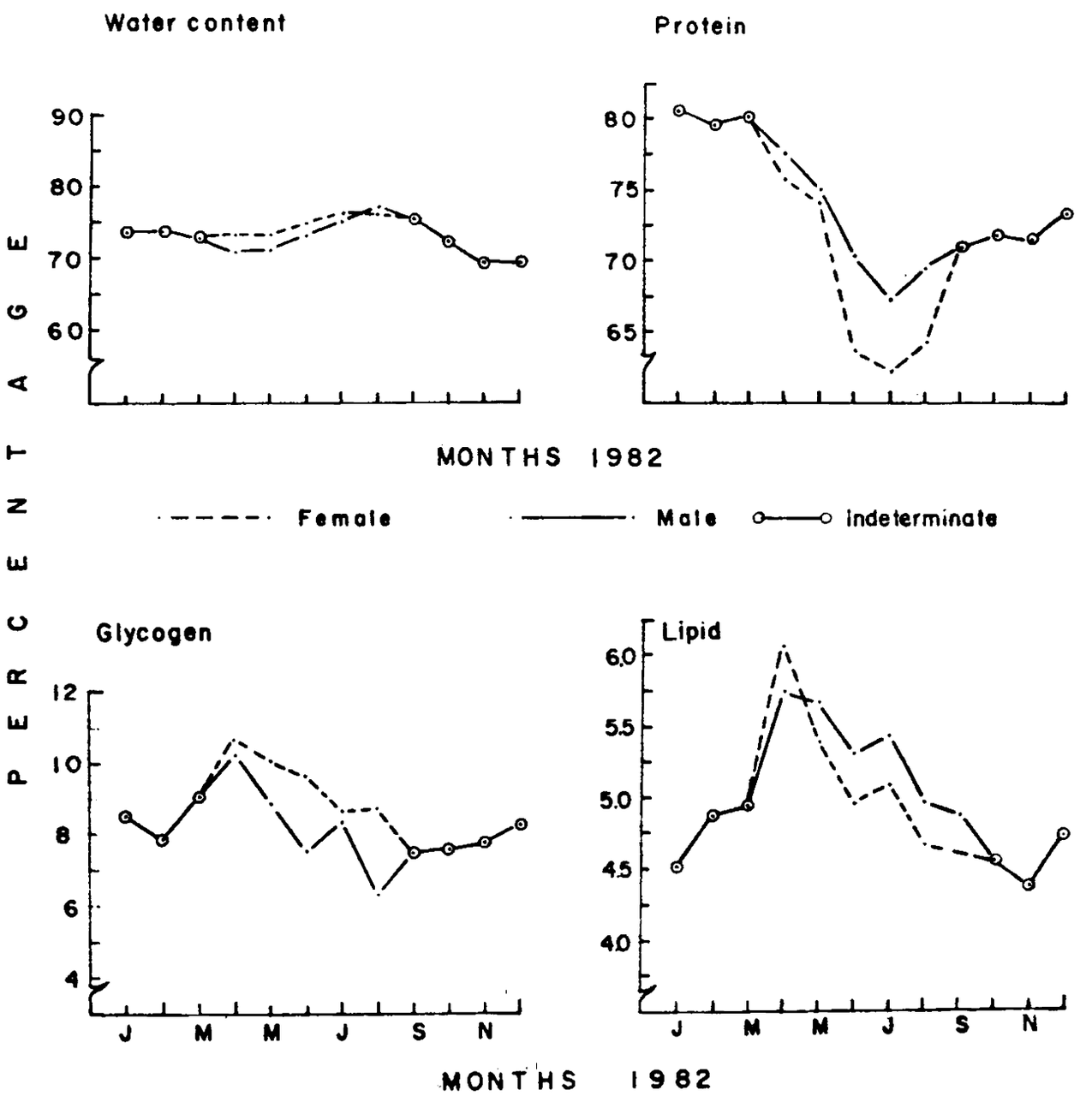
Protein: Protein level was comparatively higher in the adductor muscle than any other tissue in the body of the mussel. Also it was higher in the non-reproductive period. It gradually increased from October 1981 to March 1982 followed by a reduction to a minimum of 67.25 % in males and 62.16 % in females in July (Table 14, Fig. 9). The seasonal variations in protein level are correlated with the reproductive activity of the animal. As the protein content decreased, the adductor muscle was found to lose its colour and texture.

Glycogen: The glycogen level in the adductor muscle was comparatively low (Table 14). The maximum glycogen level was noticed in April (10.36 % in males and 10.71 % in females). After April the glycogen level gradually decreased and reached 6.31 % in males and 8.73 % in females by August 1982 (Fig. 9). After spawning, the level of glycogen in the adductor muscle was found to increase.

Table 14 Seasonal variations in the percentage water content, protein, glycogen and lipids in the adductor muscle of the mussel Perna indica.

Month	Dominant maturity stage	Sex	Water content	Protein	Glycogen	Lipid
1981						
Oct	0	-	70.48 ± 1.19	73.18 ± 2.04	9.81 ± 1.72	4.32 ± 0.75
Nov	0	-	71.32 ± 2.86	77.20 ± 3.04	8.80 ± 1.21	4.14 ± 0.96
Dec	0	-	72.11 ± 2.21	80.10 ± 2.17	8.65 ± 1.15	4.16 ± 0.48
1982						
Jan	0	-	73.62 ± 1.76	80.84 ± 3.11	8.54 ± 1.38	4.52 ± 0.76
Feb	0	-	73.92 ± 3.34	79.84 ± 4.36	7.90 ± 1.42	4.86 ± 0.65
Mar	0	-	72.84 ± 2.57	80.16 ± 3.16	9.04 ± 1.24	4.92 ± 0.71
Apr	1	M	71.24 ± 1.68	77.57 ± 3.86	10.36 ± 1.31	5.74 ± 0.36
		F	73.68 ± 2.13	75.68 ± 3.30	10.71 ± 0.94	6.12 ± 0.43
May	2	M	71.72 ± 3.76	75.00 ± 3.32	8.86 ± 1.52	5.68 ± 1.67
		F	73.61 ± 4.11	74.27 ± 2.96	10.01 ± 1.30	5.37 ± 0.56
June	3	M	73.71 ± 3.11	70.16 ± 3.96	7.50 ± 1.72	5.31 ± 0.34
		F	74.81 ± 2.74	63.87 ± 3.65	9.68 ± 1.31	4.96 ± 0.30
July	3	M	75.48 ± 2.96	67.25 ± 1.73	8.40 ± 1.35	5.46 ± 0.69
		F	76.81 ± 2.01	62.16 ± 2.55	8.58 ± 1.38	5.13 ± 0.84
Aug	4	M	78.18 ± 2.13	69.42 ± 3.40	6.31 ± 1.32	4.94 ± 0.49
		F	77.09 ± 1.98	64.37 ± 2.57	8.73 ± 1.26	4.68 ± 0.94
Sep	0	-	76.42 ± 1.14	71.19 ± 3.31	7.46 ± 1.46	4.84 ± 0.84
Oct	0	-	73.33 ± 1.96	71.82 ± 2.71	7.52 ± 1.26	4.57 ± 0.39
Nov	0	-	69.86 ± 2.44	71.61 ± 1.87	7.76 ± 0.32	4.39 ± 0.24
Dec	0	-	70.17 ± 2.86	73.39 ± 1.61	8.21 ± 0.71	4.74 ± 0.32

Fig 9 SEASONAL VARIATIONS IN WATER CONTENT AND BIOCHEMICAL COMPOSITION OF ADDUCTOR MUSCLE OF *PERNA INDICA*



Lipid: The lipid level was comparatively low in the adductor muscle than in other tissues. The amount ranged from 4.94 % to 5.74 % in males and 4.68 % to 6.12 % in females (Table 14). The lipid level was relatively high during reproductive phase (Fig. 9).

Digestive gland

Water content: The water content of the digestive gland did not show much noticeable fluctuations in response to the reproductive stages of the animal (Fig. 10). There was a slight increase in water content during July-August associated with spawning phase (Table 15).

Protein: The range of protein content was 59.46 - 62.66 % during the non-reproductive phase, 61.40 - 67.23 % in males and 60.97 - 65.11 % in females. In the reproductive phase there was reduction in protein content from gametogenesis to spawning (Table 15, Fig. 10).

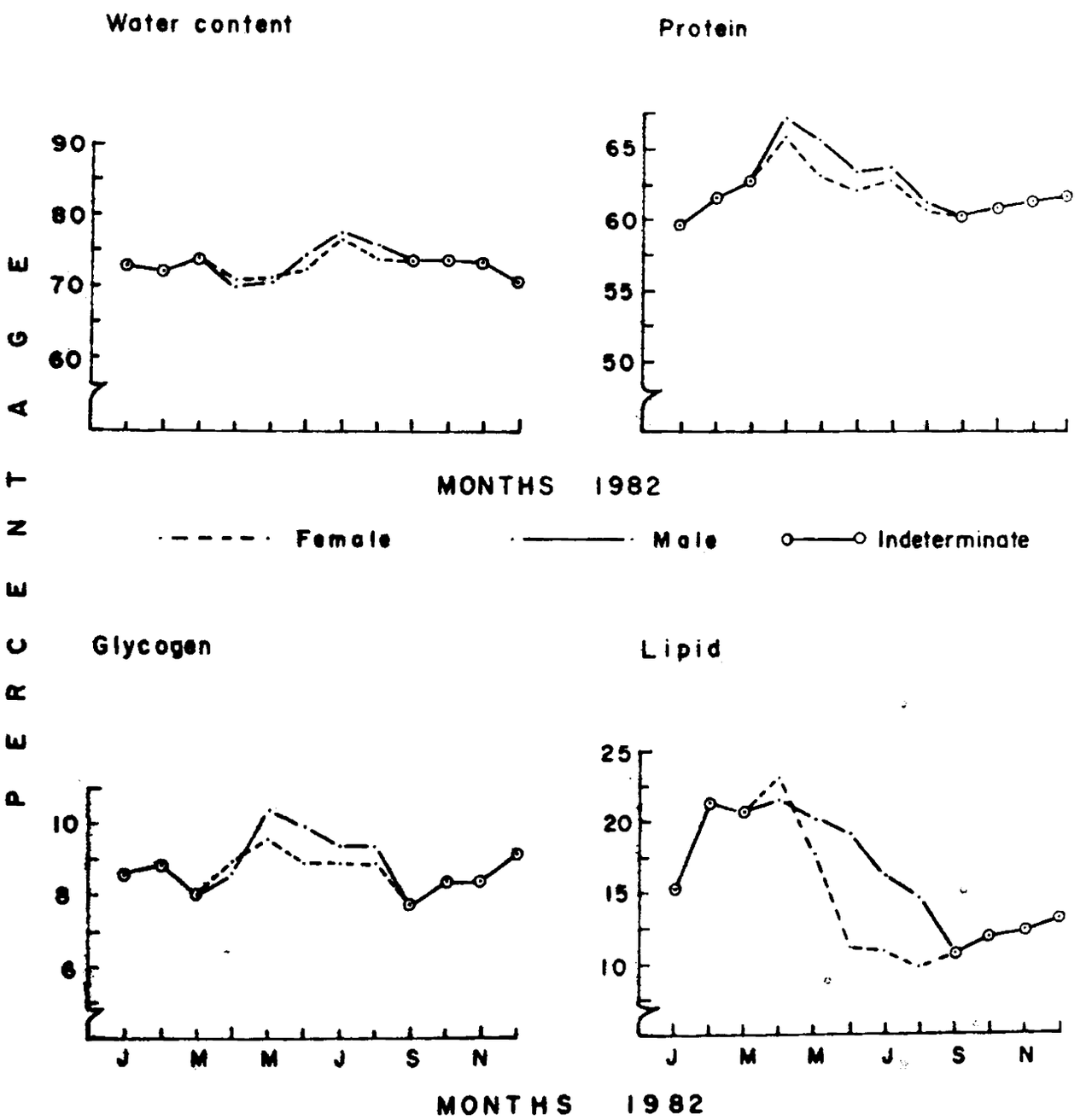
Glycogen: The glycogen content was the highest in May associated with gametogenesis. It got progressively reduced during maturation and spawning. In the non-reproductive phase, the glycogen content fluctuated between 6.83 - 9.17 % (Table 15, Fig. 10).

Lipid: The lipid content of the digestive gland of the mussel showed significant variation in relation to maturation and spawning (Fig. 10). The lipid level increased from 13.16 % in October 1981 to 21.82 % in males and 23.31 % in females in April 1982 (Table 15). With increase in

Table 15 Seasonal variations in the percentage water content, protein, glycogen and lipids in the digestive gland of the mussel *Perna indica*

Month	Dominant maturity stage	Sex	Water content	Protein	Glycogen	Lipid
1981						
Oct	00	-	70.63 ± 1.72	59.81 ± 1.58	7.62 ± 0.95	13.16 ± 2.14
Nov	0	-	69.84 ± 1.82	59.74 ± 1.09	6.83 ± 0.60	13.08 ± 1.43
Dec	0	-	68.52 ± 2.06	59.46 ± 1.80	8.81 ± 0.89	14.05 ± 1.51
1982						
Jan	0	-	73.34 ± 1.43	59.68 ± 2.19	8.52 ± 0.84	15.16 ± 1.09
Feb	0	-	72.18 ± 2.98	61.74 ± 1.75	8.79 ± 0.72	21.20 ± 1.51
Mar	0	-	74.37 ± 1.95	62.66 ± 0.98	8.00 ± 1.13	20.94 ± 2.04
Apr	1	M	69.82 ± 2.72	67.23 ± 2.07	8.58 ± 0.67	21.82 ± 1.14
		F	72.28 ± 1.43	65.11 ± 2.81	8.94 ± 0.48	23.31 ± 1.93
May	2	M	70.81 ± 1.76	65.09 ± 2.22	10.46 ± 1.56	20.43 ± 2.01
		F	71.29 ± 1.84	63.00 ± 2.84	9.53 ± 0.91	17.54 ± 1.81
June	3	M	74.70 ± 3.48	63.42 ± 1.42	9.94 ± 0.67	19.18 ± 1.15
		F	72.61 ± 2.37	62.37 ± 1.95	8.97 ± 0.75	11.21 ± 2.01
July	3	M	78.24 ± 2.23	63.84 ± 1.93	9.35 ± 0.66	16.51 ± 1.43
		F	77.19 ± 2.48	62.61 ± 2.44	8.81 ± 0.71	10.82 ± 2.08
Aug	4	M	76.38 ± 1.81	61.40 ± 1.25	9.31 ± 0.91	14.84 ± 1.76
		F	74.44 ± 2.04	60.97 ± 2.66	8.87 ± 0.78	9.87 ± 2.11
Sep	0	-	74.14 ± 1.74	60.12 ± 1.94	7.72 ± 0.66	10.93 ± 1.86
Oct	0	-	73.92 ± 1.78	61.20 ± 1.52	8.37 ± 0.57	11.95 ± 1.51
Nov	0	-	74.32 ± 1.75	61.18 ± 1.22	8.37 ± 0.71	12.34 ± 0.98
Dec	0	-	70 98 ± 1.98	61.90 ± 1.62	9.17 ± 0.55	13.33 ± 1.83

Fig 10 SEASONAL VARIATIONS IN WATER CONTENT AND BIOCHEMICAL COMPOSITION OF DIGESTIVE GLAND OF *PERNA INDICA*



reproductive activity the lipid level gradually decreased to 14.84 % in males and 9.87 % in females in August.

Perna viridis

Whole body

Dry weight: The data on dry weight of *P. viridis* are presented in Table 16, Fig. 11. The monthly mean ranged from 0.448 g in January 1982 (post-spawning) to 1.104 g in August 1982 (active gametogenesis). There was a steady increase from January through June during the indeterminate phase and further increase upto September coinciding with gamete maturation. With spawning in October, the dry weight drops down by about 50 %. Females showed slightly higher values than males.

Water content: High water content was observed from December 1981 to May 1982 and from October to December 1982 (80.14 - 85.02 %), coinciding with the spawning, recovery and indeterminate stages of the animal (Table 16, Fig. 11). With the onset of gametogenesis, water content decreased and with maturation it decreased further (68.74 % in November 1981). The males generally had a higher water content than the females.

Mantle

Water content: In the indeterminate phase during January-June, the water content was high (73.57 - 84.28 %) (Table 17, Fig. 12). During gametogenesis and maturation

Table 16 Stages of maturity, total body dry weight and percentage water content in *Perna viridis*

Month	Dominant maturity stage	Sex	Whole body dry weight (g)	Water content %
1981				
Oct	3	M	0.782 ± 0.216	73.17 ± 2.18
		F	0.834 ± 0.119	69.72 ± 1.74
Nov	3	M	0.893 ± 0.256	72.19 ± 1.96
		F	0.940 ± 0.150	68.74 ± 1.57
Dec	4	M	0.512 ± 0.196	85.02 ± 3.17
		F	0.483 ± 0.206	83.49 ± 2.76
1982				
Jan	0	-	0.448 ± 0.120	84.58 ± 3.76
Feb	0	-	0.534 ± 0.176	84.87 ± 2.94
Mar	0	-	0.682 ± 0.217	81.98 ± 3.82
Apr	0	-	0.629 ± 0.182	82.02 ± 1.86
May	0	-	0.715 ± 0.113	80.14 ± 2.39
Jun	0	-	0.817 ± 0.146	72.24 ± 8.71
Jul	1	M	0.993 ± 0.107	73.11 ± 1.29
		F	1.023 ± 0.096	70.90 ± 2.11
Aug	2	M	0.925 ± 0.231	71.84 ± 2.38
		F	1.104 ± 0.231	71.84 ± 2.38
Sep	3	M	1.030 ± 0.075	69.84 ± 1.92
		F	1.101 ± 0.112	68.70 ± 2.10
Oct	4	M	0.522 ± 0.284	84.01 ± 1.77
		F	0.596 ± 0.147	80.35 ± 1.98
Nov	0	-	0.578 ± 0.112	80.38 ± 3.38
Dec	0	-	0.728 ± 0.189	80.74 ± 4.82

Fig. 11 SEASONAL VARIATION IN THE BODY DRY WEIGHT AND PERCENT WATER CONTENT OF *PERNA VIRIDIS*

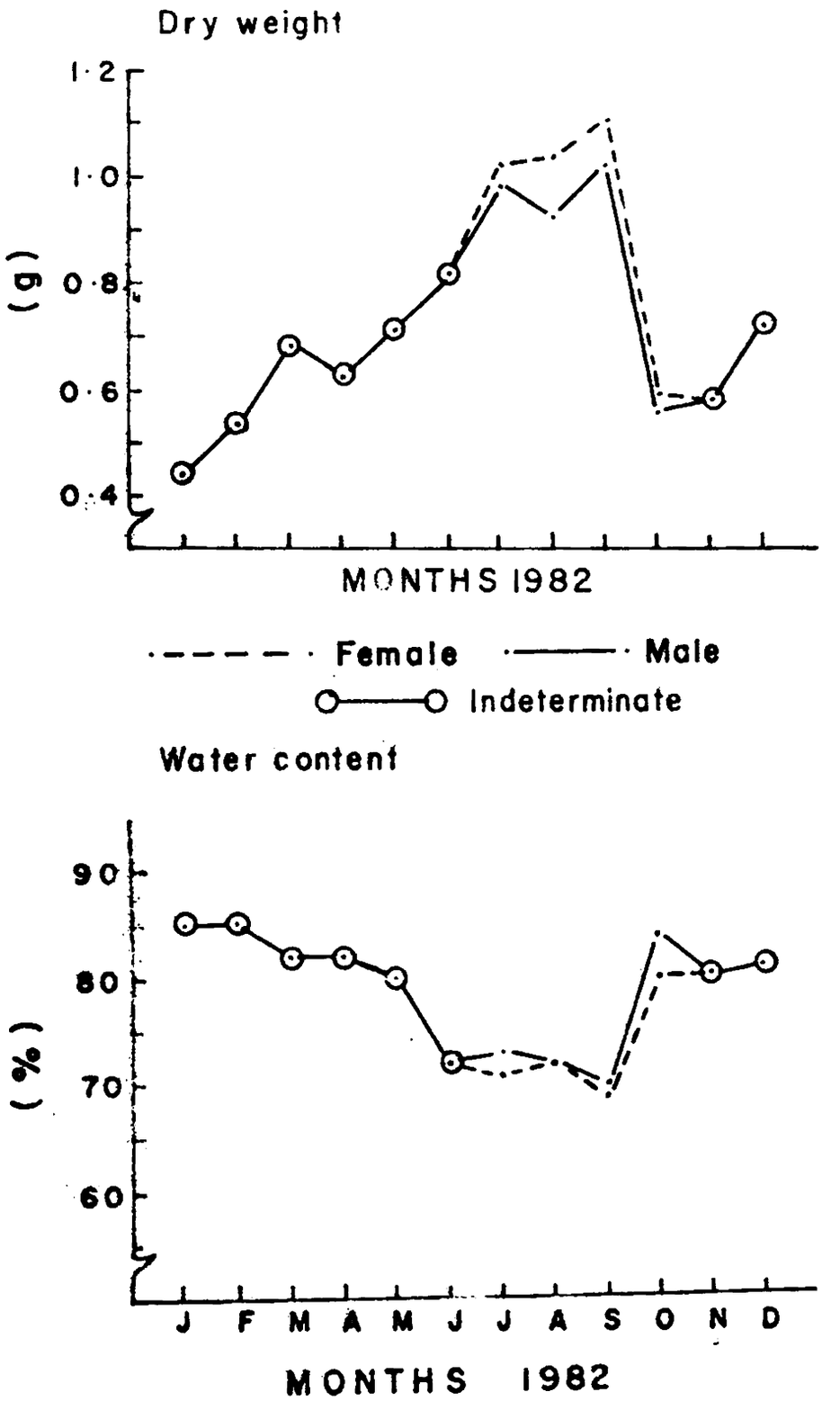
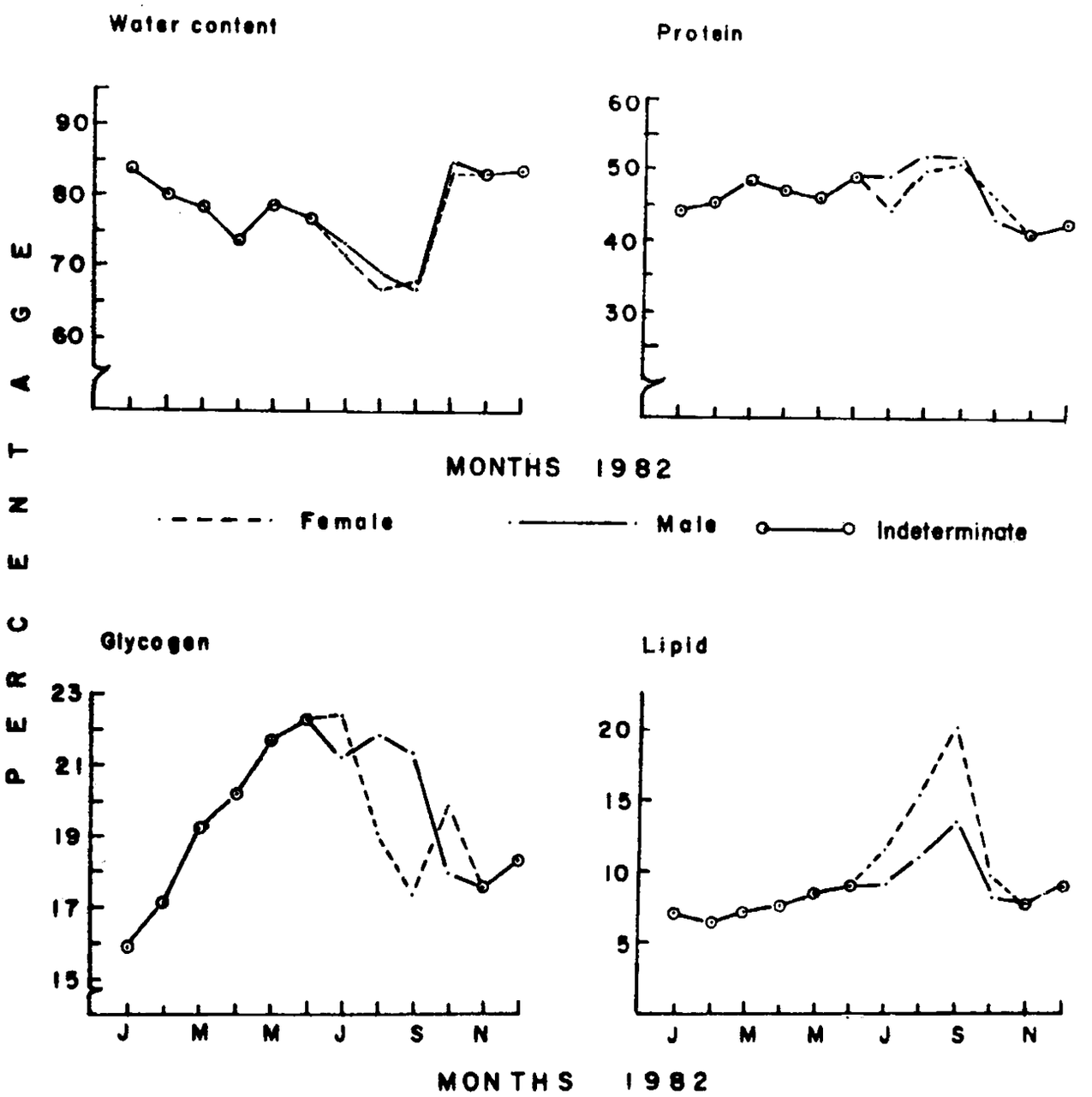


Table 17 Seasonal variations in the percentage water content, protein, glycogen and lipids in the mantle tissue of the mussel Perna viridis.

Month	Dominant maturity stage	Sex	Water content	Protein	Glycogen	Lipid
1981						
Oct	3	M	69.34 ± 0.31	52.54 ± 1.05	21.12 ± 0.97	12.44 ± 1.50
		F	71.36 ± 2.94	51.36 ± 3.97	16.60 ± 0.81	17.55 ± 1.92
Nov	3	M	74.82 ± 2.21	53.92 ± 1.43	22.27 ± 1.38	12.25 ± 1.74
		F	72.57 ± 1.97	49.34 ± 1.69	16.81 ± 2.11	16.84 ± 2.08
Dec	4	M	81.62 ± 4.81	46.78 ± 1.31	16.24 ± 1.52	6.59 ± 1.28
		F	84.71 ± 3.39	42.71 ± 1.94	15.42 ± 1.76	7.82 ± 1.19
1982						
Jan	0	-	84.28 ± 3.84	44.21 ± 3.48	15.86 ± 1.65	6.92 ± 1.71
Feb	0	-	80.18 ± 6.11	45.31 ± 1.52	17.10 ± 1.31	6.57 ± 2.04
Mar	0	-	78.48 ± 2.48	48.17 ± 1.36	19.23 ± 1.69	7.12 ± 0.86
Apr	0	-	73.57 ± 3.40	47.56 ± 2.08	20.13 ± 1.08	7.50 ± 2.71
May	0	-	79.23 ± 2.84	46.64 ± 1.27	21.71 ± 1.68	8.29 ± 0.96
June	0	-	77.38 ± 1.62	49.63 ± 1.53	22.26 ± 2.01	8.47 ± 0.84
July	1	M	73.81 ± 1.89	49.75 ± 1.52	21.21 ± 0.84	8.95 ± 1.52
		F	72.34 ± 1.68	44.68 ± 1.37	22.40 ± 1.31	11.16 ± 1.67
Aug	2	M	69.82 ± 2.04	52.75 ± 2.20	20.83 ± 1.86	11.30 ± 1.84
		F	67.68 ± 1.78	50.92 ± 1.37	19.10 ± 1.39	15.26 ± 1.31
Sept	3	M	67.13 ± 2.16	52.24 ± 1.39	21.30 ± 3.76	13.68 ± 1.31
		F	68.32 ± 1.74	51.68 ± 1.48	17.34 ± 2.11	20.70 ± 2.71
Oct	4	M	85.21 ± 3.68	43.31 ± 1.71	17.92 ± 1.88	8.18 ± 1.41
		F	83.68 ± 2.51	46.37 ± 1.38	19.82 ± 1.76	9.43 ± 1.52
Nov	0	-	83.64 ± 1.62	41.15 ± 2.02	17.56 ± 1.74	7.54 ± 1.63
Dec	0		83.92 ± 2.48	42.50 ± 1.31	18.36 ± 0.92	8.61 ± 1.18

Fig 12 SEASONAL VARIATIONS IN WATER CONTENT AND BIOCHEMICAL COMPOSITION OF MANTLE TISSUE OF *PERNA VIRIDIS*



from July to September, water content was low in males and females (67.13 - 73.81 %) and, with spawning in October 1982, hydration reached the highest level (85.21 % in males).

Protein: The protein level fluctuated between 44.21 - 49.63 % in stage-0 animals and increased with reproductive activity reaching 52.24 % in males and 51.68 % in females (Table 17, Fig. 12). With spawning there was a drop in protein level to the minimum 41.15 % in November 1982.

Glycogen: Glycogen builds up in the mantle tissue increasing from 15.86 % in January to 22.26 % in June in the vegetative phase (Table 17, Fig. 12). Gametogenesis draws upon the reserve resulting in fall in glycogen level during July-October 1982.

Lipids: The lipid level slowly increases from 6.92 % to 8.47 % during January-June and shows a sharp rise with reproductive activity, reaching 13.68 % in males and 20.70 % in females by September (Table 17, Fig. 12). Spawning in October brings about an equally rapid fall in lipid level to 8.18 % in males and 9.43 % in females.

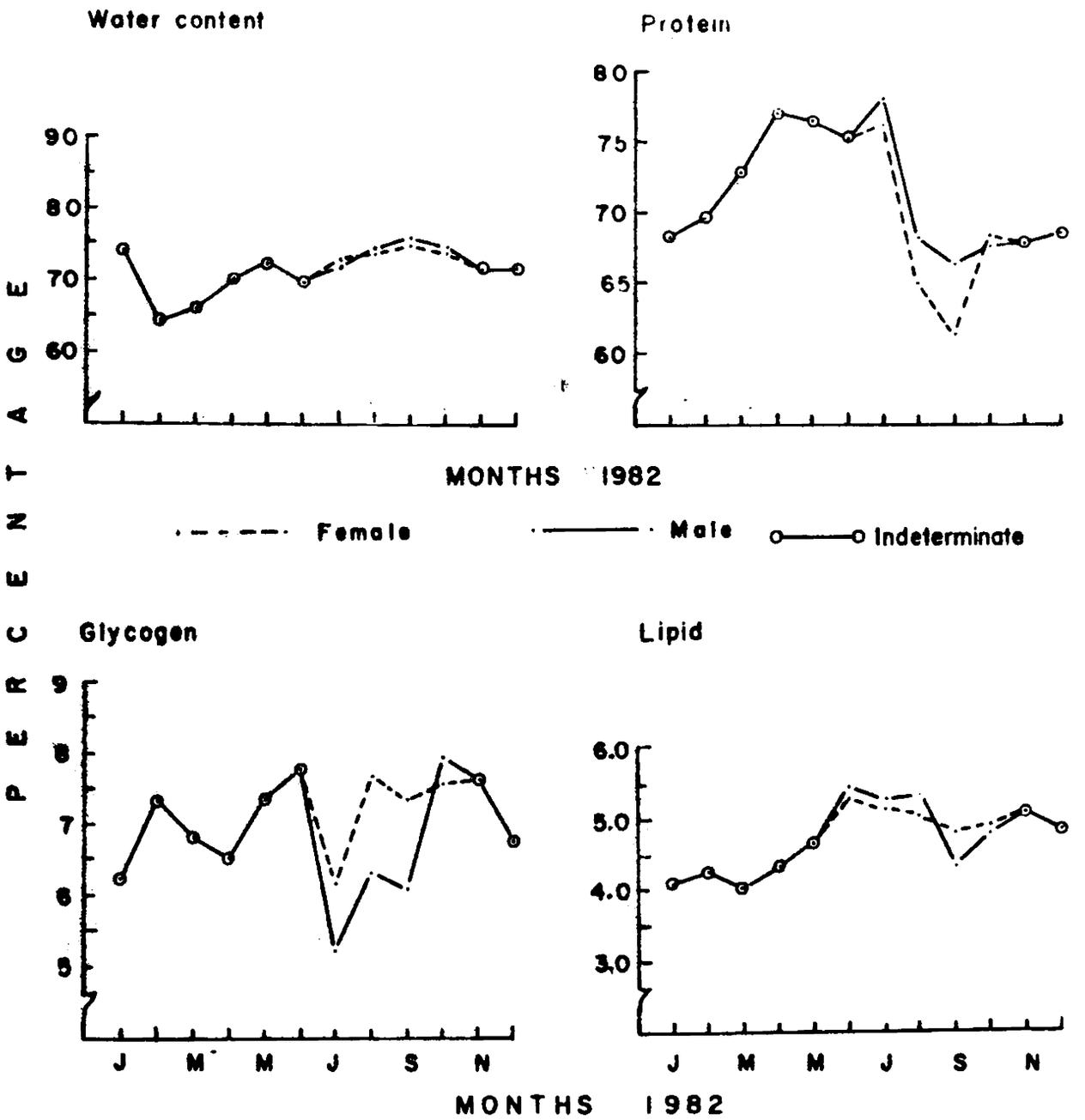
Adductor muscle

Water content: The vegetative phase shows a fluctuation between 64.21 - 73.62 % and reproductive phase has a higher level of water content (71.82 - 76.21 %) (Table 18, Fig. 13).

Table 18 Seasonal variations in the percentage water content, protein, glycogen and lipid in the adductor muscle of the mussel Perna viridis

Month	Dominant maturity stage	Sex	Water content	Protein	Glycogen	Lipid
1981						
Oct	3	M	76.18 ± 1.83	64.50 ± 1.35	7.72 ± 0.97	3.90 ± 0.60
		F	74.92 ± 2.72	61.80 ± 1.22	8.31 ± 0.92	3.21 ± 0.61
Nov	3	M	72.47 ± 1.59	64.52 ± 1.43	7.35 ± 0.85	4.37 ± 1.83
		F	73.84 ± 3.42	63.15 ± 1.39	7.88 ± 0.97	3.96 ± 1.64
Dec	4	M	74.81 ± 3.08	66.50 ± 1.53	6.74 ± 1.10	3.71 ± 1.49
		F	73.28 ± 1.33	64.80 ± 1.71	6.29 ± 1.11	3.78 ± 0.69
1982						
Jan	0	-	73.62 ± 2.27	67.82 ± 2.44	6.21 ± 0.49	4.10 ± 1.58
Feb	0	-	64.21 ± 1.16	69.56 ± 1.12	7.34 ± 1.12	4.26 ± 2.88
Mar	0	-	66.14 ± 1.72	72.64 ± 2.48	6.81 ± 1.32	4.04 ± 0.06
Apr	0	-	70.41 ± 1.35	77.00 ± 2.36	6.57 ± 0.67	4.31 ± 0.55
May	0	-	72.86 ± 1.19	76.50 ± 2.36	7.37 ± 0.88	4.67 ± 1.06
June	0	-	70.41 ± 1.85	75.06 ± 2.59	7.74 ± 0.39	5.32 ± 1.14
July	1	M	71.82 ± 2.39	78.14 ± 1.28	5.26 ± 1.55	5.40 ± 0.61
		F	72.84 ± 2.05	76.24 ± 1.41	6.17 ± 0.68	5.56 ± 1.27
Aug	2	M	74.58 ± 2.53	68.25 ± 1.62	6.37 ± 0.61	5.32 ± 0.47
		F	73.81 ± 1.47	65.00 ± 1.83	7.71 ± 1.06	5.00 ± 0.97
Sept	3	M	76.21 ± 1.92	66.27 ± 1.63	6.06 ± 1.82	4.34 ± 0.36
		F	75.24 ± 2.61	61.64 ± 1.38	7.31 ± 0.74	4.76 ± 0.53
Oct	4	M	73.81 ± 3.18	67.43 ± 2.19	7.92 ± 0.57	4.81 ± 0.77
		F	73.81 ± 2.64	67.68 ± 2.51	7.56 ± 1.08	4.12 ± 0.99
Nov	0	-	71.81 ± 2.83	67.81 ± 1.47	7.61 ± 0.63	5.17 ± 0.49
Dec	0	-	72.28 ± 2.69	68.35 ± 3.12	6.74 ± 1.86	4.86 ± 0.56

Fig 13 SEASONAL VARIATIONS IN WATER CONTENT AND BIOCHEMICAL COMPOSITION OF ADDUCTOR MUSCLE OF *PERNA VIRIDIS*



Protein: The highest protein levels (78.14 % in males and 76.24 % in females) are noticed in July 1982 at the beginning of reproductive phase (Table 18, Fig. 13). There is considerable reduction with maturation reaching 66.27 % in males and 61.64 % in females in September 1982 indicating utilisation of protein reserves of adductor muscle during gamete maturation.

Glycogen: The glycogen levels in adductor muscle are very low compared to mantle tissue. Glycogen increases from 6.21 % to 7.74 % during January-June and declines to some extent during reproductive activity (Table 18, Fig. 13).

Lipids: In the vegetative phase, the lipid level ranges between 4.04 - 5.32 %. In the initial gametogenic phase the level remains slightly high (5.40 % in males and 5.56 % in females) but drops marginally with maturation and spawning (Table 18, Fig. 13).

Digestive gland

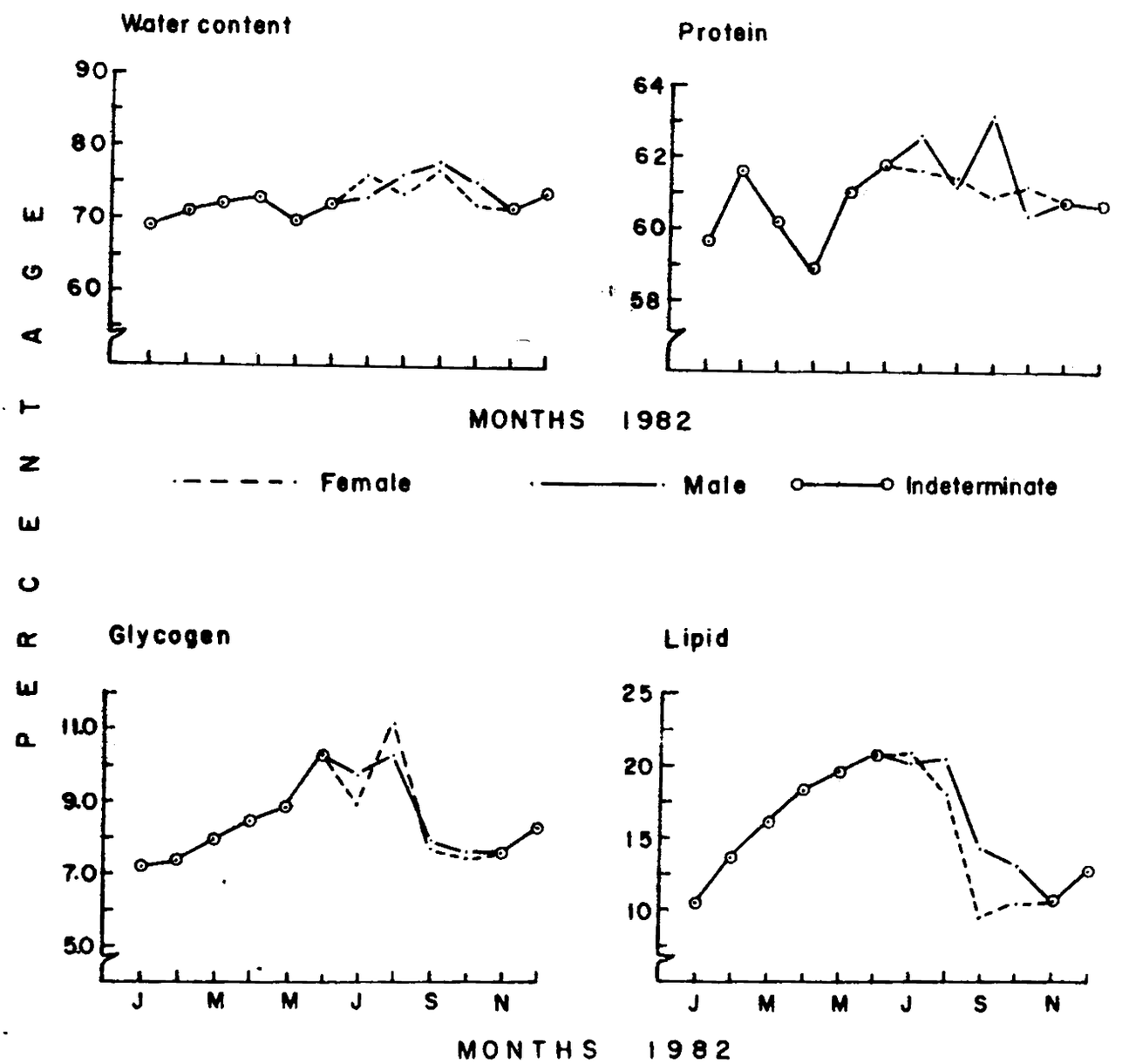
Water content: Gametogenesis and maturation bring about an increase in water content, from 68.81 - 73.28 % in the vegetative phase to 73.32 - 78.43 % (Table 19, Fig. 14). With spawning there is slight reduction in water content.

Protein: The total range of protein level during the observation period remains narrow between 58.90 - 64.14 % (Table 19, Fig. 14). The values are slightly higher in the reproductive phase than in the vegetative phase within the above range, with the highest levels in stage-3 animals associated with gamete maturation.

Table 19 Seasonal variations in the percentage water content, protein, glycogen and lipid in the digestive gland of the mussel *Perna viridis*

Month	Dominant maturity stage	Sex	Water content	Protein	Glycogen	Lipid
1981						
Oct	3	M	74.48 ± 1.83	60.18 ± 1.05	9.69 ± 0.36	13.12 ± 1.32
		F	72.91 ± 1.62	60.70 ± 0.92	9.87 ± 0.59	9.37 ± 0.68
Nov	3	M	73.56 ± 2.60	64.14 ± 0.96	9.07 ± 0.47	14.18 ± 1.11
		F	72.62 ± 1.84	61.28 ± 1.71	10.12 ± 0.53	8.67 ± 1.76
Dec	4	M	68.71 ± 2.17	59.62 ± 1.31	7.69 ± 0.76	9.86 ± 1.14
		F	70.64 ± 2.84	60.84 ± 0.94	7.72 ± 0.97	9.43 ± 1.76
Jan	0	-	68.81 ± 1.30	59.60 ± 2.21	7.20 ± 0.62	10.30 ± 1.18
Feb	0	-	71.41 ± 2.70	61.62 ± 1.52	7.34 ± 0.64	13.32 ± 2.17
Mar	0	-	72.36 ± 3.60	60.17 ± 1.13	7.92 ± 1.07	16.20 ± 2.28
Apr	0	-	73.28 ± 2.48	58.90 ± 1.56	8.43 ± 0.98	16.49 ± 1.02
May	0	-	69.51 ± 4.70	61.07 ± 2.02	8.82 ± 0.87	19.76 ± 2.21
June	0	-	71.78 ± 2.07	61.80 ± 2.10	10.34 ± 0.62	20.86 ± 1.39
July	1	M	73.32 ± 2.60	62.64 ± 1.58	9.76 ± 0.74	20.20 ± 1.15
		F	76.11 ± 2.37	61.69 ± 1.45	8.92 ± 0.59	20.81 ± 1.14
Aug	2	M	76.17 ± 3.50	61.24 ± 1.53	10.34 ± 0.73	20.43 ± 1.81
		F	73.48 ± 2.39	61.46 ± 1.72	11.27 ± 0.77	18.19 ± 2.17
Sept	3	M	78.43 ± 4.40	63.26 ± 1.90	7.82 ± 0.93	14.47 ± 1.39
		F	77.35 ± 3.17	60.92 ± 1.82	7.64 ± 0.82	9.65 ± 2.44
Oct	4	M	74.82 ± 2.11	60.45 ± 1.90	7.57 ± 0.86	13.36 ± 1.60
		F	72.15 ± 2.45	61.25 ± 1.21	7.40 ± 0.55	10.54 ± 1.62
Nov	0	-	71.61 ± 3.20	60.82 ± 1.52	7.52 ± 0.76	10.94 ± 1.84
Dec	0	-	73.63 ± 1.60	60.76 ± 1.15	8.23 ± 0.87	12.79 ± 2.02

Fig 14 SEASONAL VARIATIONS IN WATER CONTENT AND BIOCHEMICAL COMPOSITION OF DIGESTIVE GLAND OF *PERNA VIRIDIS*



Glycogen: Glycogen builds up from 7.20 % in January to 10.34 % in June which drops to 9.76 % in males and 8.92 % in females in July with onset of gametogenesis indicating glycogen utilisation in the process (Table 19, Fig. 14). This is followed by another spell of glycogen increase in August 1982 to 10.34 % in males and 11.27 % in females, but suddenly drops again to 7.82 % in males and 7.64 % in females in September concomitant with gamete maturation. Spawning brings about a further marginal reduction in glycogen.

Lipids: The lipid levels are high in the digestive gland as compared to mantle and adductor muscle. In the non-reproductive phase, it doubles up from 10.30 % in January to 20.86 % in June and maintains the higher level during July-August (Table 19, Fig. 14). Maturation and spawning bring a reduction in lipid level during September-October.

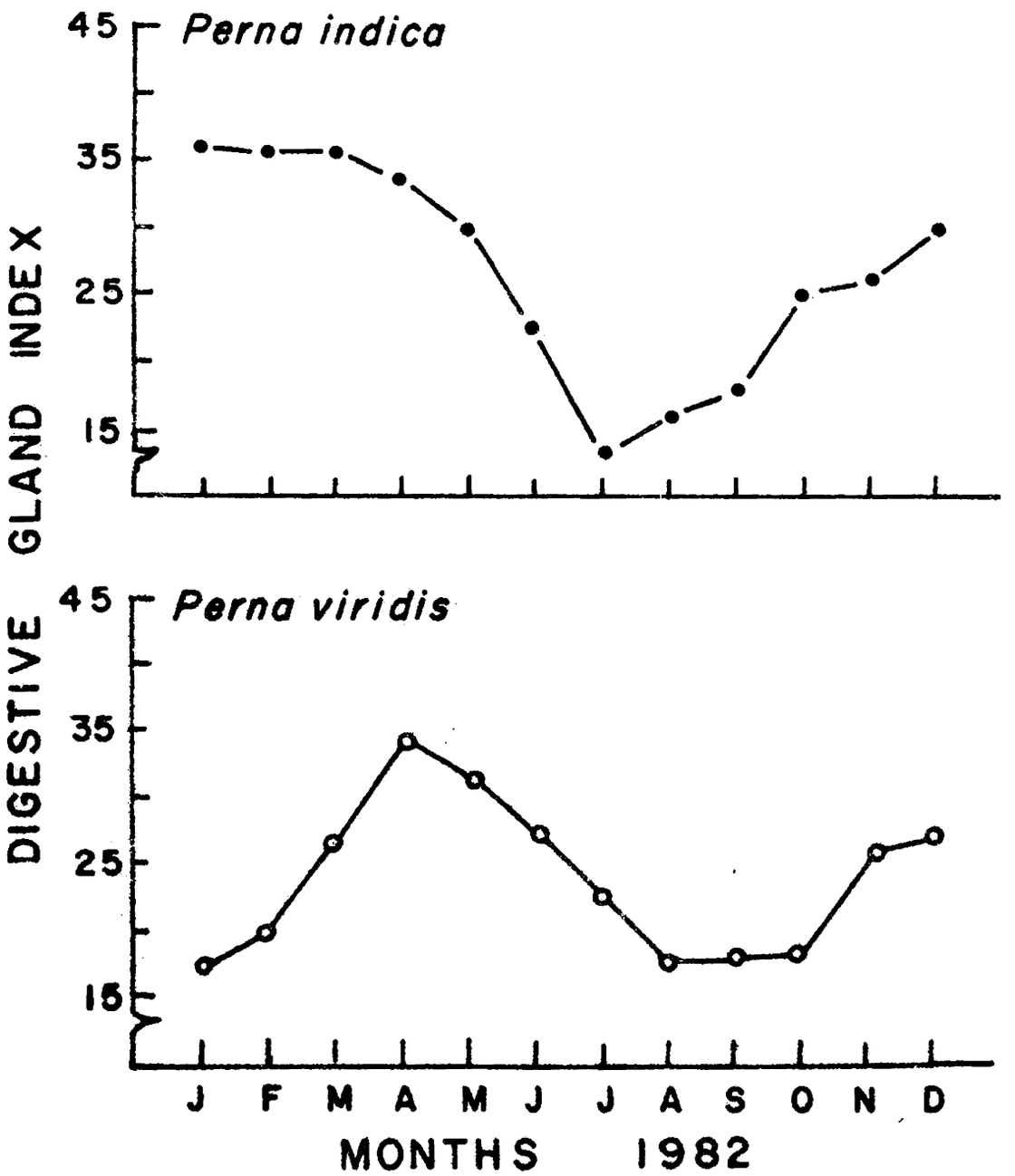
Digestive gland index

The digestive gland index of Perna indica and Perna viridis showed significant variations seasonally in relation to reproduction (Table 20 and Fig. 15). The digestive gland index was high in the indeterminate stage animals with 36.26 in January 1982 in P. indica and 33.96 in April 1982 in P. viridis. The index dropped during sex differentiation and maturation. The reduction in the digestive gland index was highly pronounced during vitellogenesis which suggests the possible role of extra ovarian synthesis of lipid in Perna.

Table 20 Digestive gland index of *Perna indica* and *P. viridis*

Month	Digestive gland index	
	P. indica	*P. viridis*
Oct 1981	27.16	16.14
Nov 1981	32.73	16.28
Dec 1981	34.31	19.69
Jan 1982	36.26	17.53
Feb 1982	35.71	20.07
Mar 1982	35.64	26.48
Apr 1982	33.52	33.96
May 1982	29.87	31.34
Jun 1982	22.14	27.26
Jul 1982	13.17	22.67
Aug 1982	16.39	17.89
Sep 1982	18.2[illegible]	18.36
Oct 1982	25.04	18.43
Nov 1982	26.25	25.16
Dec 1982	29.66	26.71

Fig. 15 SEASONAL VARIATION IN THE DIGESTIVE GLAND INDEX OF *PERNA INDICA* AND *PERNA VIRIDIS*



DISCUSSION

The seasonal variations in the biochemical composition of invertebrates are dependent upon many factors such as food availability, reproductive condition of the animal and different stress conditions (Bayne, 1976 a, Sastry, 1979; Taylor and Venn, 1979; Pieters et al., 1980; Zandee et al., 1980).

The body weight of brown mussel P. indica was minimum during December-February and it started increasing with an increase in the phytoplankton biomass, as also initiation of reproduction. The decrease in dry weight in August and September coincides with spawning. After spawning the dry weight was found to increase due to the accumulation of the nutrients resorbed from the residual gametes. There was also elaboration of interfollicular connective tissue in the mantle and accumulation of glycogen in the tissue. The build up of lipids in the digestive gland and protein in the adductor muscle was also observed in the spent animals.

Similar trends were observed in the green mussel P. viridis. The phytoplankton biomass was high preceding germ cell proliferation and gonad development. In P. viridis two peaks of dry weight increment were observed coinciding with reproductive activity. These observations lead to the assumption that changes in dry weight can be associated with food abundance and sexual activity of the animal as has been

reported for other bivalves (Gabbott, 1975; Mane and Nagabhushanam, 1975; Bayne, 1976 a; Velez and Epifanio, 1981; Lowe et al., 1982). The dry weight of the females was found to be higher than that of the males which may be due to the high lipid content of the eggs in females or due to the high amount of reproductive material in the females (Bayne et al., 1975; Zandee et al., 1980).

The percent water content in the body of both *P. indica* and *P. viridis* showed fluctuations in relation to the reproductive activity. The water content increased sharply following spawning. After spawning, as the connective tissue proliferates and nutrients accumulate, the water content decreases reaching the minimum in stage-3 animals. The reduction in water content, maturity of gonad and lipid accumulation coincide. The hydrophobic nature of lipids in the gonad also may account for the reduction in the water content (Pieters et al., 1980; Zandee et al., 1980).

The biochemical constituents glycogen, proteins and lipids varied seasonally in relation to the reproductive activity of the animal. The glycogen and lipid levels were significant in the gonad and digestive gland while in the adductor muscle the protein was the dominant factor.

The glycogen content was higher in the mantle than in the other tissues studied. It fluctuated in relation to the food abundance and reproductive activity. The mantle glycogen started increasing from January and reached the maximum in March 1982. During this period sex differentiation

was not noticed. As gametogenesis commenced in April the glycogen level began to decline. The reduction of the glycogen content was more marked in the females than in males, perhaps due to the accumulation of glycogen in the sperms in follicles and the conversion of glycogen into lipids in females. The yolk particles in the eggs of bivalves have been shown to be composed of lipid material (Sastry, 1979). As spawning commenced, the reduction in the glycogen content in males was more rapid than in females. This again confirms that there is glycogen accumulation in the sperms. After spawning the glycogen level increases along with new cell proliferation and glycogen accumulation in the mantle. These observations in P. indica and P. viridis agree with those reported earlier in other bivalves (Gabbott, 1975; Bayne, 1976 a; Bayne et al., 1982). Williams (1969) and Bayne and Thompson (1970) observed increase in the protein and lipids and decrease in carbohydrates during winter and spring and correlated the changes with gonad development. They also observed reduction in protein and lipid level during spawning.

The role of mantle tissue as a storage site during the non-reproductive season and as a fertile area in the reproductive period was suggested by Bayne and Thompson (1970). The present observations confirm the above and also agree with those of Gabbott (1975, 1976) in M. edulis.

The protein content in the mantle of P. indica increased from March onwards and was found to be the highest during June 1982. Later there was a slight reduction in the

protein content and this coincided with active gametogenesis and vitellogenesis. As spawning commenced the protein content of the mantle decreased. The pattern of mantle protein variations was similar in P. viridis.

Compared to the other tissues, the protein level in the adductor muscle was high as has also been reported by Zandee et al. (1980) and Pieters et al. (1980). As gametogenesis progressed the protein content in the adductor muscle was found to decrease while that in the mantle increased. This suggests that protein from the adductor muscle is mobilized and stored in the gonadal tissue. The adductor muscle's contribution of protein to the developing gametes has been reported by Zandee et al. (1980) in M. edulis. The protein content in the adductor muscle increased immediately after spawning.

The glycogen and protein level in the digestive gland did not show much seasonal variations, though the lipid level fluctuated significantly in the digestive gland and gonad. The observed reduction in the lipid level in the digestive gland and subsequent accumulation in the gonad shows that the digestive gland functions as a storage site for lipids during the non-reproductive season and with the onset of gametogenesis, the lipid is transported to the gonad. A similar view has been expressed by Ansell et al. (1964), Widdows (1972), Gabbott and Bayne (1973), Thompson et al. (1974), Bayne (1975, 1976 a) and Thompson (1977). Gabbott (1975) showed that the storage of nutrients in the digestive gland of M. edulis during the

non-reproductive season is related to the food abundance and the energy is mobilised for gametogenesis. Reciprocal relationship of the size of an organ and/or its lipid level with those of the gonads constitutes the bulk of the evidence for transfer of lipid from somatic storage to the gonads during gonad development (Giese, 1969; Lawrence, 1976). The present data on digestive gland index of P. indica and P. viridis support the reciprocal relationship suggested above. In P. indica species lower DGI levels, down to the minimum of 13.17, correspond with the reproductive phase and the higher levels with non-reproductive phase. In P. viridis too, the lower values of 17.89 - 22.67 correspond with the reproductive phase during July-October. Lawrence (1976), while reviewing relationship between pattern of storage of lipids in marine invertebrates and the reproductive cycle, suggested that 'there may be an obligation in the animal to store reserves for reproduction if there is a seasonality in food intake'.

CHAPTER VIII

HISTOCHEMICAL CHANGES IN REPRODUCTIVE TISSUE

INTRODUCTION

Histochemical investigation aids in qualifying further the chemical nature of the organic substances present in the different cell layers. Compared to the literature in this specialisation in other invertebrates such as crustaceans and echinoderms, studies on marine bivalves have been a few. Histochemical studies in relation to reproduction of the mussels have been carried out by Lubet (1959) in Mytilus edulis and M. californianus, Lunetta (1969) in Perna perna and Costanzo (1966) in M. galloprovincialis. Lubet (1959) investigated oogenesis and vitellogenesis in M. edulis and confirmed the occurrence of neutral lipids, phospholipids, glycogen and acid mucopolysaccharides in the oocytes. The storage of glycogen in the adipogranular tissue and occurrence of lipid droplets and protein granules in the oocytes were reported by Lubet (1959, 1976).

Glycogen accumulation in the mantle during non-reproductive season and its utilisation for gametogenesis was confirmed histochemically in P. perna (Lunetta, 1969) and M. galloprovincialis (Costanzo, 1966). Seasonal variations

in protein, carbohydrate and lipids in the non-reproductive and gametogenic phases were studied (Lubet, 1959; Lunetta, 1969). The occurrence of lipid in the sperm and oocytes of Perna perna was reported by Lunetta (1969).

The histochemical variations consequent to ganglion ablation were investigated by Lubet (1959) in M. edulis and M. californianus. The cytochemical characterisation of the adipogranular and vesicular cells was studied in M. edulis by Moore (1976) and Bayne et al. (1982). Lysosomal activity and its seasonal changes in the connective tissue of M. edulis were reported by Lowe et al. (1982).

During the present study, the histochemical characterisation of mantle tissue and changes associated with reproductive cycle in Perna indica and P. viridis were investigated to amplify the biochemical data on reproduction.

MATERIALS AND METHODS

Samples of Perna indica and P. viridis collected and fixed in 5 - 7 % neutral beffered formaldehyde and Baker's Formal-Calcium were used for the histochemical studies (Pearse, 1968). Mantle tissue from the animals was removed, sex noted and processed and sectioned as for histology using methods described in Chapter IV. The sections were stained for histochemical localisation of proteins, carbohydrates and lipids using standard histochemical staining techniques (Pearse, 1968; Subramoniam, 1982).

The standard scheme of stains/tests followed in the present investigation is given below :

Proteins

Basic proteins	..	Aqueous Bromophenol blue test. Aqueous Bromophenol blue after deamination.
Acidic proteins	..	Toluidine blue test Toluidine blue after methylation.
Tyrosil group	..	Millon's test Millon's test after iodination
SH group	..	Ferric - ferrycyanide test Ferric - ferrycyanide after mercaptide.
-S-S- group	..	Alcian blue test Alcian blue after performic acid
Tryptophan	..	DMAB test DMAB test after formaldehyde

Carbohydrates

Free aldehydes	..	PAS test - Schiff alone
Carbohydrates	..	PAS test - Periodic Acid Schiff
1,2 glycols and muco substances	..	PAS test - Schiff after acetylation and deacetylation
Glycogen	..	PAS test - Schiff after diastase.
Glycogen and mucoproteins	..	PAS test - Schiff after pyridine and methanol - chloroform
Muco substances	..	Toluidine Blue at different pH - 1.09; pH - 3.0; pH - 5.2 ; pH - 7.0

Carboxylated and sulphated acid mucosubstances	..	Alcian blue at critical electrolyte concentration 0.2 M; 0.6 M; 0.8 M; 1.0 M
Sulphated acid mucosubstances	..	Bracco curti's test
Diastase	..	Best Carmine test
<u>Lipids</u>		
Presence of lipids	..	Sudan Black B. Sudan Black B after pyridine
Neutral lipid	..	Oil Red O Oil Red O after pyridine.
Neutral and acid lipids	..	Nile Blue Nile Blue after pyridine.

As histochemical tests were performed only to understand the changes in the chemical nature of the gonad during different stages of reproduction, studies were limited to the mantle tissue along (Lunetta, 1969). The reproductive stages have been classified into the following 5 stages (Chapter IV): stage 0 - indeterminate; stage 1 - gonial cell differentiation and gametogenesis; stage 2 - active gametogenesis; stage 3 - fully mature gonad with ripe gametes; and stage 4 - spawning and recovery.

The staining properties and colour intensity were considered in histochemical characterisation. Lipid histochemistry of the gonad was also carried out on fresh tissue crushes and spawned sperms and eggs to confirm the observations on the preserved samples.

RESULTS

On examining the histochemical results of Perna indica and P. viridis at the end of the study period, it was found that the data for different reproductive stages were identical for the two species. The histochemical data presented here are common for P. indica and P. viridis.

Protein histochemistry

The protein histochemistry of the mantle tissue of P. indica and P. viridis during different stages of reproduction is given in Tables 21 and 22.

The coplasm of P. indica and P. viridis in the stage 1 shows positivity to general protein as evidenced by the positivity to Bromophenol blue reaction. Reactive groups such as tyrosil, tryptophenyl and the SH groups were found to be predominant in the coplasm. In the stage 2 and stage 3 oocytes, the -SS- and SH groups also became abundant as the cytoplasm reacts intensively only to those tests meant for -S-S- and SH, namely Ferric-ferricyanide and performic acid alcian blue tests. In the stage 3 oocytes, though no changes in the staining property were noticed, discrete granules were observed in the coplasm. The presence of granules may suggest that the secretory products may be concentrated in these granules in the final stages of maturity.

Tests for presence of aromatic amino acids in the oocytes proved positive. Almost similar aromatic amino acids

Table 21 Protein histochemistry of the mantle tissue of male *Perna indica* and *P. viridis*

Stages	Epithelial cells	Connective tissue	Sperm Bundle
0	NH_2, -S-S- and SH groups present	NH_2, COOH, tyrosyl, SH and trypto-phenyl group present	-
1	NH_2, COOH, tyrosyl, SH, -S-S- and tryptophenyl groups present	NH_2, COOH, tyrosyl, SH -S-S- and tryptophenyl groups present	NH_2, COOH, tyrosyl, SH, -S-S- and tryptophenyl groups present
2	NH_2, COOH, and tyrosyl groups present	NH_2, COOH, tyrosyl and tryptophenyl groups present	NH_2, COOH tyrosyl, -S-S- and tryptophenyl present
3	NH_2, COOH, and tyrosyl groups present	NH_2, COOH, and tyrosyl groups present	NH_2, COOH, tyrosyl, -S-S- (?) and tryptophenyl groups present
4.	NH_2, COOH and tryptophenyl groups present. COOH is rich	NH_2, COOH and tryptophenyl groups present	NH_2, COOH, tyrosyl and tryptophenyl groups present

Table 22 Protein histochemistry of the mantle tissue of female *Perna indica* and *P. viridis*

Stages	Epithelial cell	Connective	Nucleus	Cytoplasm
0	NH_2 and -S-S- and SH group present	NH_2, COOH, tyrosyl, SH and Trypto-phenyl groups present		
1	NH_2, COOH, tyrosyl, SH and -S-S groups present	NH_2, present tyrosyl and -S-S- groups show high positivity	NH_2 present COOH, tyrosyl groups pro-minent	NH_2 present SH, very light positivity.
2	NH_2, COOH, tyrosyl and Tryptophenyl groups present	NH_2, COOH, tyrosyl, -S-S- and Tryptophenyl groups present	COOH, tyrosyl, -S-S- and Tryptophenyl groups present	COOH, tyrosyl, -S-S- and tryptophenyl groups present
3	NH_2, COOH, -S-S- and tyrosil groups present	NH_2, COOH -S-S- groups present	NH_2, and -S-S- groups present	NH_2, COOH and -S-S- groups present
4	NH_2, COOH, tryptophenyl and tyrosyl groups present in good quantity	NH_2, COOH, tyrosyl, SH -S-S-, and tryptopheyl group present	COOH, tyrosyl and tryptophenyl present	NH_2, COOH tryptophenyl present NH_2 shows positivity

were identified in the protein components in the connective tissue as well as epithelial cells of the mantle. The reduction in the distribution of these protein fractions in the mantle epithelium and connective tissue during the oocyte development suggests that these two tissues may contribute protein precursor substances for the yolk formation.

Identical results were obtained in the case of the males. The protein of the male follicles is also reactive to tyrosyl, -S-S- and tryptophenyl groups. The possibility of the derivation of protein precursor substances from the connective tissue and epithelial cells of the mantle is also revealed by the identical histochemical nature.

In the spent animals, the residual ova and developing connective tissue showed positivity to basic protein, acidic protein, -S-S- and tryptophenyl groups. The elaboration of amoebocytes was also high in these stages suggesting that the residual gametes are lysed and resorbed into the body and stored in the connective tissue.

Carbohydrate histochemistry

The carbohydrate histochemistry of the mantle tissue of P. indica and P. viridis is given in Tables 23 and 24.

The developing oocytes in stage 1 showed positivity to glycogen in the ooplasm as revealed by the Braeco-curtis test and PAS tests, and to sulphated mucopolysaccharides (SMP) as evident from toluidine blue (in different pH) and alcian blue (in different critical electrolytic concentrations) tests.

Table 23 Carbohydrate histochemistry of the mantle tissue of male *Perna indica* and *P. viridis*

Stages	Epithelial cell	Connective tissue	Sperm Bundle
0	Free aldehydes, SAMP and glycogen present	Free aldehydes, SAMP and glycogen present. The glycogen level was very high.	-
1	Free aldehydes, glycogen and SAMP present	Free aldehydes, glycogen and SAMP present. glycogen level was low	Free aldehydes SAMP and glycogen present
2	Free aldehydes, glycogen and SAMP show possitivity	Free aldehydes, glycogen and SAMP present	Free aldehyde glycogen 1:2&1 and glycon and SAMP present
3	Free aldehyde, glycogen and SMP present. SAMP in high quantity	Free aldehyde, glycogen and SAMP noticed.	1:2 glycol and Free aldehyde noticed. AMP present
4	1:2 glycol and free aldehydes present	Free aldehyde, glycogen and 1:2 glycol present	Free aldehyde, glycogen, and SAMP present

Table 24 Carbohydrate histochemistry of the mantle tissue of female Perna indica and P. viridis

Stages	Epithelial cell	Connective tissue	Nucleus	Cytoplasm
0	Free aldehyde, glycogen and SAMP present	Free aldehyde, Glycogen and SAMP present (Glycogen (+‡)	-	-
1	Free aldehyde, SAMP and glycogen present	Free aldehyde glycogen and SAMP present	AMP present	Free aldehyde and glycogen show positivity
2	Free aldehyde, SAMP and glycogen present 1:2 glycols suspected	1:2 glycol Free aldehyde present glycogen level reduced considerably	Free aldehyde and glycogen present	Free aldehyde 1:2 glycol, AMP present SMP suspected
3	1:2 glycols and Free aldehyde noticed	Free aldehyde and 1:2 glycols noticed.	1:2 glycol present	1:2 glycol and AMP present
4	1:2 glycols and free aldehyde present	Free aldehyde 1:2 glycols and glycogen noticed	1:2 glycols and glycogen present	2 glycols AMP and SAMP are present

As the oocyte growth proceeds, in the ooplasm of the stage 2 and 3 animals, the appearance of acid mucopolysaccharides was noticed along with increase in the unsubstituted 1:2 glycol groups. The nucleus of the developing oocytes in the stage 2 animals showed high positivity for acid mucopolysaccharides and 1:2 glycols, but the latter was noticed in the mature oocytes in the stage 3 animals.

The connective tissue and the epithelial cells revealed a very close relationship in the changes in their carbohydrate reactive sites. The disappearance of SAMP in the connective tissue and in the epithelial cells and their immediate appearance in the ooplasm of the developing oocytes suggests a possible transformation of the materials from these tissues to the ooplasm. The presence of free aldehydes was noticed in all stages, with greater abundance in the stage 1 and 2 animals, as evidenced by the positivity to Schiff's reagent with periodic acid after delipidation.

In the case of males the presence of free aldehyde in the epithelial, connective and follicular tissues was noticed in all stages. In the male follicles the sulphated acid mucopolysaccharides (SAMP) are gradually changed to acid mucopolysaccharides (AMP) as growth proceeds. The 1:2 glycols are comparatively high in quantity in the male follicles of mature animals suggesting glycogen deposition. In the case of males too, transfer of carbohydrate components from the connective and epithelial tissues to the male follicles was evident from the reduction in different carbohydrate reactive

sites in the former and their appearance and accumulation in the latter.

In the spent gonad, the reappearance of SAMP was noticed in the residual sperm and ova. In the connective tissue, which starts proliferating after the elaboration of amoebocytes was found to contain free-aldehyde and glycogen in good quantity. The sperms released were highly positive to the AMP with free-aldehyde and glycogen.

Lipid histochemistry

The lipid histochemistry of the mantle tissue of P. indica and P. viridis is given in Tables 25 and 26.

Acidic and neutral lipids are low in the ooplasm in the initial stages of development. Before the gonial cells appear the connective tissues contains highly acidic lipids. As oogenesis commences and proceeds, the intensity of acidic lipids gets reduced and neutral lipids accumulate as globules in appreciable quantity, as evidenced by the staining reaction with Oil red 'O' and Nile blue. In the connective tissue and epithelial cells, the neutral lipid content gets reduced with a gradual build up of the same in the ooplasm of the oocytes. This suggests an extraovarian precursor involved in vitellogenesis. The enormous accumulation of neutral lipids in the female follicles in the vitellogenic stage also suggests that lipid is transferred from some other body tissue to the developing gonad. The staining intensity of the follicular stalked ova with intact germinal epithelium was

Table 25 Lipid histochemistry of the mantle tissue of male *Perna indica* and *P. viridis*

Stages	Epithelial cell	Connective tissue	Sperm Bundle
0	SB (++) Acidic and neutral lipids present	SB (++) Acidic lipids and trace neutral lip lipids present	-
1	SB (++) Acidic and neutral lipids present	SB (++) Acidic and neutral lipids present	SB (++) Acidic and neutral lipids present
2	SB (+) Acidic and neutral lipids present	SB (+) Acidic and neutral lipids present	SB (+‡) Acidic and neutral lipids present
3	SB (+) Acidic lipids present. Neutral lipids (trace)	SB (+) Acidic and neutral lipids present	SB (+‡) Acidic and neutral lipids present
4	SB (+) Acidic and neutral lipids present	SB (+) Neutral lipids in abundance. Acidic lipids present	SB (+) Acidic and neutral lipids present

Table 26 Lipid histochemistry of the mantle tissue of female *Perna indica* and *P. viridis*

Stages	Epithelial cell	Connective tissue	Nucleus	Cytoplasm
0	SB (++) Acidic, & neutral lipids present	SB (++) Acidic lipid and neutral lipid present		
1	SB (++) Acidic and neutral lipids present	SB (+) High acidic and trace neutral lipids present	SB (+) Neutral lipids alone present	SB (+) Acidic and neutral lipids present
2	SB (+) Acidic and neutral (less) present	SB (++) Acidic neutral lipids present	SB (+) Neutral lipid alone present	SB (++) Acidic and neutral lipids present
3	SB (++) Acidic and neutral (less) present	SB (++) Neutral lipids present (less intensive)	SB (+) Neutral lipids alone pfesent	SB (++) Acidic and neutral lipids present
4	SB (+) Acidic lipid and neutral lipids present	SB (+) Neutral lipids in abundance and acidic lipids present	SB (+) Neutral lipids present	SB (++) Acidic and neutral lipids present

observed to be comparatively high, especially in the stalks. This suggests that the yolk precursors are transported into the egg through the stalk.

In the case of males the distribution of acidic and neutral lipids was uniform in stage 0 and stage 1 gonads. There was a gradual increase of neutral lipids in stage 2 animals. But in the stage 3 animals the ratio of acidic and neutral lipids was almost the same as revealed by Oil-red 0 and Nile blue sulphate tests. However, the staining intensities of the male follicles was high to the Sudan Black B. The uniform distribution of both acidic and neutral lipids in the epithelial and connective tissues was also noticed.

In the spent animals the staining property of the follicle was diminished and, on the proliferation of connective tissue, the intensity of the lipids increased. The neutral lipids were observed to be high in this stage compared to other stages, which suggests resorption of the lipids from the residual eggs and sperms after spawning.

DISCUSSION

The histochemical studies revealed that the mantle (gonadal tissue) of *Perna indica* and *P. viridis* is glycolipoprotein in nature. In the non-reproductive phase, the mussels accumulate glycogen and lipids in mantle tissue and the glycogen level is comparatively high. Similar observations were made on *Perna perna* by Lunetta (1969). The mantle tissue

is comprised of epithelial layers and connective tissues of adipogranular (ADG) and vesicular (VC) in nature (Lubet, 1959; Lubet et al., 1976 - 1978; Bayne et al., 1982). As primordial germ cell differentiation and active gametogenesis proceed, disintegration of the adipogranular cells occurs. Lubet et al., (1976 - 1978) and Bayne et al. (1982) reported that during gametogenesis, there is a decline in the volume of ADG cells coincident with an increase in the volume of gametes and they confirmed that the loss in ADG cell mass is due to lysosomally mediated autolysis. Biochemical studies (Chapter VII) also confirm the observation that during germinal cell differentiation and proliferation there is reduction in the glycogen content in the mantle tissue.

The occurrence of acid mucopolysaccharides (AMP) in the gonad follicles agrees with the findings of Lubet (1959) in M. edulis and Lunetta (1969) in Perna perna. Lunetta (1969) reported the disappearance of AMP at the end of Vitellogenesis in P. perna, but the present observation differs in that in P. indica and P. viridis, AMP is retained even after vitellogenesis in the ripe ova.

The transfer of yolk precursor substances from the connective tissue to the developing oocytes in the follicles was observed by Lowe et al. (1982) and Bayne et al. (1982). Houtteville (1974) and Mathieu (1979) have shown that the ADG cells in the connective tissue are an important source of proteins, lipids and glycogen for gametogenesis. The nutrient reserve in the mantle is depleted during gametogenesis with an

increase in the lysosomal activity. The simultaneous accumulation of nutrients in the follicles and gametes suggests that the lysed nutrients are transferred to the developing follicles.

The protein reactive sites in the mantle of *P. indica* and *P. viridis* are rich in tyrosyl, tryptophenyl and -S-S- groups. This suggests that the protein content in the connective tissue has a rich amino acid profile. The protein level in the mantle was high during the stage 0 animal and was depleted from the connective tissue and transferred to the gonial follicles for gametogenesis (Lunetta, 1969; Houtteville, 1974; Mathieu, 1979). The biochemical studies (Chapter VII) also revealed that protein level in the mantle during the resting phase of the animal started increasing although slowly. This may be due to the build up of protein in the developing oocytes and spermatocytes. Biochemical studies revealed that the protein level in the male gonad was relatively higher than in the female. This was confirmed histochemically with the increase in the staining intensity in the stage 2 and stage 3 animals. The occurrence of tyrosyl and tryptophenyl groups in the sperm bundle and the abundance of tryptophan in the fully mature gonad of *P. indica* and *P. viridis* are similar to the findings of Krishnan (1968) in *Holothuria scabra*.

The lipid histochemistry revealed that the lipids accumulated in the mantle tissue of *P. indica* and *P. viridis* are both acidic and neutral. The lipid content was comparatively high in the resting phase of the animal. Lunetta (1969) also reported the occurrence of acidic and neutral lipids in the

mantle tissue of M. perna. As gametogenesis begins the neutral lipid content in the follicles started increasing. This coincided with the slight reduction in the lipid level in the connective tissue. The staining of stalked oocytes is very high for the specific lipid stains. This suggests that, other than the lipid stored in the connective tissue, lipid is also contributed from some other source. Biochemical studies (Chapter VII) have indicated that the lipid in the digestive gland is mobilised into the gonad during vitellogenesis.

The appearance of the granules in the stage 3 animals and the occurrence of lipid droplets agree with the observations of Lubet (1959) in M. edulis. He reported accumulation of yolk platelets, lipid droplets and mucopolysaccharides during vitellogenesis and correlated the colour change in the stage 2 and 3 animals (females) with lipid accumulation.

CHAPTER IX

EFFECT OF RATION ON NUTRIENT RESERVE AND GAMETOGENESIS IN PERNA INDICA

INTRODUCTION

The reproductive strategy of marine bivalves has been studied extensively and the literature has been reviewed from time to time (Giese, 1959; Fretter and Graham, 1964; Giese and Pearse, 1974; Bayne, 1976 a; Sastry, 1979). Most of the studies have been based on observations on the natural populations of these molluscs which are of considerable economic importance, particularly oysters, mussels, scallops and clams. A new dimension to these researches was added with the development of techniques for artificial production of spat in the laboratories wherein control of reproduction came to be recognised as one of the important aspects. A good deal of scientific effort has been devoted to induced maturation and spawning at several shellfish laboratories, particularly at Milford, U.S.A. (Loosanoff and Davis, 1963) and Kesennumma, Japan (Imai, 1971).

Giese and Pearse (1974) considered temperature, photoperiod, salinity, abundance of food and chemical factors such as trace metabolites as important factors in the exogenous

regulation of reproduction in marine invertebrates including pelecypods. The influence of temperature and salinity on reproduction of marine bivalves has already been discussed in the earlier chapters of the present study. The role of photoperiod in reproduction of bivalves has not yet been elucidated except for some evidence on scallop (Sastry, 1970). The effect of trace metabolites has been very little understood (Giese and Pearse, 1974).

In the recent years more attention has been paid to understand food availability, assimilation, nutrient storage and energy release for various metabolic activities, particularly for gonad growth and gametogenesis, of marine bivalves (Gabbott, 1975; Bayne, 1976 a; Bayne et al., 1982; Thompson et al., 1974; Mann, 1979). For some species, e.g. Argopecten irradians (Sastry, 1968) and Placopecten magellanicus (Thompson, 1977) the periods of food abundance and gonad development are nearly coincident. For many others, gonad development is linked with storage and utilisation of reserves accumulated during the preceding period of phytoplankton blooms, e.g. Crassostrea virginica (Loosanoff, 1965) and Mytilus edulis (Chipperfield, 1953). Mann (1979) found that C. gigas has a temperature related sequence of storage metabolism. Lubet (1959) stated that the development of storage tissue and the germinal tissue goes in two opposite directions.

In the tropical waters, there has been very little work on the dependence of reproduction on temperature and food

availability and the few results show that this dependence is less certain (Veles and Epifanio, 1981). Veles (1977) found that, in Crassostrea rhizophorae, while peak reproduction occurs during the period of minimal phytoplankton density coinciding with maximal seasonal temperature, most somatic growth occurs during the period of highest phytoplankton density coinciding with upwelling and minimal temperature. But in the tropical mussel Perna perna spawning peaks take place when temperature has declined to 22°C after a maximum of 28°C (Lunetta, 1969). Veles and Epifanio (1981) experimentally observed that gametogenesis in Perna perna was generally inhibited by high temperature irrespective of the amounts of ration.

Among the various exogenous factors that control reproduction of Indian marine bivalves, many authors have shown that primarily salinity, in conjunction with temperature, govern the reproductive cycle (Chapter V). Since the effect of salinity and temperature is so pronounced and decisive in nature, the factor of nutrition has so far been overlooked.

During the present investigation it was noticed that gonad growth and gametogenesis in Perna indica are preceded by intense phytoplankton production and accumulation of nutrient reserves (Chapter V). In the area of the natural distribution of the brown mussel P. indica, the annual range of chlorophyll a concentration was found to be 2.0 - 25.0 mg/m^3 with the highest value in May and the lowest in October-November. Peak spawning

was during July-August coinciding with low values of chlorophyll *a* concentration. In order to test if reproductive activity in the species could be induced out of season by artificial feeding, an experiment was designed and carried out, the results of which are presented here.

MATERIALS AND METHODS

In February 1982, the brown mussel (*Perna indica*) was collected from the natural beds at Vizhinjam and transported to Tuticorin by road, over a distance of 220 km. The Tuticorin Research Centre of Central Marine Fisheries Research Institute has established facilities for the supply of filtered sea water and mass culture of algal food and, hence, the experiments were carried out in this laboratory. The mussels were acclimated to the laboratory conditions for two days before commencing the experiments.

Mussels in the size range 40 - 50 mm were segregated and divided into nine groups of 40 animals each. Ten animals from the same lot, but outside the nine groups, were sacrificed to collect the data on initial conditions and the parameters noted were reproductive stage, digestive gland index, mean gonad index, total weight, wet meat weight and dry meat weight. The reproductive stage and mean gonad index were estimated by procedures laid down in Chapter IV and the other parameters as stated in Chapter VII. Each experimental group was placed in a plastic tub containing 40 litres of seawater filtered to remove particles larger than 10 µm. The water was aerated and changed every day.

The experiments were conducted under ambient conditions and during the 45 days of experiment, the temperature ranged from 28.3° to 29.1°C and salinity from 37.6 to 38.3 ppt.

For feeding the mussels, mixed phytoplankton blooms were raised in outdoor fibreglass tanks of 500 litres capacity by fertilising filtered (10 µm) seawater with commercial fertilisers urea and NPK (nitrate, phosphate and potassium). Axenic cultures of Isochrysis galbana and Chlorella ovalis were inoculated into the medium to supplement the natural forms of algae like coccoid green and blue-green algae, small diatoms and other nanoplankters. The chlorophyll a content of the phytoplankton blooms occuring on the third day from inoculation was estimated spectro-photometrically after correcting for pheopigments, following the method of Strickland and Parsons (1968). The mean chlorophyll a content of the third day blooms was estimated to be 12.00 ± 1.68 µg/litre.

Of the nine experimental groups of 40 animals each, one group was maintained as control without feeding. The other eight groups were fed with appropriate amount of phytoplankton which would give the pre-determined level of chlorophyll a as indicated in Table 27. The ration was given once a day at 09.00 h for some groups or divided up to four equal volumes and given at equal intervals between 09.00 - 21.00 h (Table 27).

Table 27 Experimental feeding levels of *Perna indica* for induction of maturation. The amount of phytoplankton fed is expressed as chlorophyll *a* content per animal per day.

Experimental group	Feeding schedule in a day	Total ration supplied chlorophyll *a* μg animal^{-1} day^{-1}		
		days 1-15	days 16-30	days 31-45
1 (control)	0	0	0	0
2	once	0.6	0.8	1.2
3	once	0.9	1.2	1.8
4	once	1.2	1.6	2.4
5	twice	1.8	2.4	3.6
6	twice	2.4	3.2	4.8
7	thrice	3.6	4.8	7.2
8	thrice	4.8	6.4	9.6
9	four times	6.0	12.8	19.2

The experimental groups were examined at fortnightly intervals and, from each, a sub-sample of 10 animals was sacrificed to obtain data on maturity stage, digestive gland index, gonad index, total weight, wet meat weight and dry meat weight. Glycogen content in the mantle was examined histochemically by Lugol's iodine method (Pearse, 1968). The experiment was terminated on the 45th day from initiation.

RESULTS

Body dry weight

The data on growth of *P. indica* in terms of dry weight of the soft tissue in response to different chlorophyll *a* concentrations in the phytoplankton supplied, as sampled on days 15, 30 and 45, are presented in Table 28 and Fig. 16. In contrast to the control, there was positive growth in all the treatments which was directly correlated with the ration levels. In the control there was progressive decrease in weight from the initial 0.504 g to final 0.210 g, the net loss being 58.3 %. The wet weight of animals in control showed a 27.3 % loss from the initial 2.67 g to final 1.94 g. It indicates that the nutrient reserves in the body tissues were being depleted fast to overcome the stress and support the basal metabolism in the control animals.

In treatments 2 (T-2) and 3 (T-3) with chlorophyll *a* concentration ranging 0.6 - 1.8 µg/animal/day, an initial reduction in dry weight was noticed on day 15 indicating that the initial feeding levels of 0.6 µg and 0.9 µg chlorophyll *a* were inadequate even for overcoming the initial stress and for maintenance metabolism and, therefore, reserves from the body tissues had been utilised, resulting in reduction of body weight. However, when the ration was increased to 0.8 µg and 1.2 µg on day 16 and 1.2 µg and 1.8 µg on day 31 (Table 28) the animals registered weight increase, the net gain being 18.7 % in T-2 and 25.4 % in T-3 on day 45.

Table 38 Effect of different feeding levels of phytoplankton on somatic growth and reproductive activity in the brown mussel *Perna indica*

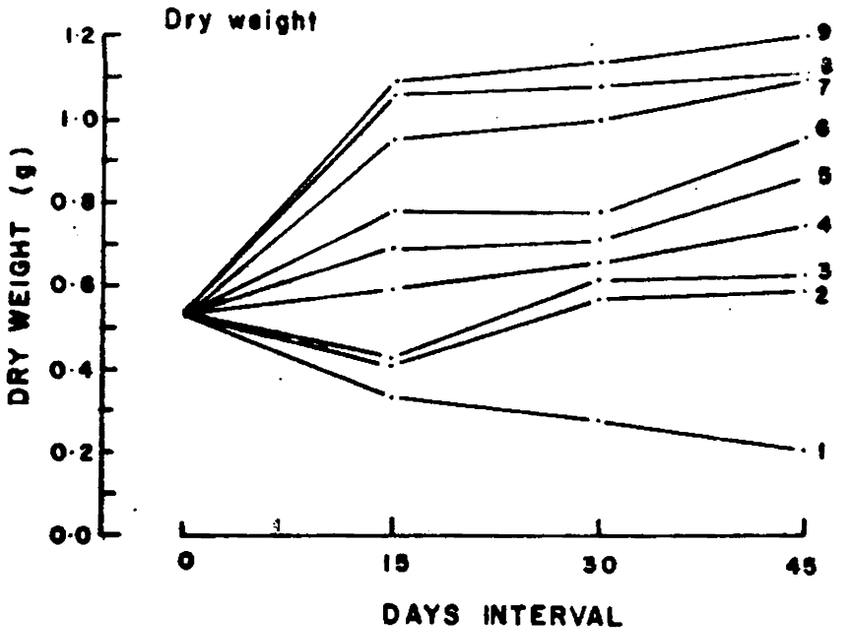
Sl. No.	Chlorophyll a animal^{-1} day^{-1}	Day from start								
		Dry wt (g)			Wet weight(g)			Maturity stage		
		15	30	45	15	30	45	15	30	45
1.	0 control	0.340	0.280	0.210	2.48	2.12	1.94	0	0	0
2.	0.6 - 1.2	0.412	0.571	0.598	2.68	2.74	2.94	0	0	0
3.	0.9 - 1.8	0.432	0.621	0.632	2.71	2.80	2.92	0	0	0
4.	1.2 - 2.4	0.599	0.660	0.753	2.85	2.79	2.93	0	0	0
5.	1.8 - 3.6	0.694	0.716	0.872	2.79	2.84	2.85	0.1	0.1	1.2
6.	2.4 - 4.8	0.789	0.786	0.971	3.00	2.95	3.01	1	1.2	2
7.	3.6 - 7.2	0.954	1.020	1.110	3.38	3.41	3.53	1.2	2.3	3
8.	4.8 - 9.6	1.060	1.090	1.121	3.74	3.84	3.94	1.2	3	3
9.	9.6 -19.2	1.090	1.140	1.210	3.88	3.98	4.13	2	3	3

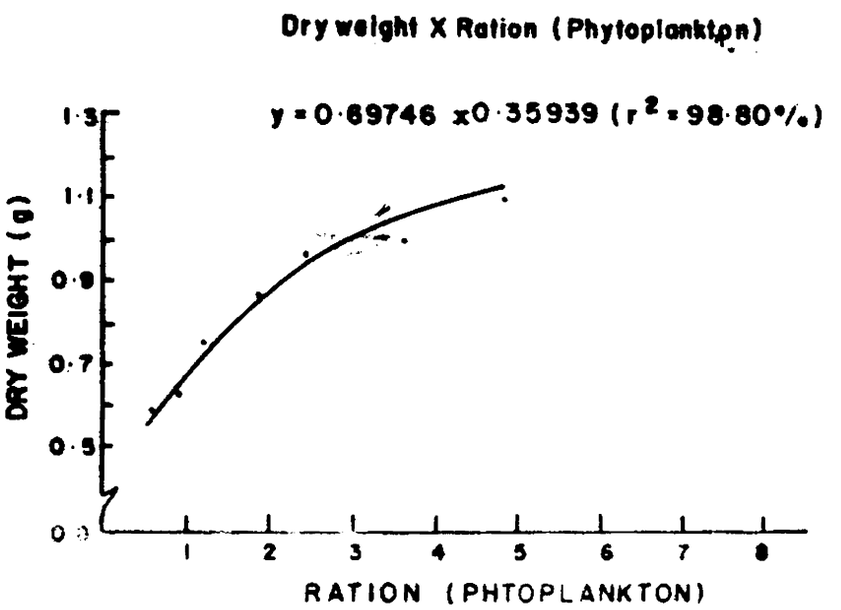
Note: Mean values at initiation (day 1) were : dry wt. - 0.504 g; maturity stage - 0

Fig.16 EFFECT OF RATION (PHYTOPLANKTON) ON DRY WEIGHT IN *PERNA INDICA*

Rations: chlorophyll *a* (μg) animal^{-1}; day^{-1}

[1] 0 [2] 0·6–1·2 [3] 0·9–1·8
[4] 1·2–2·4 [5] 1·8–3·6 [6] 2·4–4·8
[7] 3·6–7·2 [8] 4·8–9·6 [9] 9·6–19·2





The treatments 4, 5 and 6 which received rations in the range 1.2 µg - 4.8 µg chlorophyll *a* showed moderate increase in dry weight, in direct relation to feeding levels up to day 15, (Fig. 16) the percentage increase being 18.8 %, 37.7 % and 56.5 % from the initial mean of 0.504 g. Between days 15 - 30, the net increase in growth was respectively 10.2 %, 3.2 % and 0.4 % in T-4 through T-6. However, in the next fortnight, the net growth improved to 14.1 %, 21.8 % and 23.5 % respectively in the treatments.

The growth response to feeding levels 3.6 µg - 19.2 µg chlorophyll *a* was very high in the first fortnight, the increase in dry weight being 83.3 %, 110.3 % and 116.3 % on the initial mean weight of 0.504 g in treatments T-7, T-8 and T-9 respectively. As in the case of the group T-4 to T-6, the net growth in the second fortnight was minimal (6.9 %, 2.8 % and 4.6 %) followed by a slight improvement (8.8 %, 2.8 % and 6.1 %) during the third fortnight. It is observed that doubling of the ration from 4.8 - 9.6 µg (T-8) to 9.6 - 19.2 µg chlorophyll *a* (T-9) had only a marginal effect on growth.

The data on dry weight attained on the conclusion of the experiment (day 45) were statistically tested by analysis of variance (Snedecor and Cochran, 1967) for significance of the variations due to different feeding levels and the results presented in Table 29 showed high significance at $P < 0.01$ level.

Table 29 Analysis of variance of animal weight (dry weight) on day 45 at different feeding levels (data from Table 28) in *Perna indica*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatment	7	1.532036	0.218862 **
Error	24	0.647260	0.002697
Total	31		

** Highly significant $P < 0.01$

When the mean values of dry weight in different treatments were compared, it was seen that they were significantly different from one another at $P < 0.05$ level, except between T-1 and T-2, the pair of these mean values being not significantly different.

For different chlorophyll *a* concentrations (T-1 to T-8) the growth of mussel, as represented by dry weight, showed a logarithmic relationship (Fig. 16) and the equation is

$$y = 0.69747\, x^{0.35939}$$

with $r^2 = 98.80\ \%$, where x = chlorophyll *a* concentration and y = dry weight.

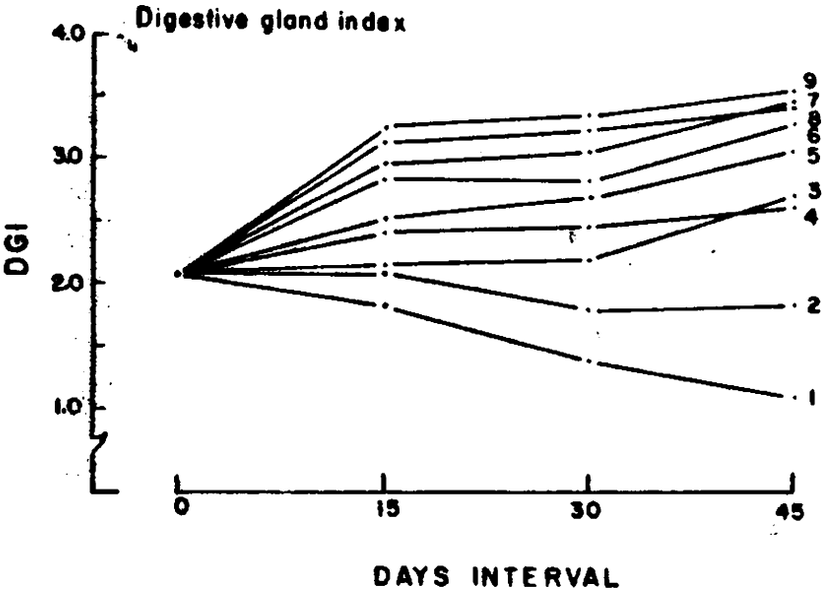
Digestive gland index (DGI)

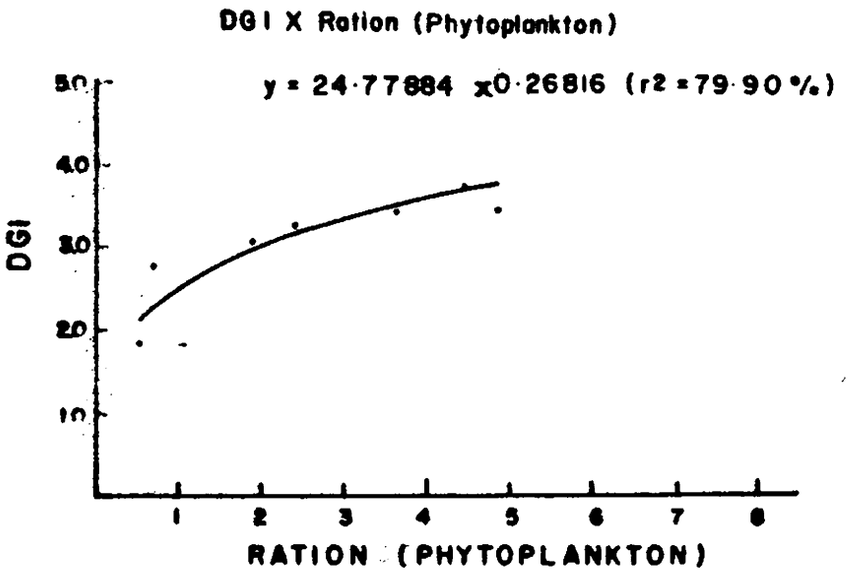
The data on digestive gland index (DGI) of *Perna indica* for different feeding levels are presented in Table 30 and Fig. 17. In the control (T-1) there was steady decline in

Fig 17 EFFECT OF RATION (PHYTOPLANKTON) ON DIGESTIVE GLAND INDEX IN *PERNA INDICA*

Rations: chlorophyll a (µg) animal^{-1}, day^{-1}

[1] 0 [2] 0·6–1·2 [3] 0·9–1·8
[4] 1·2–2·4 [5] 1·8–3·6 [6] 2·4–4·8
[7] 3·6–7·2 [8] 4·8–9·6 [9] 9·6–19·2





DGI, from 20.84 on day 1 to 11.17 on day 45, the loss being 46.40 %. Thus, the animals which did not receive any ration survived (no mortality during experiment), fastly depleting the energy reserves from the digestive gland and other parts of the body (58.3 % loss of dry weight of animal).

Treatment-2 (0.6 - 1.2 µg chlorophyll a/animal/day) also showed progressive decrease in DGI, the net loss being 12.43 % and the ration was obviously inadequate for maintenance metabolism of the animals. From Fig. 17 it is seen that in T-3, there might have been an experimental error in the calculation of DGI on day 45, as the value exceeds that of T-4 with a higher ration. The animals showed a slight improvement in DGI on day 45 in T-4, the net increase being 29.03 %. Even at this level, as can be seen later, growth of gonad has not commenced.

In treatments T-5 to T-9, there was consistent increase in DGI, with a spurt of accumulation of nutrient reserves between days 1-15, moderate increase between days 15-30, and further improvement between days 30-45. The net increase of index ranged from 49.09 % in T-5 to 72.07 % in T-9 on day 45.

Statistical treatment of data on digestive gland index for different feeding levels, by analysis of variance, showed high significance (Table 31).

Table 30 Effect of different feeding levels of phytoplankton on energy storage and reproductive activity in the brown mussel *Perna indica*

Sl. No.	Chlorophyll *a* (μg animal^{-1} day^{-1})	DGI on day			MGI on day		
		15	30	45	15	30	45
1.	0 control	18.61	14.12	11.17	0	0	0
2.	0.6 - 1.2	21.00	18.14	18.25	0	0	0
3.	0.9 - 1.8	21.22	21.96	27.24	0	0	0
4.	1.2 - 2.4	24.07	24.72	26.89	0	0	0
5.	1.8 - 3.6	25.29	26.45	31.07	0.15	0.41	1.31
6.	2.4 - 4.8	27.91	27.65	32.90	0.54	1.41	2.00
7.	3.6 - 7.2	29.60	30.69	34.77	1.46	2.50	3.00
8.	4.8 - 9.6	31.69	32.04	34.16	1.92	3.00	3.00
9.	9.6 - 19.2	32.17	33.11	35.86	2.00	3.00	3.00

Note: Mean values at initiation (day 1) were: DGI - 20.84; MGI - 0. DGI - digestive gland index; MGI - mean gonad index.

Table 31 Analysis of variance of digestive gland index of *Perna indica* on day 45 at different feeding levels (data from Table 30).

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	7	918.94850	131.27837 **
Error	24	129.204550	5.3835229
Total	31		

** Highly significant $P < 0.01$

Testing the DGI mean values statistically, it was found that T-3, T-5 and T-6 values do not differ significantly from one another at $P =< 0.05$ level, so also T-6, T-7, T-8 and T-9 values which had resulted in gonad growth and gametogenesis.

The increase in DGI for different feeding levels is exponential (Fig. 17) and the relationship is described by the equation

$$y = 24.77884\ x^{0.26816}$$

with r^2 value of 79.90 %, where x = feeding level and

y = digestive gland index.

Mean Gonad index and gametogenesis

The mean gonad index (MGI) and stage of maturity of the animals have been shown in Table 30. The animals in control, as well as treatments T-2, T-3 and T-4 did not show

any reproductive activity till the end of experiment. Gonad growth commences with treatment T-5 at the ration of 1.8 - 3.6 µg chlorophyll a/animal/day and MGI values increase in tune with increasing feeding levels as can be seen from Fig. 18. On day 15, all the animals under these treatments had entered the gametogenic phase, the highest MGI value of 2.00 was achieved in T-9 with the highest ration of 9.6 - 19.2 µg chlorophyll a/animal/day, closely followed by the gonad index of 1.92 in T-8 with half the ration of that of T-9. On day 30, the MGI values have increased further indicating intense gametogenic activity and the highest value of 3.00 was achieved both in T-8 and T-9. On day 45, with further increase in gametogenesis, the animals in T-7 receiving ration of 3.6 - 7.2 µg chlorophyll a/animal/day, had also reached MGI 3, and those in T-8 and T-9 had maintained at MGI 3 level. In T-5, with a ration of 1.8 - 3.6 µg a chlorophyll, and T-6, with 2.4 - 4.8 µg chlorophyll a concentration in the food, the animals had attained MGI values of 1.31 and 2.00 respectively.

The mean gonad index has a linear relationship with the dry weight (Fig. 18) described by the equation

$$y = 10.2546 + 21.7211\ x$$

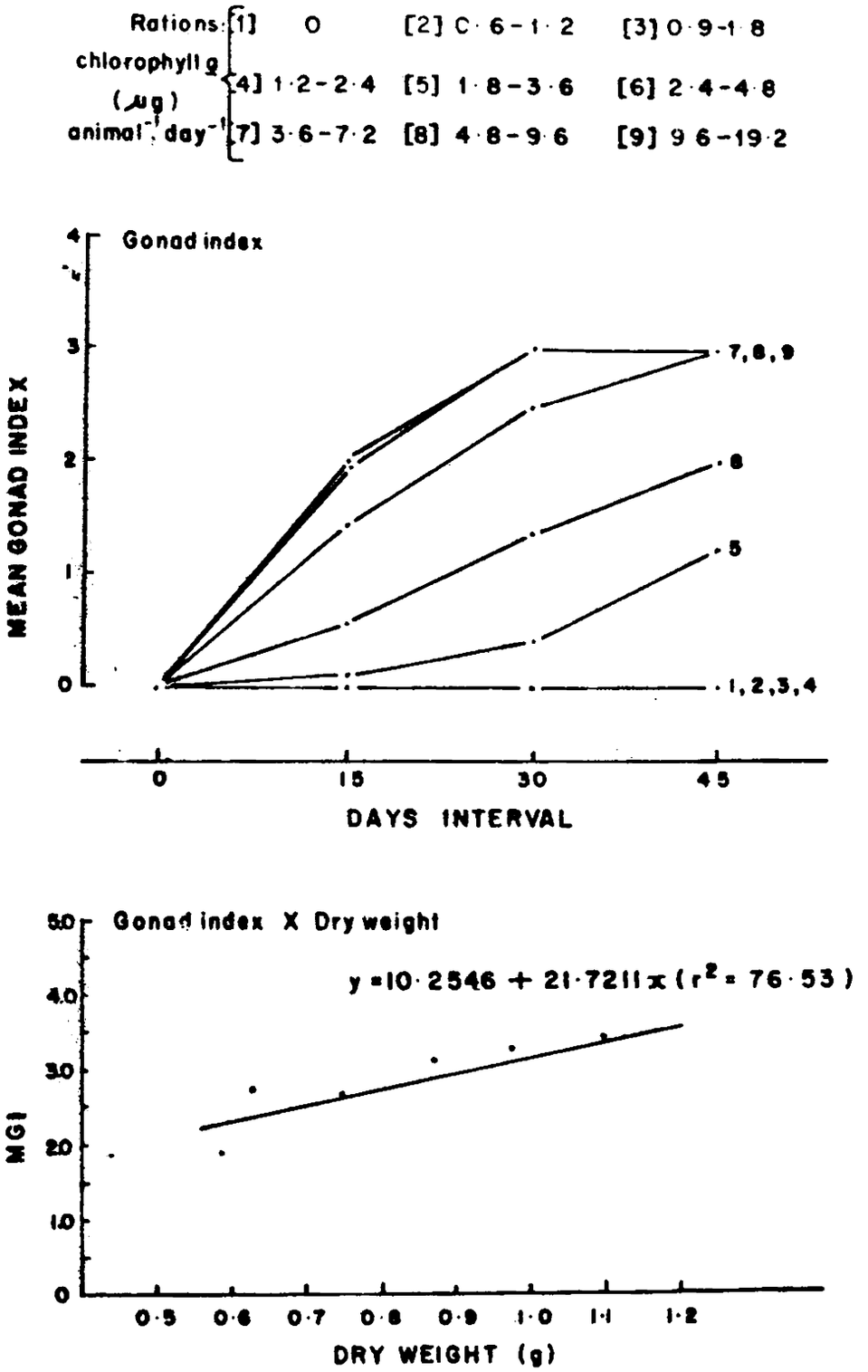
with r^2 = 76.53 %, where x = dry weight (g) and

y = mean gonad index.

Glycogen storage and utilisation

The glycogen content in the mantle of the experimental animals on day 45 was studied histochemically and the

Fig.18 EFFECT OF RATION (PHYTOPLANKTON) ON MEAN GONAD INDEX IN *PERNA INDICA*



results, along with percentages of animals in different stages of maturity, are given in Table 32. In the control, there was no trace of glycogen. In treatments T-2 to T-4, when the mussels were in the vegetative phase, glycogen started accumulating in the mantle. With increase in feeding levels, there was corresponding increase in glycogen level, with high positivity in T-4 at a ration of 1.2 - 2.4 µg chlorophyll *a*/ animal/day.

Gonad growth with gametogenesis commenced in T-5 with 1.8 - 3.6 µg chlorophyll *a* concentration and the highest accumulation of glycogen was noticed in this group. The stages of maturity are distributed with 16.7 % of mussels in stage 0, 45.8 % in stage 1, 16.7 % in stage 2 and 20.8 % in stage 3. Glycogen accumulation and utilisation goes concurrently with more reserves. From T-6 onwards, with higher rate of gametogenesis with increasing feeding levels, there is faster utilisation of glycogen to maintain this active phase. The treatment in which 100 % of the mussels reached stage 3 on day 45 is T-7 with a ration of 3.6 - 7.2 µg chlorophyll *a* concentration and the mantle glycogen level is as high as in T-6. In treatments T-8 and T-9 which did not show glycogen levels any higher than T-6 and T-7, the reserve accumulation could have been in other nutrients such as proteins and lipids which were not looked into in this experiment. Since the objective of the experiment was restricted to investigating the influence of ration on maturation of the mussels, it was not continued beyond day 45, when positive results were obtained.

Table 32 Development of ration-induced maturity and level of glycogen in the mantle tissue of brown mussel *Perna indica* in 45 days of treatment.

S.No.	Feeding level chlorophyll *a* (μg) animal^{-1} day^{-1}	Percentage of animals in maturity stage				Glycogen
		0	1	2	3	
1.	0 control	100	-	-	-	-
2.	0.6 - 1.2	100	-	-	-	±
3.	0.9 - 1.8	100	-	-	-	± +
4.	1.2 - 2.4	100	-	-	-	+ +
5.	1.8 - 3.6	16.7	45.8	16.7	20.8	± + +
6.	2.4 - 4.8	-	29.2	20.8	50.0	+ +
7.	3.6 - 7.2	-	-	-	100	+ +
8.	4.8 - 9.6		-	-	100	+ +
9.	9.6 - 19.2	-	-	-	100	+ +

Therefore, the relation of nutrient storage cycle and spawning (stage 4) has not been studied.

DISCUSSION

The results show a good correspondency among the three factors investigated, namely the growth of the brown mussel *P. indica* (dry weight increase), digestive gland index and mean gonad index for different levels of ration. Starvation of the animal (control) leads to a weight loss of 58.3 % on day 45. Fox and Coe (1943), as reported by Thompson *et al.* (1974), found an approximate 50 % loss of initial somatic tissue weight in starved *Mytilus californianus* and the weight loss in the present case appears to be more rapid. Figures 15 and 16 show the diminishing changes taking place in the mussel due to stress of starvation. The feeding level which can sustain the basal metabolism of the animal appears to be the ration of T-4, that is 1.2 - 2.4 µg chlorophyll *a*/animal/day (Table 28 and 30, dry weight and DGI data). Rations below this level might result in under-nutritional stress and above this level lead to growth, reserve accumulation and gametogenesis.

Growth (dry weight increase) and digestive gland index show increase with increasing levels of ration. Once the nutritional level for basal metabolism is provided for, growth depends on the additional amounts of ration at the levels of treatment in the experiment.

Figures 15 and 16 would show that growth is considerable during the first fortnight, but generally tends towards a plateau during the second fortnight in spite of an increase in ration and shows a rise during the third fortnight. The second fortnight is a period of intense gametogenic activity and it would appear that much of the energy is utilised for this. Further increase in gametogenic activity is related to the higher levels of ration. This trend in growth is reflected in the mean gonad index too (Fig. 17). Treatments 8 (chlorophyll a 4.8 - 9.6 µg/animal/day) and 9 (9.6 - 19.2 µg/animal/day) do not differ in promoting gonad growth and gametogenesis (Fig. 17) and would show that, under the given experimental conditions, additional amount of phytoplankton may not be assimilated by mussels as in T-9. The present observations on growth and gametogenesis, as related to ration, corroborate the finding of Velez and Epifanio (1981) in Perna perna that the greatest growth and gonadal development occurred when fed a high ration (0.050 - 0.018 mg-algae mg-mussel^{-1}). But their observation that at high ration gametogenesis is inhibited by high temperature (28°C) but most advanced in low temperature (21°C) could not be substantiated in the present study (temperature range 24.8 - 28.4°C).

An interesting feature observed during the present investigation is that all the mussels in the gametogenic phase were males. Velez and Epifanio (1981) found for Perna perna that, in all the experimental groups, the majority of sexually differentiated individuals were males, particularly those

-ed with high temperature (28°C). However, their data show that even at low temperature (21°C) all animals fed low ration developed into males (stage 1 and stage 2) except 6 % of the animals in sexual regression (stage 4) in the female phase. Tranter (1958 b) observed in the Australian pearl oyster Pinctada albina that the species, which is a protandric hermaphrodite, responds differentially to achieve the sexual phase suited for its nutritional condition; oysters with low food reserves may reproduce less efficiently as females than as males. It was considered that germ cell rudiments might respond to the food reserve level, developing toward maleness if at a lower level. Sastry (1968) observed in the case of Argopecten (= Aequipecten) irradians that at 20°C oocyte growth is suppressed if food is absent but both spermatozoa and oocytes develop in the presence of food. Thus, there is evidence that in some of the marine bivalves development of gonad toward either sex is dependent on food reserves.

Fretter and Graham (1964) considered Mytilus californianus as gonochoristic and Ahmed and Sparks (1970) considered that M. edulis and M. californianus reproduce as true males and females possibly due to crossing over and recombination of genes concerned with sex determination.

During the present study, the natural populations of P. indica were observed to be either true males or true females and hermaproditic condition was never observed. Nutrient reserve-dependence seems to be the only plausible

explanation that can be attributed to the present case where all the experimental animals developed into males. It is probable that the ration supplied to the mussels, although meet the requirements for gonad growth and male gametogenesis, was not adequate to provide the threshold energy for the differentiation of oocytes which require higher energy reserves. The data of Velez and Epifanio (1981) should also be taken to corroborate this statement, although these authors were inclined to attribute the male dominance to higher temperature. Another piece of supporting evidence coming from the observation of wild populations of P. **indica** in the area of study is that during March 1982 which is the month of low phytoplankton productivity (chlorophyll *a* mg/m^3), there were more males than females in the ratio 80:20. With gradual increase in phytoplankton, reaching the peak value of 25.6 mg/m^3 in May 1982, the sex ratio shifted to 55:45, subsequently reaching a 50:50 ratio in June, which is the first peak month of spawning (Chapters III and IV).

The present study has shown that gametogenic process in the tropical mussesl can be induced outside the normal reproductive season through appropriate feeding levels. Further detailed investigations would be required, particularly towards finding the threshold levels of ration for cogenesis, for application of the induced maturation principle in practical aquaculture of mussels.

CHAPTER X

NEUROSECRETION AND REPRODUCTION

INTRODUCTION

Scharrer (1935), for the first time, reported the occurrence of neurosecretory cells (NSC) in the molluscs, based on a study of opisthobranchiate molluscs. After two decades, Gabe (1955) reported the presence of secretory neurons in 20 species of lamellibranchs. Since then several workers have studied the structure, cytochemistry and distribution of neurosecretory cells in the central nervous system, particularly in the cerebral (cerebropleural), visceral and pedal ganglia and their probable role in metabolism and reproduction of lamellibranchs.

Lubet has made substantial contributions to the studies on neurosecretion, starting from his base-line experimental studies in Mytilus edulis and Chlamys varia (Lubet, 1955 a, b; 1956) to his more recent works on M. edulis involving ganglion extirpation, grafts and organ cultures (Lubet and Mathieu, 1982). The ultrastructure of the neurosecretory cells of M. edulis has been studied with electron microscope by Illanes (1979). Among others, Fahrenmann (1961) reported neurosecretory granules in the freshwater mussel Unio tumidus; Antheunisse (1963) worked on the

neurosecretory cycle in Dreissena polymorpha; Semaya (1965) postulated a relationship between neurosecretory and reproductive cycles in M. (Perna) perna ; Umiji (1969) reported neurohaemal area in cerebral commissure of Mytilus perna and experimented on ganglion ablation; and Blake (1972) examined the effect of temperature and starvation on neurosecretory activity and oogenesis in Argopecten (= Aequipecten) irradians.

The Indian workers have made significant contributions to the studies on lamellibranch neurosecretion, both in marine and freshwater bivalves, pioneered by Nagabhushanam on Crassostrea virginica (1962 a; 1963a,b), Spisula solidissima (1962 b), Bankia (Bankiella) gouldii (1962 c), Voldia limatula (1963 c), Modiolus demissus (1964 a), Mulinia lateralis (1964 b), Martesia striata (1969), Meretrix casta (1969), Diplothyra smithii (1970) and Parreysia corrugata (1972), which include some of the earlier works carried out in the United States of America. The neurosecretory activity in Katelysia opima was studied by Nagabhushanam and Mane (1973), in Crassostrea cucullata by Nagabhushanam and Bidarkar (1975) and in Donax cuneatus by Nagabhushanam and Talikhedkar (1975). Similar contributions have been made in India on freshwater bivalves: on Lamellidens corrianus (Lomte and Jadhav, 1981), Parreysia corrugata (Nagabhushanam and Lomte, 1973) and Indonaia caerulea (Hanumante et al., 1979).

Endocrine glands or neurohaemal organs have not been identified in bivalve molluscs. Based on morphological, cytological and cytochemical studies, experimental evidences

and the cyclical nature of neurosecretory products exhibiting close correlation with metabolic functions of the bivalves, certain cells in the ganglia of the central nervous system have been identified as neurosecretory cells with implicit endocrine functions. Such cells have been reported in the dorsal cell caps of cerebral ganglia and the dorsal cell layer of visceral ganglia (Lubet, 1955 b; Nagabhushanam, 1963 a, b, c and others). Neurosecretory cells are not usually found in the pedal ganglia of bivalves (Sastry, 1979), although such cells are reported in the pedal ganglia of freshwater bivalves (Fahrmann, 1961; Antheunisse, 1963).

The presence of more than one kind of neurosecretory cells, based on size, in the ganglia of M. edulis was reported by Lubet (1959). Later Nagabhushanam (1962 a) demonstrated two cell types, Type I and Type II, in C. virginica based on differential staining techniques. Such typing was followed by later workers and, more recently, Lubet and Mathieu (1962) described three neurosecretory cell types, designated Type a_1, Type a_2 and Type a_3, in M. edulis based on signaletic stains for neurosecretion. The stages in neurosecretory cycle have been described by Nagabhushanam (1968) for Martesia striata and by Nagabhushanam and Sidaraar (1975) for C. cuculiata. Axonal transport of neurosecretory material has been suggested in Mercenaria mercenaria (Welsh, 1961), Martesia striata (Nagabhushanam, 1968) and Diplothyra smithii (Nagabhushanam, 1970). However, as stated by Sastry (1979), the chemical

nature, transport and fate of neurosecretory products have not so far been clearly established.

Besides other metabolic functions such as growth, glycogen metabolism, osmoregulation and water filtration (Nagabhushanam, 1964 a; Lubet, 1966), neurosecretion has been found to play a predominant role in the control of reproduction of bivalves. This control has been inferred from the cyclical activity of the neurosecretory cells (NSC) which shows a close correlation with and which is superimposable on the seasonal or annual reproductive cycle (Lubet, 1955, 1956, 1966; Fahrmann, 1961; Antheunisse, 1963; Nagabhushanam, 1962 a, 1968; Lucas, 1965). The integrity of cerebral ganglia was found indispensable for the normal development of the reproductive cycle in M. edulis (Lubet 1955 a, 1959), C. virginica (Nagabhushanam, 1962 a), D. polymorpha (Antheunisse, 1963) and K. opima (Nagabhushanam and Mane, 1973). Neurosecretory material builds up in the NSC of the cerebral ganglion during active gametogenic phase and is evacuated just before spawning. Neurosecretion from the visceral ganglia has been found to be responsible for the metabolic activities (Lubet, 1966; Nagabhushanam, 1968). Lubet (1966) has drawn attention to the 'very short' neurosecretory cycles which are due to experimental stresses (thermal, osmotic) and the 'long' cycles which are associated with regular biological and environmental cycles such as reproduction, metabolism and seasonal variations of factors such as temperature and salinity and laid emphasis on taking special precautions in the study of annual neurosecretory

cycle as sudden disturbance of external factors can considerably modify cytological properties of neurosecretion.

Considerable experimental work has been done in several species of bivalves on ganglion ablation and its effects on reproduction: on M. edulis and C. varia (Lubet, 1956), C. virginica (Nagabhushanam, 1964 a), M. edulis (Lubet, 1966), K. opima (Nagabhushanam and Mane, 1973), P. corrugata (Nagabhushanam and Lomte, 1973), P. cumeatus (Nagabhushanam and Talikhedkar, 1975) and C. cucullata (Nagabhushanam and Bidarkar, 1975). Cerebralectomy has shown specific response to reproductive cycle and visceralectomy to metabolism, as a rule, in the marine bivalves that have been experimented upon. Lubet (1959, 1966) reported that bilateral cerebral ablation on M. edulis in the resting (stage 0) and gametogenic (stages 1 and 2) phase leads to significant delay in sexual phenomenon, and when the cerebral ganglia are extirpated at the end of gametogenesis, discharge of gametes was more rapid.

Lubet and Mathieu (1982) observed that achievements of gametogenesis in adult M. edulis, Crassostrea gigas and Ostrea edulis, during the annual reproductive cycle, depends upon the action of several neuroendocrine factors. They further stated that the neuroendocrine factors seem to be neither sexualised nor specific. Lubet and Mathieu (1982) concluded that 'It is therefore impossible to rule out the complication that the neurohormone may not act directly upon

the germ cells but may instead act through intermediary cells in the storage tissue'.

During the course of the present study, efforts were made to map the neurosecretory cells in the central nervous system, study the annual neurosecretory cycle in relation to the reproductive cycle and obtain experimental evidence on the role of neurosecretion in spawning by ganglion extirpation in the two species of mussel Perna viridis and P. indica.

MATERIALS AND METHODS

Identical methods were used in the study of neurosecretion in the two species of mussels, Perna viridis from Elathur and P. indica from Vizhinjam. Ten mussels, in the size range 45 - 60 mm, were collected every fortnight from October 1981 to December 1982 and the whole animals were fixed in Bouin's fluid immediately in the field.

In the laboratory the cerebral (cerebropleural), visceral and pedal ganglia were carefully dissected out from the mussels with part of the commissures (in the case of cerebral and visceral ganglia) and the surrounding tissues were removed to the maximum extent possible. The ganglia were processed for histological studies using standard techniques as described in Chapter IV. Serial sections of 6 - 8 µm thickness were prepared.

Mallory's triple stain (Mallory, 1944) was used for one slide in each series to differentiate the ganglion from

the surrounding tissue. Regular staining was done using one of the three methods, namely (1) Gomori's (1939) Chrome-Hematoxylin Phloxine method, (2) Gomori's (1950) Paraldehyde Fuchsin method and (3) Dogra and Tandan's (1964) modified Performic Acid/Victoria Blue method. In the third method the sections were stained at 50 % alcohol level. For each sample of animals, the ganglia were stained independently in all the three methods for detailed cytological studies. After staining, the sections were dehydrated in the ascending series of alcohol grades, cleared in Xylol and mounted in DPX. The sections were examined under light microscope.

Ganglion ablation experiments were carried out during July-August, 1982 on Perna indica at Vizhinjam and during May-June 1983 on P. viridis (obtained from Ennore backwaters) at the Kovalam Field Laboratory near Madras, when the animals were in sexually ripe condition. Fifty mature animals were used in each of the experiments. Identical procedures were followed for both the species.

The animals were acclimated in the laboratory by keeping them for 24 h in tubs containing well aerated seawater. Sex and ripeness of gonad were confirmed by examining smear from the mantle taken after inserting a wooden peg between the shells without causing any tissue damage. Ripe males and females were segregated. Ganglion ablation was performed, after narcotising the animals in menthol sprinkled seawater for 2-4 h, using a surgical kit originally developed for nucleus implantation in pearl oyster (Alagarswami and

Sivarajan, 1975). Inserting the speculum between the valves a controlled shell gap of about 1 cm was obtained and the animal was mounted on a stand with the speculum in position. Using surgical tools, unilateral and bilateral extirpations of cerebral, visceral or pedal ganglia as per experimental protocol were carried out. In all cases tissue damage, which was unavoidable, was kept to the minimum. A batch of mussels was sham operated at the sites of cerebral, visceral and pedal ganglia together and maintained as control I, as it was difficult to make concurrent observations on shams for individual treatments. Since the combined effect will be more than individual effect, the data can be considered for comparison. Animals in the natural state were kept as control II.

After ganglion extirpation, the mussels were returned to continuously aerated seawater and observations were made on recovery. Fully recovered mussels were transferred to 20-l plastic tubs and maintained with aeration and change of sea water for 72 hours for observations on mortality and spawning response. They were so arranged in the tubs that reaction of each individual mussel could be noted.

The temperature and salinity ranges of the experimental medium were 28° - 30°C and 36 - 37 ppt for *P. viridis*, and 26° - 28°C and 34 - 35 ppt for *P. indica*.

RESULTS

Neuroendocrine control of reproduction in wild populations

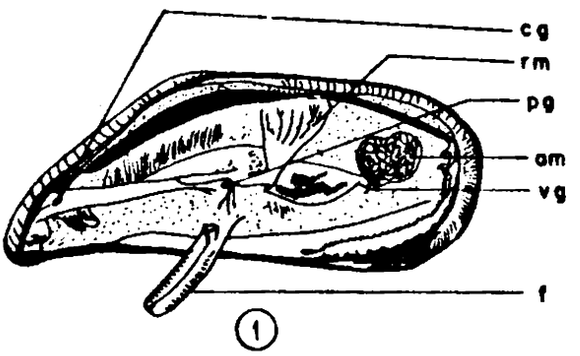
Nervous system

The nervous system of Perna indica and P. viridis consists of three pairs of ganglia, namely the cerebral (cerebropleural) ganglia, visceral ganglia and pedal ganglia joined by paired connectives (Fig. 19). The pairs of cerebral and visceral ganglia are well separated and are connected by intercerebral and intervisceral commissures respectively. The cerebral ganglia are located anteriorly dorsal to the labial palps. The visceral ganglia are located posteriorly on the ventral side below the posterior adductor muscle. The pedal ganglia are placed above the origin of foot. The cerebral and visceral ganglia are connected directly by the cerebrovisceral connectives. The pedal ganglia are not connected directly to either of the cerebral or visceral ganglia, but the cerebro-pedal connective joins the cerebro-visceral connective posterior to the cerebral ganglia (Fig. 19).

Neurosecretory cells

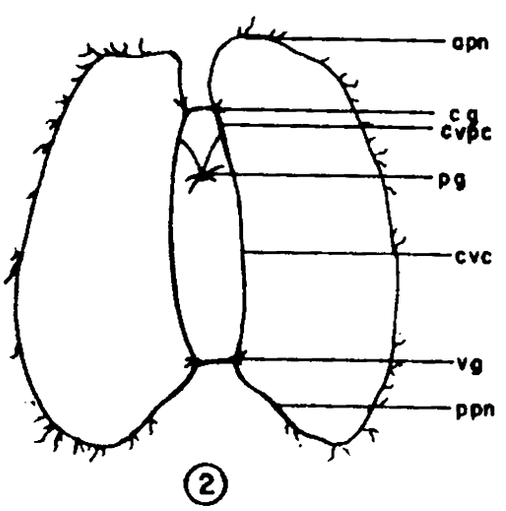
The presence of neurosecretory cells (NSC) in the nervous system of P. indica and P. viridis was ascertained histologically by their staining properties. The cerebral, visceral and pedal ganglia were examined and the results were identical for both species. The characteristic pattern of staining of nucleus, cytoplasm and neurosecretory material in the neurosecretory cells is given in Table 33.

FIG. 19 Dissection showing the location of ganglia and connectives in the brown mussel Perna indica



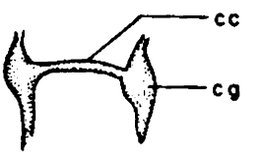
①

Schematic representation of ganglia and nervous system of the mussel Perna indica

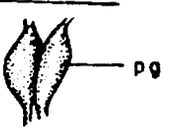


②

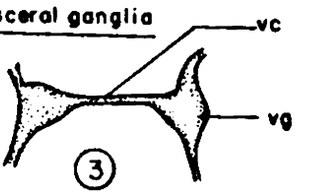
Cerebral ganglia



Pedal ganglia



Visceral ganglia



③

am – adductor muscle ; apn - anterior pallial nerve ; c c – cerebral commissure ; cg-cerebral ganglion ; cpc - cerebro pedal connective ; pg – pedal ganglion ; cvpc - cerebro visceral pedal connective ; cvc - cerebro visceral connective ; f – foot ; vc – visceral commissure ; vg – visceral ganglion , ppn - posterior pallial nerve ; rm – retractor muscle.

Table 33 Characteristic staining properties of neurosecretory cells of *P. indica*

Stain	Nucleus	Cytoplasm	Neurosecretory material
Mallory's triple stain	dark pink	pink	reddish pink
Chrome-hematoxylin phloxine	purple	reddish pink	deep purple or blue black
Victoria blue	dark blue	light blue	greenish blue
Paraldehyde fuchsin	pink	purple	deep purple

Neurosecretory cells were present in the cerebral ganglia and visceral ganglia. Pedal ganglia of *P. indica* and *P. viridis* did not show presence of neurosecretory cells.

Cerebral ganglia: In the cerebral ganglia, the neurosecretory cells are distributed in the peripheral area (Pl. 8, A). The inner area is occupied by neurons which stain lightly. In the peripheral distribution of NSC, a greater aggregation is found on the dorsolateral surface of the ganglia (Pl. 8, C and D). NSC are absent in the cerebral commissure.

Visceral ganglia: The distribution of NSC in the visceral ganglia follows the same pattern as in cerebral, with peripheral occupation and aggregation in the dorsolateral surface. The presence of NSC has been detected in the visceral

PLATE 8

A. Cerebral ganglion of *P. indica*. The neurosecretory cells are distributed in the peripheral area of ganglion. The NSC are absent in the cerebral commissure.

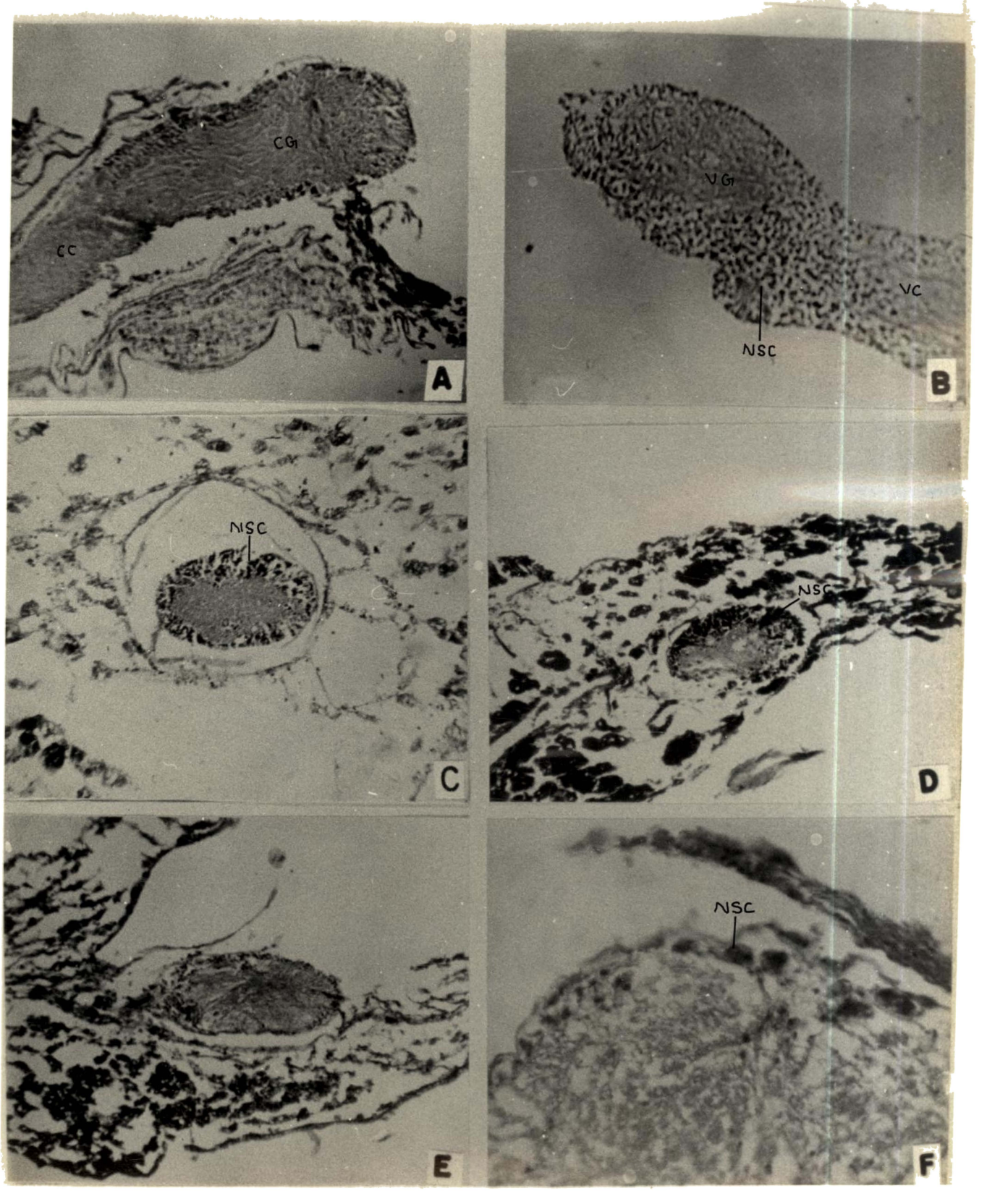
B. Visceral ganglion of *P. indica*. The NSC are distributed in the ganglion as well as visceral commissure.

C&D Cerebral ganglion of *P. indica*. The NSC are seen peripherally with greater aggregation in the dorsolateral region.

E&F Cerebral ganglion of *P. indica*. The NSC are fewer in the non-productive phase.

CC - Cerebral commissure; CG - Cerebral ganglion; NSC - Neurosecretory cells; VC - Visceral commissure; VG - Visceral ganglia.

PLATE 8



commissure also, the cells being distributed peripherally throughout the length of the commissure (Pl. 8, B). Staining of the NSC in the visceral commissure is prominent immediately after the spawning of the mussels.

Neurosecretory cell type

The neurosecretory cells in the cerebral ganglia of the mussel P. indica and P. viridis conform to a single cell type. They are pyriform and unipolar and measure 15 to 20 µm in length (Pl. 9, B). Variations within the general pyriform shape of NSC noticed are illustrated in Fig. 20 (A-D). The single spherical nucleus is either central or eccentric in position. Vacuoles are found in the cytoplasm of the NSC depending on the stage of neurosecretory activity. The neurosecretory material (NSM) is in the form of fine granules.

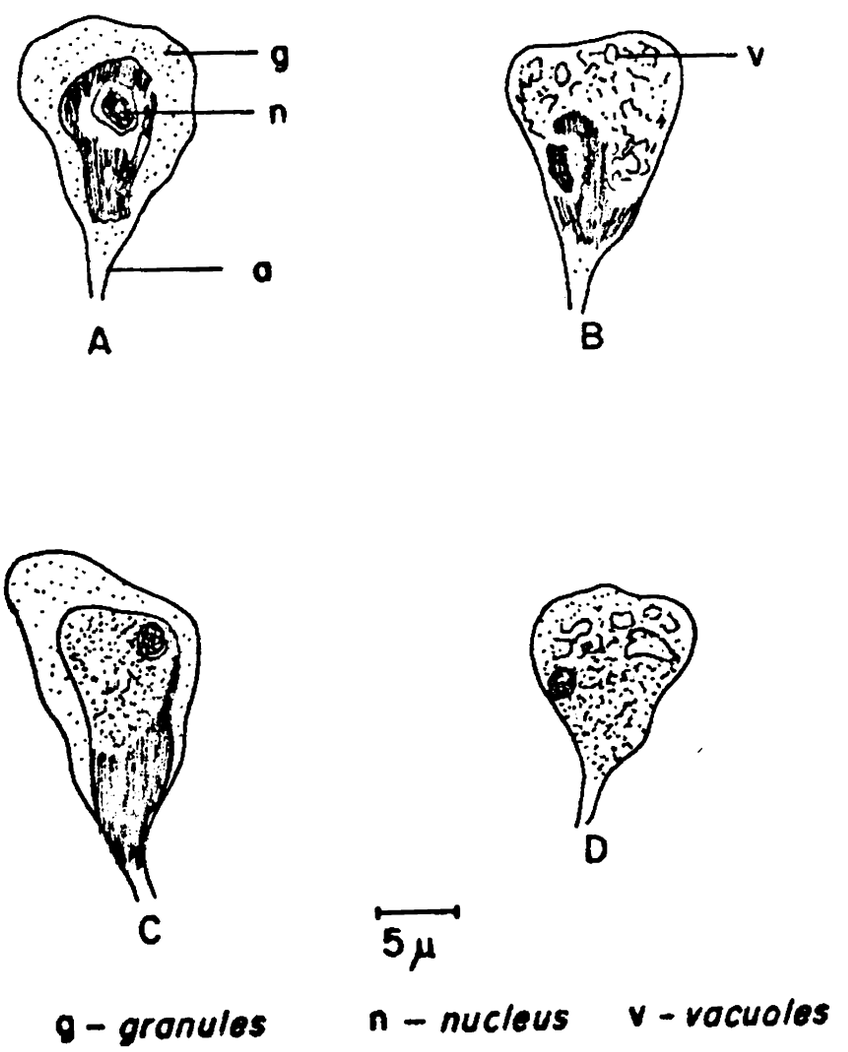
Neurosecretory stages of NSC

The characteristic staining property of neurosecretory material (with Mallory's triple stain) in the NSC of cerebral ganglia of P. indica and P. viridis made it possible to determine four successive stages of neurosecretory activity, related to the stage of maturity of the gonad, which are described below (Fig. 21):

Stage 1: Neurosecretory material (NSM) distributed uniformly throughout NSC with light staining property; vacuoles not generally present.

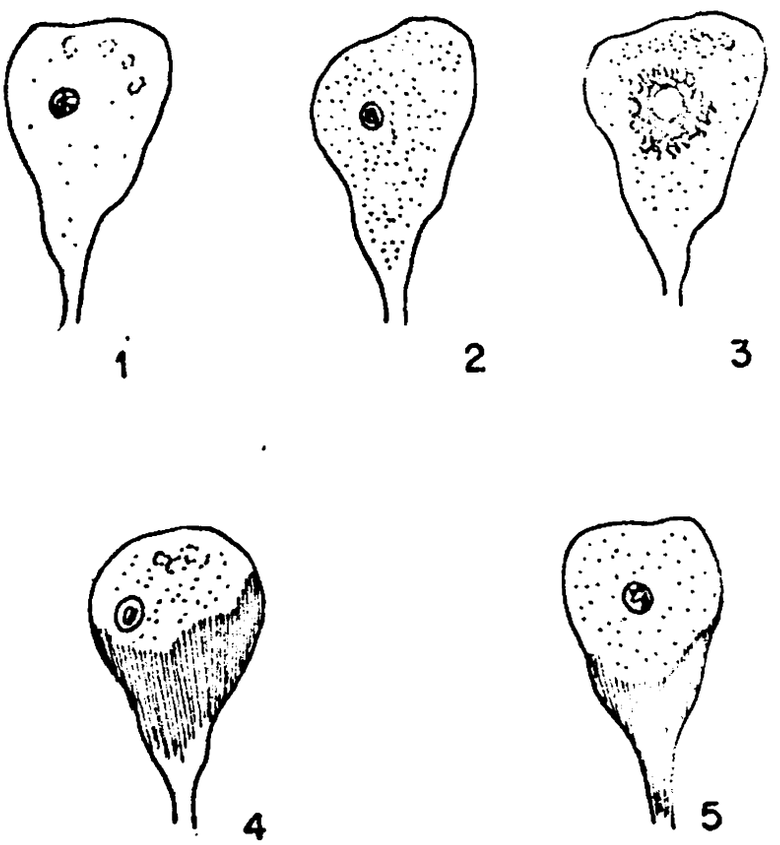
FIG. 20 Single type of pyriform neurosecretory cells present in the cerebral ganglia of Perna indica and Perna viridis. Within the general pyriform shape variations (A, B, C, D) are seen.

(Figures drawn with camera lucida)



a – *axon* g – *granules* n – *nucleus* v – *vacuoles*

FIG. 21 Different stages of neurosecretory activity in the NSC of Perna indica and Perna viridis as deduced from their staining property. The stages 1 - 5 are arbitrary as the process of N S M. secretion, accumulation and evacuation is continuous in a given cycle of activity.



Stage 2: NSM is concentrated around the nucleus with deep staining property; vacuoles may or may not be present in the peripheral area of cytoplasm.

Stage 3: NSM accumulates in the axonal hillock with deep staining property; vacuoles are generally present.

Stage 4: NSM enters the axon of NSC, implying axonal transport of neurosecretory material; NSC stains very lightly except in the axonal region where stain is deep; vacuoles are present.

Beyond stage 4, when NSM has been fully discharged, the NSC in the inactive phase can be recognised only by the pyriform shape and the presence of vacuoles.

Generally, stage 3 of neurosecretory activity can be more readily recognised as it is the most common stage during the peak period of maturation of gonad. The transitional stages are commonly seen with the NSM going through the processes of secretion, accumulation and evacuation.

Cytochemistry of NSM

The chemical nature of the NSM was characterised by staining the sections with specific cytochemical stains. The results of this study are presented in Table 34.

Table 34 Cytochemical characterisation of the neurosecretory material in the cerebral ganglia of *P. indica* and *P. viridis*.

Stain	Observation	Inference
Mercuric Bromophenol Blue	+ + + (dark blue)	Presence of Acidic proteins
Methylation + MBB	-	
Aqueous Bromophenol Blue	+ (blue)	Presence of basic proteins
Deamination + ABB	-	
Toluidine Blue	++ (purple)	Presence of acidic proteins
Methylation + TB	-	
Alcian Blue	+ + (blue)	Presence of acidic proteins
PAAB	-	
Schiff	-	No free aldehydes
Periodic acid + Schiff	++ (magenta)	Presence of glycogen and Vicenyl glycols
Diastase + PAS	-	Presence of glycogen
Bracco-Curtis test	+ +	
Sudan Black	+ (black)	Presence of lipids
Pyridine + SB	-	Confirms the presence of lipids
Nile Blue	+ + (blue)	Acidic lipids
Pyridine + NB	-	Confirms the presence of acidic lipids

The positivity to Mercuric Bromophenol blue indicated the presence of acidic proteins and aqueous bromophenol blue revealed basic proteins. The proteinaceous nature of NSM was further confirmed by Toluidine blue and Alcian blue stains. The presence of glycogen and vicenyl glycols was shown by the positivity to periodic acid-schiff and diastase + PAS reaction. The occurrence of glycogen in the NSM was further confirmed by Bracco-curtis test. The positive staining to sudan black indicated the presence of lipids. The NSM was also positive to Nile blue sulphate which confirms the acidic nature of the lipid moiety in the NSM. The cytochemical study revealed that the NSM is acidic and is glycolipoprotein in nature.

Neurosecretory cycle in relation to reproduction

The neurosecretory activity (NSA) in the NSC of cerebral ganglia of *P. indica* and *P. viridis* has been found to have some correlation with the annual reproductive cycle of the mussels. In this study animals of 45 - 60 mm length alone have been used and, therefore, only a single annual reproductive cycle of both species has been traced. Apart from seasonal differences in the reproductive cycle of *P. indica* and *P. viridis* as discussed in Chapter IV, the neurosecretory cycle was similar in both species. Therefore, *P. indica* is taken as the typical case and its neurosecretory cycle is described here.

October - January: The animals are in indeterminate stage (repr. stage 0). Neurosecretory cells are very few

(gg. 2-5 cells per section of ganglion) and stain light purple (NSA stage 1).

February - April: Gametogenesis commences in February (repr. stage 1) and intensive gametogentic activity is noticed in April (repr. stage 2). Increase in neurosecretory activity becomes evident during this period. The number of NSC is more (gg. 12-28 cells/section) and the neurosecretory material stains deep purple. The NSA stage 1 is dominant in February and neurosecretory activity progresses to stage 2 with concentration of NSM around the nucleus in April (Pl. 9, A-D).

May - July: Most of the mussels attain fully mature condition (repr. stage 3) during this period. The neuro-secretory activity is at a peak (NSA stage 3) with accumulation of NSM at the axonal hillock; the number of NSC is high (gg. 60-72 cells/section). The NSM stains deep orange to red. The nucleus is not visible due to the deep staining of NSM (Pl. 9, E). While NSA stage 3 is predominant during these months, some NSC show lower activity (NSA stage 2).

August: This is the period of intense spawning activity (repr. stage 4). The vast majority of the animals are in spent condition but a few are in fully ripe condition and some are partially spent. In the ripe animals the NSA is in stage 3. Prior to spawning there is evacuation of NSM (Pl. 9, F). In partially spent ones the NSA is in stage 4 with NSM only in the axonal region. In fully spent animals the NSM is faintly recognisable in the axon.

PLATE 9

A & B. Neurosecretory cells are fewer and stain deep at the initiation of gametogenesis in *Perna indica*. The pyriform cell type is distinctly seen in B.

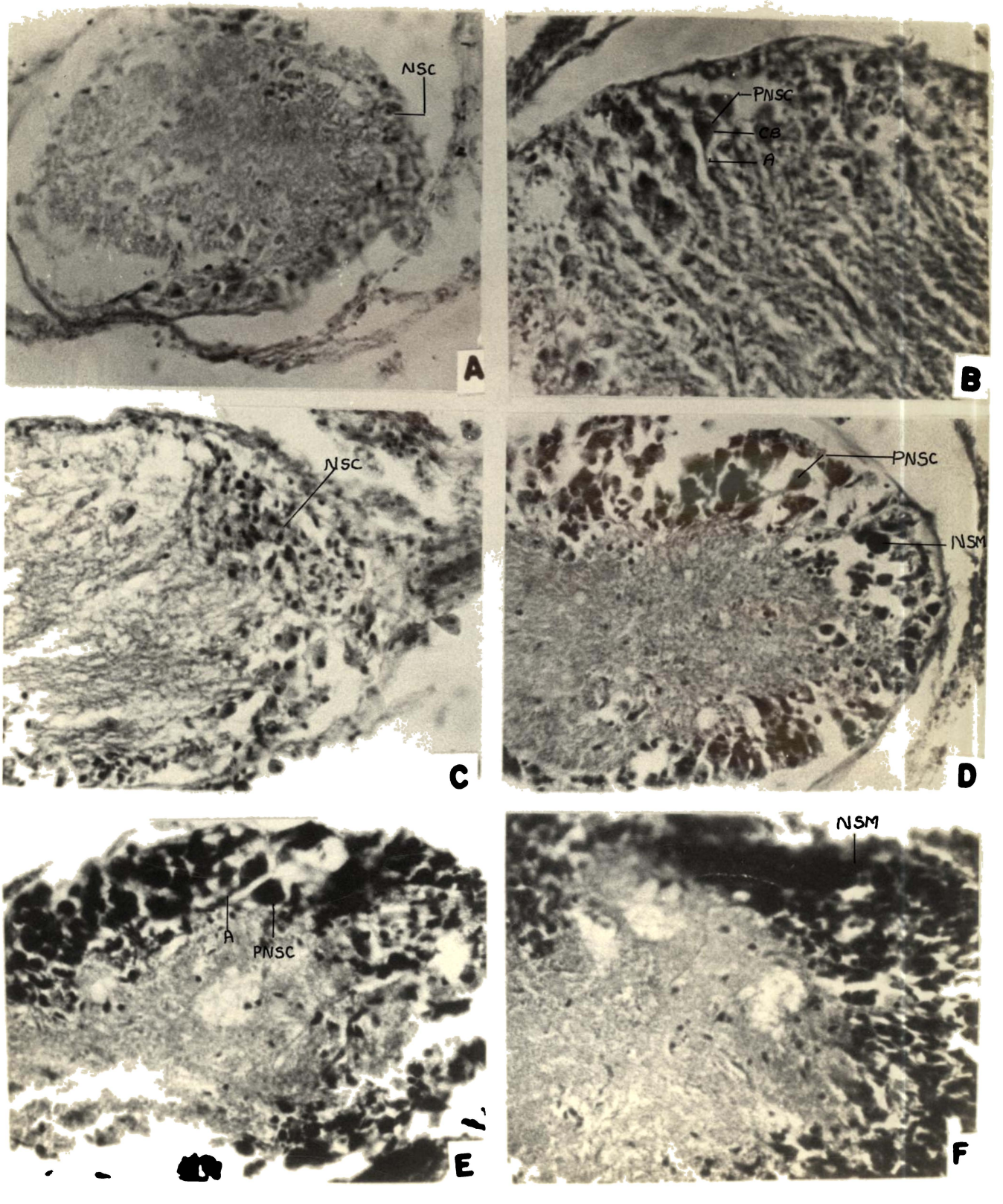
C & D. Successive stages of increase in NSC and neurosecretory activity in the active gametogenic phase.

E Deep staining NSC with intense neurosecretory activity in the ripe animals.

F NSC in pre-spawning phase showing commencement of evacuation of neurosecretory material.

A - Axon; CB - Cell body; NSC - Neurosecretory cells; NSM - Neurosecretory material; PNSC - Pyriform neurosecretory cell

PLATE 9



September: In September, the animals are in the spent-recovery stage with traces of NSM in the axonal region of NSC.

The above observations show the typical cycles of reproduction and neurosecretory activity in the species studied during the year 1982, as represented by majority of the animals in the samples. However, both the activities fall in a range of stages, rather than in a particular stage in any given period, especially during the active reproductive period. But the evidence of correlation between the stages of reproductive activity and neurosecretory activity is unmistakable. During the active gametogenic and maturation phase, the neurosecretory material builds up in the neurosecretory cells of the cerebral ganglia. In those animals which have entered the spawning phase the NSM has been evacuated from the neurosecretory cells. During the indeterminate phase, when there is no reproductive activity in the animals, the neurosecretory cells are also dormant in activity.

Effect of Ganglion ablation on spawning

Ablation of cerebral ganglia

The results of cerebralectomy are given in Table 35. Bilateral cerebralectomy elicited strong positive spawning response in both the species, as compared to unilateral ablation. The females showed a slightly greater response than the males

in both species. Spawning was seen in both controls on a much lower scale, Control I animals (sham operated) giving a little more response than Control II animals. It would appear that basically a stress factor might have been responsible for spawning in the controls. When sexually ripe animals are transferred from the natural beds to the laboratory, occasionally natural spawning occurs. However, a dominant spawning response in the cerebralectomised animals is evident and is attributable to the extirpation of cerebral ganglia.

Spawning response due to bilateral ablation was observed within 3 hrs from treatment in 38 % males and 52 % females in P. viridis, and 36 % males and 60 % females in P. indica. This was followed by similar quick response in the next 3 hrs. The spawning response in both the species as per data in Table 35 for bilateral cerebralectomy was complete within 12 hrs from treatment. On the other hand, in unilateral cerebralectomy first spawning response was delayed and seen at the 12th hour in both species and was complete at the 48th hour in P. viridis and 24th hour in P. indica. Thus , it is evident that unilateral ablation brings about a delayed and limited response than bilateral ablation. The approximate ratio of response is 4:1 in bilateral and unilateral cerebralectomy in both the species.

The spawning response to cerebralectomy was tested statistically by Z-test of significance by means of the normal deviate (Snedecor and Cochran 1967). The results of Z-test are presented in Table 36.

Table 35 Spawning response in cerebralectomised *P. indica* and *P. viridis*

Species	Percentage of animals spawned upto 72 hrs.							
	Unilateral		Bilateral		Control I		Control II	
	M	F	M	F	M	F	M	F
P. indica	20	24	70	86	12	12	10	8
P. viridis	16	22	72	88	10	14	8	12

Table 36 Z-test results on spawning response of *P. indica* and *P. viridis* to cerebralectomy.

Species	Unilateral	Bilateral	Unilateral x Bilateral		Unilateral x control 1		Bilateral x control 1		Control 1x Control II	
	M x F	M x F	M	F	M	F	M	F	M	F
P. indica	N.S.	N.S.	*	*	N.S.	N.S.	*	*	N.S.	N.S.
P. viridis	N.S.	*	*	*	N.S.	*	*	*	N.S.	N.S.

* Significant at P 5 %

N.S. Not significant

M = Males

F = Females

Table 36 shows that the spawning response in control I and control II animals is not significantly different. Bilateral and unilateral ablation brings about significant differences in spawning response in respect of males and females. Also response to bilateral ablation is significantly different from that obtained for sham operated animals. Unilateral ablation marks a significant difference from control I only for females of P. viridis which is absent in P. indica.

Observations on mortality of animals during the experiment are presented in Table 37. The data would show that although the greatest spawning response was obtained in bilateral cerebralectomy, the mortality was also the highest, all animals dying within 36 hrs. Initial mortality was observed within 6 hrs. In the case of unilateral ablation mortality was moderate. Mortality among sham operated animals was slightly more than that among control control II animals. P. indica and P. viridis gave similar results on mortality. In the treatment and controls, mortality was observed after spawning response.

Cerebralectomised animals (unilateral as well as bilateral) showed fast recovery, within 60 - 80 minutes of returning them to sea water. They appeared to show normal shell activity. Attachment by byssal threads was seen within 20 minutes. Spawning, when it occurred, was vigorous and the gametes were extruded continuously. During the initial phase of spawning the shell movements were normal. As time passed,

Table 37 Progressive mortality of cerebralectomised animals (both sexes) of Perna indica and P. viridis during 72 hrs of experiment (Total No. of animals 100).

Treatment & Species	Progressive mortality %							
	Observation period in hours							
	3 h	6 h	12 h	24 h	36 h	48 h	60 h	72 h
Unilateral								
P. indica	-	-	11	27	31	40	40	40
P. viridis	-	-	-	17	49	52	52	52
Bilateral								
P. indica	-	9	24	85	100			
P. viridis	-	12	39	69	100			
Control - I								
P. indica	-	-	-	4	6	8	16	28
P. viridis	-	-	-	6	9	17	25	36
Control - II								
P. indica	-	-	-	-	6	7	12	16
P. viridis	-	-	-	-	-	13	18	22

Table 38 Spawning response in visceralectomised Perna indica and P. viridis

Species	Percentage of animals spawned upto 72 hrs			
	Unilateral		Bilateral	
	M	F	M	F
P. indica	16	20	30	40
P. viridis	18	16	34	42

the spawning activity was reduced along with the reduction in shell movement (shell opening and closure).

Ablation of visceral ganglia

Data on spawning response of visceralectomised animals are presented in Table 38. Since control I and II were common for all the ganglion ablation experiments and the results have already been presented in Tables 35 and 37 (this Chapter), these have not been repeated here. Bilateral ablation of visceral ganglia gave more response of spawning than unilateral ablation, the ratio being approximately 2:1. Unilateral ablation results are slightly higher than those in Control I and moderately higher than those in control II (Table 35). As in the case of cerebralectomy, there was 'background spawning' which can again be attributed to a stress factor. Females showed a slightly greater response than males. The spawning response was observed between 6 - 12 hrs in bilateral and between 24 -48 hrs in unilateral visceralectomy in the case of P. viridis. In P. indica the unilateral ablation response was advanced to 12 - 24 hrs.

The Z-test results on visceralectomised animals are presented in Table 39. The results show that response to bilateral ablation was significantly different from that to unilateral ablation. Sexually, the differences in response between females and males were significant only in bilateral ablation and not in unilateral. While the response was significantly different between bilateral and control I, it

was not so between unilateral and control I. Both *P. indica* and *P. viridis* gave identical results, answering the Z-test of significance.

Data on progressive mortality of animals subjected to visceralectomy are presented in Table 40. In bilateral ablation, mortality commenced from the third hour, while the spawning response is caused in the sixth hour. By 12th hour when most of spawning response was completed, the death toll was 48 % and 37 % in *P. viridis* and *P. indica* respectively which increased sharply in the next 24 hrs to total mortality. In unilateral ablation too, mortality set in before spawning response and reached 46 % in *P. viridis* and 36 % in *P. indica* between 36-48 hrs and remained at that level till 72 hrs. The data show that compared to bilateral ablation, spawning response and mortality was more moderate in unilateral ablation. Mortality in unileteral ablation was slightly higher than that in controls I and II (Table 37).

The visceralectomised animals appeared to show a greater effect on general metabolism, as compared to cerebralectomised animals. Recovery from treatment was poor and slow and the valves remained in half open condition till death or till the end of experiment in the survivors. Byssal attachment appeared normal. Bilaterally ablated animals showed greater effect than the unilaterally ablated ones. In the animals where spawning was observed, the spawning activity was not intense. After the initial spawning there was

Table 39 Z-test results on spawning response of Perna indica and P. viridis to visceralectomy.

Species	Unilateral M x F	Bilateral M x F	Unilateral x Bilateral M	Unilateral x Bilateral F	Unilateral x Control I M	Unilateral x Control I F	Bilateral x Control I M	Bilateral x Control I F
P. indica	N.S.	*	*	*	N.S.	N.S.	*	*
P. viridis	N.S.	*	*	*	N.S.	N.S.	*	*

* Significant at P 5 %

N.S. Not significant

M = Male

F = Female

Table 40 Progressive mortality of visceralectomised animals (both sexes) of Perna indica and P. viridis during 72 hrs of experiment (Total no. of animals 100).

Treatment & Species	Progressive mortality % Observation period in hours 3	6	12	24	36	48	60	72
Unilateral								
P. indica	-	-	9	15	28	36	36	36
P. viridis	-	7	15	39	46	46	46	46
Bilateral								
P. indica	-	26	37	95	100	-	-	-
P. viridis	3	18	48	82	100	-	-	-

Table 41 Spawning response in pedalectomised *Perna indica* and *P. viridis*.

Species	Percentage of animals spawned upto 72 hrs			
	Unilateral		Bilateral	
	M	F	M	F
P. indica	10	12	18	26
P. viridis	12	8	14	20

Table 42 Progressive mortality of pedalectomised animals (both sexes) of *Perna viridis* and *Perna indica* during 72 hrs of experiment(Total no. of animals 100)

Treatment & Species	Progressive mortality %							
	Observation period in hours							
	3	6	12	24	36	48	60	72
Unilateral								
P. indica	-	-	-	5	16	30	30	30
P. viridis		-	-	-	18	32	32	32
Bilateral								
P. indica	-	-	16	33	92	100		
P. viridis	-	-	-	21	73	100		

accumulation of gametes with heavy mucus in the exhalent siphon area, following which heavy mortality was observed.

Ablation of pedal ganglia

Spawning response in pedalectomised animals is shown in Table 41. Bilateral ablation gave a slightly higher response than unilateral ablation and the females responded slightly more than the males in most cases. The degree of response was less, being closer to that of control I, as compared to the results of cerebralectomy and visceralectomy. The Z-test of significance by means of normal deviate showed that except those of bilateral ablation between females and males, among females between bilateral and unilateral as also between bilateral and control I, all other treatments had no significant differences.

Mortality data of pedalectomised animals are given in Table 42. In bilateral pedal ganglion ablation, mortality commenced by 12 hrs and was total by 48 hrs. Unilateral ablation showed a lower mortality rate (30 - 32 %) which was greater than in the control I and II. Both P. indica and P. viridis suffered similar mortality rates.

Recovery of pedalectomised animals was quick. While byssus secretion was totally absent in bilateral ablation, it was much reduced in unilateral ablation.

The spawning activity of the pedalectomised animals was normal initially, but prolonged spawning was not observed. The shell movements were affected by pedalectomy.

Table 41 Spawning response in pedalectomised Perna indica and P. viridis.

Species	Percentage of animals spawned upto 72 hrs			
	Unilateral		Bilateral	
	M	F	M	F
P. indica	10	12	18	26
P. viridis	12	8	14	28

Table 42 Progressive mortality of pedalectomised animals (both sexes) of Perna viridis and Perna indica during 72 hrs of experiment(Total no. of animals 100)

Treatment & Species	Progressive mortality %							
	Observation period in hours							
	3	6	12	24	36	48	60	72
Unilateral								
P. indica	-	-	-	5	16	30	30	30
P. viridis		-	-	-	18	32	32	32
Bilateral								
P. indica	-	-	18	33	92	100		
P. viridis	-	-	-	21	73	100		

DISCUSSION

Lubet (1955 a, 1959) found neurosecretory cells (NSC) in the dorsal caps of cerebral ganglia and the dorsal cell layer of visceral ganglia of Mytilus edulis and Chlamys varia. In the former species he described two types of NSC which could be differentiated by size (Lubet, 1959). Nagabhushanam (1962 a) observed neurosecretory cell types I and II in the cerebral and visceral ganglia of Crassostrea virginica and similar observations of two cell types were made on Martesia striata (Nagabhushanam, 1968), Meretrix casta (Nagabhushanam, 1969), Katelysia opima (Nagabhushanam and Mane, 1973), Crassostrea cucullata (Nagabhushanam and Bidarker, 1975) and Donax cuneatus (Nagabhushanam and Talikhedkar, 1975). But in Mytilus viridis, Nagabhushanam et al., (1975) reported only one cell type, pyriform in shape and measuring 15 - 25 µm in length from the cerebral, visceral and pedal ganglia. In the present study on the brown mussel Perna indica and green mussel P. viridis, the presence of only one cell type in the cerebral and visceral ganglia has been confirmed, but no neurosecretory cell could be found in the pedal ganglia. The NSC are distributed peripherally in the ganglia with a greater concentration in the dorsolateral surface. The peripheral distribution agrees with the observation of Lubet and Mathieu (1982) in M. edulis, who have also found the NSC in the medio-dorsal region. Using the method of Illanes and Lubet (1980) for identification of NSC by signaletic stains after

permanganate oxidation to distinguish neurosecretory material from chromolipoids, Lubet and Mathieu (1982), identified three types of NSC, namely type a_1, a_2 and a_3, and observed that the activity of type a_1 cells (6 - 15 µm) is closely correlated with gametogenesis. Illanes and Lubet (1980) identified a fourth cell type a_4 in M. edulis. Using the standard technique of Mallory's triple stain in the present study, it has not been possible to identify cell types other than the pyriform cells of 15 - 20 µm which would actually appear to correspond with the type a_3 cells observed by Lubet and Mathieu (1982).

Performing experiments on organ culture of M. edulis gonial explants with autologous cerebropleural, pedal and visceral ganglia, Lubet and Mathieu (1982) observed that gametogenesis in adult mussels appears to depend on several factors emitted by the cerebropleural ganglia and indicated (a) a 'mitotic factor' triggering gonial mitosis leading to multiplication of oogonia and spermatogonia; (b) a 'meiotic factor' acting on the first division of meiosis allowing the formation of spermatocytes II; (c) a 'previtellogenic factor' preceding the process of vitellogenesis by accumulation of RNA in the cytoplasm; and (d) a 'vitellogenic factor' causing vitellogenesis. They also found that these factors are neither sexualised nor species-specific. They could not conclude whether these different phenomena are due to one or several neuroendocrine factors. At this stage of knowledge, it can at best be stated that the neuroendocrine mechanism in the

bivalves in relation to reproduction is beginning to be understood, with the accumulating knowledge on the types of NSC and the different 'factors' responsible for specific functions, a small but definite improvement from the earlier studies which were based only on morphological and cytometrical criteria.

Reviewing the histochemical data on neurosecretion of molellibranchs, Martoja (1972) considered them of a rudimentary nature. Nagabhushanam (1968) observed that the neurosecretory material (NSM) of cell type I of C. virginica was strongly sudanophilic and showed a positive PAS reaction, indicating its glycolipid nature. Citing Lubet's work on M. edulis, Martoja (1972) remarked that Lubet had concluded a proteinic nature of NSM considering the PAS-negative character of the secreted product and the absence of lipids in it. In Meretrix casta, Nagabhushanam (1969) found the cytoplasm of the neurosecretory cells showing the presence of glycolipid. Nagabhushanam and Lonte (1972) found the secretory material on the neurosecretory cells of Paphyrela scorrugata was of glycolipoprotein nature. In the present study on Perna indica and P. viridis, it has been observed that the secretory product of the pyriform neurosecretory cells is acidic and is glycolipoprotein in nature.

Following the observations of Antheunisse (1963) on Dreissena polymorpha, the occurrence of four stages in the neurosecretory cell and the axonal transport of the neurosecretory material has been indicated by Nagabhushanam (1968)

in Martesia striata, Nagabhushanam and Sidaskar (1975) in Crassostrea cucullata and Nagabhushanam et al. (1975) in Mytilus viridis. In the present study on Perna indica and P. viridis, such stages could be discerned mainly based on the strength of the tinctorial property of the cytoplasm of the neurosecretory cells. The N:C stage 3 with neurosecretory material accumulated in the axonal hillock was the most common stage during the maturation phase of reproductive cycle and the evacuation of the secretory product through the axon appears implied based on sequence of events in the neurosecretory cell cycle. The process of aggregation of neurosecretory granules into droplets and drops and their flow around the nucleus to the axonal hillock as noticed by Nagabhushanam et al. (1975) in M. viridis could not be observed during the present study. Bartoja (1972), reviewing the information on the axonal transport of neurosecretory material, stated that 'we remain practically in complete ignorance of the transport and fate of the products of neurosecretion' and further opined that the disappearance of the neurosecretory material may be due to other causes involving particular mechanisms such as the role of glial cells.

While the 'ignorance' on the transport of neurohormones from the site of production to the target site continues, the role of storage tissue as an intermediary mechanism has come to be recognised through experiments on ganglion extirpations, grafts and organ culture and, according to Lubet and Mathieu (1982), the neurohormone may not act

directly upon the germ cells but may act through intermediary cells in the storage tissue.

Seasonal changes in the cyclical activity of neurosecretory cells have been related to the seasonal/annual reproductive cycle in the bivalves by many workers after Lubet (1955 a, 1959) ascribed such relationship in Mytilus edulis and Chlamys varia. The general observations in many species which have been studied so far are that in the resting phase of gonad, neurosecretory granules in the NSC of the cerebral ganglia are few or absent; neurosecretory products begin to accumulate in the NSC (type I cells in many species) with the initiation of gametogenesis; this accumulation is at maximum when the animals are fully mature; and just before spawning the neurosecretory material is evacuated from the NSC (Lubet, 1955 a, 1959 on M. edulis and C. varia; Nagabhushanam, 1962 a on C. virginica, 1968 on M. striata, 1969 on M. casta; Nagabhushanam and Mane, 1973 on K. opima; Nagabhushanam and Bidarkar, 1975 on C. cumillata; Nagabhushanam and Talikhedkar, 1975 on D. cumingtus; Blake, 1972 and Sastry and Blake, 1971 on Argopecten irradians; this study on P. indica and P. viridis). Anthoumisse (1963) considered this relation in D. polymorpha a pure coincidence as the reproductive cycle is also superimposable on the seasonal cycle with all the thermal, trophic and other variations and concluded that reproduction is independent of neurosecretion. Lubet (1966) stated that the 'integrity' of cerebral ganglia is indispensable for the normal development of the reproductive cycle,

but in the early stages of his work, as cited by Martoja (1972), considered it impossible to decide if the mechanism is hormonal or nervous in nature. However, later, Lubet ((1980-81) and Lubet and Mathieu (1982), indicated the hormonal nature of the secretion and identified four factors, as referred to earlier, in the neurohormones responsible in controlling reproduction in M. edulis, Crassostrea gigas and Ostrea edulis.

The synchronism between the neurosecretory activity of the cerebral ganglia and the reproductive cycle of the two species of mussels Perna indica and P. viridis has been well established in the present investigation. P. indica has a single annual reproductive cycle with a resting phase during October-January, active gametogenic phase during February-April, maturation phase during May-July, peak spawning phase in August and spent-recovery phase in September. The neuro-secretory cell stages show clear correspondency with the above annual reproductive cycle. P. viridis has two seasonal reproductive cycles: (1) a regular one with resting phase during January-June, gametogenic and maturation phase during July-August, maturation and spawning phase during September, peak spawning phase during October and recovery/resting phase in November-December; and (2) a short secondary reproductive cycle during January-March in which only the larger individuals (above 60 mm) participate. The secondary reproductive cycle is telescoped within a short period with almost all stages in each month and peak spawning in March.

The neurosecretory cell cycle of cerebral ganglia of P. viridis shows good correspondency with the regular reproductive cycle. In both species the neurosecretory cells become charged with neurosecretory product during the gametogenic phase, which is further intensified when the gonads are ripe. The neurosecretory material is evacuated just before spawning. In the partially spent animals the neurosecretory material is seen only in the axonal region. In fishes, the environmental factors perceived by the animal through sensory receptors act upon the hypothalamus of brain which signals the hypophysis through messenger chemicals to secrete the hormones into the blood to be carried to the target organs. But the action of environmental factors on the cerebral ganglia of the bivalve molluscs, if any, has not so far been studied.

The role of ganglia in reproduction and metabolism of the molluscs has been studied by their ablation and observing the effects. As cited by Lubet (1966), the physiology of the nervous system of bivalves was sought to be understood by such techniques as early as 1885 by Pavlov and others. But experimental work for understanding the effects of bilateral extirpation of cerebral or visceral ganglia as neuroendocrine centres was attempted only more recently (Lubet, 1955 a, 1959, 1965; Nagabhushanam, 1962 a; Antheuniesse, 1963 and others).

While Lubet (1959) opened a small window in the shell of M. edulis and C. varia for excising the ganglia, Nagabhushanam (1962 a) removed the right valve of C. virginica

completely. In the present study on Perna indica and P. viridis, the animal was narcotised and ablation of ganglia was done in the whole animal without damaging or removing the shell. The recovery with this procedure was fast. Survival of window-operated mussels reared in the breeding areas was as long as two years (Lubet, 1966), while in the shell-removed oysters it was 7 - 10 days in the controls and 3 -5 days in the experimental animals (Nagabhushanam, 1962 a). In the present study, where the animals were kept in the laboratory aquaria, mortality in bilaterally cerebralectomised animals commenced at the sixth hour and mortality was total by 36 hours. The rate of mortality was low in unilateral cerebralectomy. With visceralectomy, mortality rates were about the same as in cerebralectomy. However, mortality was also observed, though commencing late and moderate, in the sham operated and control animals. This would show that, apart from the effect of treatments, other factors might have also been responsible for the mortality observed.

Lubet (1959, 1966) found that bilateral ablation of cerebral ganglia during the resting phase and at the beginning of the gametogenesis delayed gametogenesis in M. edulis and that the few ripe oocytes underwent lysis; removal of ganglia at the end of gametogenesis resulted in more rapid spawning than in controls. Lubet (1959) assumed the presence of an inhibitory factor in the cerebral ganglia at maturation phase and interpreted that the removal of this inhibition by bilateral cerebralectomy leads to more rapid spawning.

In Crassostrea virginica, Nagabhushanam (1962 a) found that cerebralectomy on fully ripe males and females resulted in spawning and that the spawning response was far more pronounced in females than in males. Visceralectomy caused no definite spawning reaction, although partial spawning was observed in visceralectomised and control animals. Similar results were obtained by Nagabhushanam and Bidarkar (1975) in C. cucullata, Nagabhushanam and Talikhedkar (1975) in Donax cuneatus and Nagabhushanam and Lowte (1973) in the freshwater mussel Parreysia corrugata. Spawning response in the above studies were ascertained histologically by examining the gonad 1 - 2 days after ablation of the ganglia.

In the present investigation, direct observations were made on spawning of Perna indica and P. viridis at regular intervals for a period of 72 hours from the time of ganglia extirpation. Bilateral cerebralectomy elicited greater spawning response in P. viridis (72 % in males and 88 % in females) than the unilateral ablation (16 % in males and 22 % in females) and in P. indica the results were close to the above. Visceralectomy too yielded spawning response but on a much lower scale (P. viridis: bilateral - males 34 %, females 42 %; unilateral - males 18 %, females 16 %) which was closer to the spawning in the sham operated animals. Animals in which the pedal ganglia were ablated also responded but on a scale much lower than the response to visceralectomy. The results were tested statistically (Z-test) and relative significance of treatments on spawning has been brought out.

'Background' spawning and mortality was observed in all the experiments in the sham operated and natural control animals although the percentages were low. The factors responsible for the above could not be identified. However, there is sufficient experimental evidence to show that bilateral cerebralectomy on ripe animals produced an overwhelming spawning response. Bilateral visceralectomy produced about 50 % of the response obtained with cerebralectomy. Response to pedalectomy was close to the 'background' spawning due to unknown factors and, therefore, need not be taken as results of treatment. Bilateral ablation of both cerebral and visceral ganglia showed a much greater spawning response than unilateral ablation. The females responded more than the males to the treatments.

Visceral ganglia have been implied in maintenance of general metabolism of bivalves and their emission leads to considerable decrease of rate of filtration, depletion of nutrient reserve, poor tonus of adductor mussel, disturbance to structure of mantle and ostia and poor emission of gametes in M. edulis and M. galloprovincialis (Lubet, 1966). Altmann (1959), as cited by Nagabhushanam and Mane (1973), found that after extirpation of visceral ganglia, M. edulis was unable to perform normal respiration. Nagabhushanam (1968) found in C. virginica that visceralectomy results in considerable increase in body weight, and the rate of water filtration and heart beat are considerably reduced. Some of these observations were corroborated in other species by Nagabhushanam

and Mane (1973) and Nagabhushanam and Bidarkar (1975). Although observations were not made on these specific metabolic activities in the present study, it was seen that in the visceralectomised animals, recovery was poor and slow and the valves remained always partially open till death of the animal or till the end of the experiment, with heavy mucus secretion in the siphonal area. As observed by Hartoja (1972), the evacuation of gametes is certainly disturbed after visceral ganglion ablation as consequence of the disorders introduced into the essential vegetative functions and the gametes, in the present case, were accumulating embedded in the mucus in the exhalent siphon.

The results of the present study have further contributed to our understanding of neuroendrocrine control of reproduction in the Indian sea mussels P. indica and P. viridis from the earlier work of Nagabhushanam et al. (1975) on the green mussel. As stated by Bayne (1976 a), the study of neurosecretion in bivalves has been hampered because of the difficulties with present surgical procedures, diffuse distribution of neurosecretory cells and ignorance of the chemical nature of the neurohormones. An improvement on surgical procedure has been introduced during the present investigation. It is necessary to follow up the lead given by Lubet and Mathieu (1982) in these studies by adopting grafts and organ culture techniques to obtain more direct evidence on the role of neuroendrocrine hormones in the reproduction of mussel.

S U M M A R Y

1. The reproductive physiology of the two species of Indian sea mussels, namely the brown mussel Perna indica and the green mussel P. viridis, has been investigated by a comprehensive approach to the problem. The major aspects of the study include ecophysiology of reproduction linking up the annual reproductive cycle of the animals with the ecological conditions of the natural mussel beds, biochemical and histochemical changes associated with reproduction, and the neurosecretory cycle in synchrony with the reproductive cycle. Some basic studies on gamete morphology and certain aspects of gamete physiology have been taken up. The experimental work deals with the influence of different feeding levels on gametogenesis and maturation, and the effect of ganglia ablation on spawning.

2. The materials for the investigations on Perna indica were collected from the natural mussel beds at Vizhinjam, near Trivandrum and on P. viridis from Elathur, near Calicut. The period of observations extended from October 1981 to December 1982.

3. Data on temperature, salinity, dissolved oxygen, turbidity, phytoplankton production and rainfall have been presented for both the study areas. The annual range of the parameters for January-December 1982 period were: at Vizhinjam

for brown mussel - temperature 24.3 - 29.3°C, salinity 32.55 - 36.68 ppt, dissolved oxygen 3.64 - 5.19 ml/l, turbidity 180 - 540 JTU and phytoplankton (Chlorophyll a) 2.15 - 25.57 mg/m^3; at Elathur for green mussel - temperature 26.0° - 29.9°C, salinity 35.40 - 40.50 ppt, dissolved oxygen 4.16 - 5.94 ml/l, turbidity 100 - 940 JTU and phytoplankton (chlorophyll a) 3.31 to 22.0 mg/m^3.

4. Perna indica was in reproductive phase during February to September and in the resting (vegetative) phase from October to January. Sex differentiation and gametogenesis commenced in February and active gametogenesis was noticed in March and April. Progressive maturation was noticed during May-July. While spawning in a small part of the population commenced as early as May, peak spawning took place in August. In September a vast majority of the population had entered the resting phase. The mean gonad index truly reflected the reproductive cycle and the highest index of 2.77 was obtained in July 1982 at peak maturation phase.

5. Perna viridis showed a primary reproductive cycle from July to November with thetotal adult population participating in the precess and secondary cycle during January-March in which mussels of above 60 mm length alone participated. Each cycle was completed in a much shorter period than in P. indica with a greater degree of synchronisation in the population. Spawning took place from September to November with a peak in October in the primary reproductive cycle and was

observed from January to March with a peak in March in the secondary cycle.

6. Relatively lower temperatures prevailed during the active reproductive season than during the non-reproductive season for both P. indica and P. viridis. In this respect the tropical species appear to be opposed to their temperate counterparts which spawn in higher temperatures. However, the secondary spawning of P. viridis is associated with higher temperatures but the spawning is incomplete and partial.

7. Within the narrow annual variations of salinity in the open coastal waters, the reproductive phase of both P. indica and P. viridis was marked by lower salinity conditions and the non-reproductive phase by higher values. Individual processes of gametogenesis, maturation and spawning appeared to be triggered by progressive decrease in salinity.

8. A greater correlation was found between monthly rainfall and reproduction. In P. indica gametogenesis commenced in February with rainfall but proceeded with precipitation. The heaviest rainfall in June (480.9 mm) took the process of maturation to a peak and accelerated spawning. In P. viridis active gametogenesis in July-August coincided with 50 % of the total annual rainfall during these two months and spawning commenced in early September with reduced rainfall. Rainfall, through its effect on lowering the temperature and salinity, appeared to influence the different processes of reproduction.

9. Reproduction also showed a high degree of correlation with availability of food. In P. indica, commencement of

gametogenesis in February is preceded by a minor phytoplankton peak (chlorophyll a 11.02 mg/m^3) and maturation is preceded by a major peak (chlorophyll a 25.57 mg/m^3) in May. In P. viridis gametogenesis commenced in July with a subtle increase in phytoplankton biomass (chlorophyll a 5.99 mg/m^3). Maturation was accelerated with increase in phytoplankton production and spawning commenced in September with a peak chlorophyll a value of 22.80 mg/m^3). In this species, a minor secondary peak of chlorophyll a of 8.82 mg/m^3 in January appeared to support the secondary spawning. The larger animals (above 60 mm) which alone participated in this spawning might have been able to convert the food energy derived from the minor peak in January into reproductive effort as they have already attained a higher somatic growth than the smaller animals.

10. Localised pollution of coastal waters due to coconut husk retting near Elathur had an effect on the reproductive potential of P. viridis. Mussels at the site were all males without any exception and spawning was partial and incomplete. Pea-crab infestation in the indeterminate stage of P. indica resulted in non-development of reproductive tissue in the mantle. Infestation in the advanced stages of reproduction led to tissue damage at the site of "lodging" affecting the reproductive potential of the mussel.

11. The structure of sperm and egg is similar in both the species. Under scanning Electron Microscope, the sperm of P. indica showed four distinct parts, namely acrosome, nucleus, middle piece and tail. The sperm, including the tail,

measures 70 µm. The salinity range 32.7 - 35.2 ppt appeared to be the most suitable for the survival of sperms of P. indica. The sperms were motile even after 3 h 30 min in the above range. The 50 % sperm viability level was obtained approximately at 60 min in 20.6 ppt, 1 h 45 min in 27.1 ppt, 2 h 30 min in 32.7 ppt, 2 h 15 min in 35.2 ppt and 1 h 45 min in 38.7 ppt. In P. viridis, 50 % sperm viability level was recorded at 30 min, 1 h 15 min, 2 h 15 min, 2 h and 60 min in the above-mentioned salinities, respectively. The 50 % egg viability levels in the salinities 20.6, 27.1, 32.7, 35.2 and 38.7 ppt were reached, respectively, approximately at 15 min, 2 h 30 min, 3 h 30 min, 3 h 30 min and 1 h 30 min and 1 h 30 min in the case of P. indica and 15 min, 4 h, 4 h 30 min, 8 h and 2 h 30 min P. viridis. Preliminary study on sperm preservation was carried out.

12. Biochemical studies on P. indica and P. viridis revealed that, the composition varied with the stages of maturity and also seasonally. The nutrient storage occurred in different tissues of the body. The protein was largely stored in the adductor muscle, carbohydrates as glycogen in the mantle and lipids in the digestive gland. The accumulation of these components was high during the non-reproductive season and also when food was in abundance. The excess energy, after providing for the maintenance metabolism, was stored and later utilised for reproduction. The protein level in the adductor muscle was comparatively higher than in the digestive gland and mantle. The lipid level was comparatively higher in the

mantle of female than in the male, but glycogen level was higher in the latter. The lipid stored in the digestive gland was mobilised during active reproductive season especially during vitellogenesis. The glycogen stored in the mantle was used as the follicles developed in the mantle.

13. The gonad of the mussels was observed to be of glycolipoprotein in nature. The occurrence of acid mucopolysaccharides was confirmed. The lipid moiety in the gonad of mussel was of neutral lipid in nature. The male gonad contained more of glycogen than other carbohydrate components. The transfer of yolk precursors from different body tissues to the gonad was confirmed by histochemistry. The nutrients stored in the mantle connective tissue also was lysed and utilised during gametogenesis. Both adipogranular and vesicular connective tissue cells were observed in the mantle.

14. A 45-day experiment on P. indica to induce gametogenesis and maturation outside the natural reproductive season through feeding with mixed phytoplankton gave significant results. A good correspondency was observed among dry weight increase, digestive gland index and mean gonad index for different levels of ration (range 0.6 - 19.2 µg chlorophyll a/ animal/day). On a ration of 4.8 - 9.6 µg chlorophyll a/ animal/day, the mussels reached full maturity (mean gonad index 3) in 30 days. Statistical tests carried out on the data showed the relative significance of different feed levels in promoting reproductive activity, somatic growth and digestive gland development. All the experimental animals giving positive

response turned out to be males. It is inferred that oogenesis may require higher energy levels than those used in the treatments. The results of this experiment have practical value in controlled breeding of mussel for aquaculture purposes.

15. The neurosecretory cells (NSC) of a single type (pyriform) have been observed in the cerebral and visceral ganglia of P. indica and P. viridis. The pedal ganglia do not show presence of NSC. Four arbitrary stages of neurosecretory activity (NSA) have been described in the cerebral ganglia. Cytochemical study revealed that the neurosecretory material is acidic and glycolipoprotein in nature. The seasonal changes in the reproductive cycle synchronise with the changes in neurosecretory cycle. The sequences are similar in both the species although their annual cycle varies in respect to the months of the year.

16. Experimental evidence has been obtained on the role of neurosecretion in spawning of P. indica and P. viridis through extirpation of the ganglia of the central nervous system. The results were very similar for both the species. Bilateral cerebralectomy elicited greater spawning response than the unilateral ablation, approximately in the ratio of 4:1, and the females showed a slightly higher response than males. Visceralectomy too yielded spawning response but on a much lower scale as compared to cerebralectomy. In the experiments, a certain amount of 'background' spawning, perhaps due to stress, occurred in the controls, but this response was

very low. Pedalectomy elicited spawning response very close to the 'background' spawning in the controls. The spawning responses to various ganglion ablation treatments were statistically tested to understand their significance. Recovery process was poorer and slower in the visceralectomised animals than in the cerebralectomised ones, implicating the role of visceral ganglia in general metabolism. Mortality rates due to different treatments have been observed. The results of this study have provided further experimental evidence on the role of neurosecretion in the spawning of *Perna indica* and *P. viridis*.

REFERENCES

ABDUL AZIS, P.K. AND N.B. NAIR. 1976. The problem of pollution in the retting zones of the backwaters of Kerala. National Seminar on Environmental Pollution, May 6th - 8th 1976. Kerala, India.

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

AHMED, M. AND A.K. SPARKS. 1970. Chromosome number, structure and autosomal polymorphism in the marine mussels Mytilus edulis and Mytilus californianus. Biol. Bull. (Woods Hole, Mass.), 138: 1-13.

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

_____ 1980. Review on controlled breeding of bivalves of Aquaculture importance. In: T. Subramoniam and Sudha Varadarajan (eds.) Progress in Invertebrate Reproduction and Aquaculture, pp. 194-202.

ALAGARSWAMI, K. AND G.S. SIVARAJAN. 1978. Surgical equipments for pearl culture. Indian J. Fish., 22: 231-235.

ALAGARSWAMI, K., S. DHARMARAJA, T.S. VELAYUDHAN, A. CHELLAM AND A.C.C. VICTOR. 1981. Induced breeding of the Indian pearl oyster, Pinctada fucata (Gould). The First Indo-Pacific Symposium on Invertebrate Reproduction, 27-29 Nov. 1981. Madhurai University India.

ALLEN, F.E. 1955. Identity of breeding temperature in southern and northern hemisphere species of Mytilus (Lamellibranchia). Pacif. Sci., 9: 107-109.

ALTMAN, G. 1959. Atmungsphyologische untersuchungen Mytilus galloprovincialis. Experientia, 15: 192.

ANSELL, A.D., F.A. LOOSMORE AND K.F. LANDER. 1964. Studies on the hard-shell clam, Venus mercenaria in British waters. II. Seasonal cycle in condition and biochemical composition. J. Appl. Ecol., 1: 83-95.

ANSELL, A.D. AND K.F. LANDER. 1967. Studies on the hard-shell clam, Venus mercenaria, in British waters. III. Further observations on the seasonal biochemical cycle and on spawning. J. Appl. Ecol., 4: 425-435.

ANSELL, A.D. AND A. TREVALLION. 1967. Studies on Tellina tenuis da Costa. - 1. Seasonal growth and biochemical cycle. J. Exp. Mar. Biol. Ecol., 1: 220-235.

ANTHEUNISSE, L.J. 1963. Neurosecretory phenomena in the zebra mussel Dreissena polymorpha. Archives Neerlandaises De Zoologie Tome, 15: 237-314.

APHA, 1980. Standard methods. For the Examination of Water and Wastewater. 15th Ed. APHA - AWWA - WPCF., Washington, 1193 pp.

APPUKUTTAN, K.K. 1977. On the occurrence of green mussel Perna viridis (Linnaeus) in Andaman Island. Indian J. Fish., 24: 244-247.

APPUKUTTAN, K.K. AND T. PRABHAKARAN NAIR. 1980. Fishery and biology of the brown mussel, Perna indica, Kuriakose and Nair. CMFRI Bulletin No. 29, Coastal Aquaculture: Mussel Farming. Progress and Prospects, pp. 5-9.

APPUKUTTAN, K.K., T. PRABHAKARAN NAIR, M. JOSEPH AND K.T. THOMAS. 1980. Culture of brown mussel at Vizhinjam. CMFRI

BAYNE, B.L. 1964. Primary and secondary settlement in Mytilus edulis (L.). J. Anim. Ecol., 33: 513-523.

_____ 1965. Growth and the delay of metamorphosis of the larvae of Mytilus edulis (L.) Ophelia, 2: 1-47.

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

_____ (ed.). 1976 a. Marine Mussels: Their Ecology and Physiology. International Biological Programme, 16. Cambridge Univ. Press, London and New York, pp. 506.

_____ 1976 b. Aspects of reproduction in bivalve molluscs. In: M. Wiley (ed.) Estuarine Processes, Vol. I: Uses, Stresses and Adaptation to the Estuary. Academic Press, New York, pp. 432-448.

BAYNE, B.L. AND R.J. THOMPSON. 1970. Some physiological consequences of keeping Mytilus edulis in the laboratory. Helgolander wiss. Meeresuntersu., 20: 526-552.

BAYNE, B.L., P.A. GABBOT AND J. WIDDOWS. 1975. Some effects of stress in the adult and the eggs and larvae of Mytilus edulis (L.) J. mar. biol. Ass. U.K., 55: 675-689.

BAYNE, B.L., A. BUBEL, P.A. GABBOTT, D.R. LIVINGSTONE, D.M. LOWE AND M.N. MOORE. 1982. Glycogen utilisation and gametogenesis in Mytilus edulis (L.) Mar. Biol. Lett., 3: 89-105.

BERNER, L. 1952. Biologie de Pinnotheres pisum Penn. (Decapode, Brachyoure). Bull. Soc. Zool. Fr., 77: 344-349.

BILLARD, R. 1981. Short-term preservation of sperm under oxygen atmosphere in rainbow trout (Salmo gairdneri). Aquaculture. 23: 287-293.

BLAKE, N.J. 1972. Environmental regulation of neurosecretion and reproductive activity in the Bay scallop, Aequipecten irradians (Lamarck). Ph.D. Thesis. Univ. of Rhode Island. Kingston.

BOURCART, C. AND P. LUBET. 1965. Cycle sexual et evolution des reserves chez Mytilus galloprovincialis Lmk (Moll. Bivalve). Rap. P.V. Reun. Comm. Int. Expl. Sci. Medit., 18: 155-156.

BOURCART, C., R. LAVALLARD AND P. LUBET. 1965. Ultrastructure du spermatozoide de la moule (Mytilus perna Von Ehering). C.R. Acad. Sci., 260: 5096-5099.

BROOM, M.J. AND J. MASON. 1978. Growth and spawning in the Pectinid Chlamys opercularis in relation to temperature and phytoplankton concentration. Mar. Biol., 47: 277-285.

CARRIKER, M.R. 1961. Interrelation of functional morphology, behaviour and autecology in early stages of the bivalve Mercenaria mercenaria. J. Elisha Mitchell Sci. Soc., 77: 168-242.

CARVAJAL, R.J. 1969. Fluctuation mensual de las larvas Y crecimiento del mejillon Perna perna (L) Y las condiciones ambientales de la ensenada de Guatapanare, Edo, Sucre, Venezuela, Bol. Inst. Oceanogr. Univ. Oriente, Venezuela, 8: 13-20.

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

COE, W.R. 1932. Development of the gonads and the sequence of the sexual phases in the California oyster (Ostrea lurida). Bull. Scripps Inst. Oceanogr., Tech. Ser., 3: 119-144.

COMELY, C.A. 1974. Seasonal variation in the flesh weights and biochemical content of the scallop, Pecten maximus (L.) in the Clyde Sea area. J. Cons. Cons. Perm. Int. Explor. Mer., 35: 281-295.

COSTANZO, G. 1966. Some histochemical aspects of the male and female gonads of M. galloprovincialis L. Atti. Dell' Accademia Gioenia di scienze naturali, 18: 93-98.

DAN, J.C. 1962. The vitelline coat of the Mytilus egg. I. Normal structure and the effect of acrosomal lysin. Biol. Bull. (Woods Hole, Mass.), 123: 531-542.

DAN, J.C., Y. KARIKANA, H. KUSHIDA AND K. FUJITA. 1972. Acrosomal triggers. Exp. Cell Res., 72: 60-68.

DARBYSHIRE, M. 1967. The surface waters off the coast of Kerala, south-west India. Deep-Sea Res., 14: 295-320.

DARE, P.J. 1973. Seasonal changes in meat condition of sublittoral mussels (Mytilus edulis L.) in the Conwy fishery, North Wales. I.C.E.S., C.M. 1969, Shellfish Comm., Doc. No. 31: 1-6.

DAVIS, H.C. 1969. Shellfish hatcheries - present and future. Trans. American Fish. Soc., 98 (4): 743-750.

DOGRA, G.S. AND B.K. TANDAN, 1964. Adaptation of certain histological techniques for in situ demonstration of neuroendocrine system of insects and other animals. Anark. J. Biocrom. Soc., 105: 455-466.

DURFORT, M. 1973. Sur la formation des lamelles cuticulaires dans les oocytes des Mytilus edulis L. Comptes Rendus. Academie des Sciences, Paris, 276: 3175-3177.

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

_____ 1965. On the seasonal gonadal changes and spawning in the adult oyster Crassostrea cucullata (Schlotheim). J. mar. biol. Ass. India, 7: 328-344.

EASTERSON, D.C.V. AND S. MAHADEVAN. 1980. Review of open sea environmental conditions along Indian coast. CMFRI Bulletin, No. 29. Coastal Aquaculture: Mussel Farming. Progress and Prospects. pp. 17-21.

FAHRMANN, W. 1961. Licht - und electronenmikroskopische untersuchungen des nervensystems von Unio tumidus (Philipsson) unter besonderer berucksichtigung der neurosekretion. Z. Zellforsch., Berlin, 54: 689-716.

FIELD, I.A. 1922. Biology and economic value of the sea mussel Mytilus edulis. Bull. U.S. Bur. Fish., 38: 127-259.

FLORKIN, M. 1966. Nitrogen metabolism. In: K.M. Wilbur and C.M. Yonge (eds.) Physiology of Molluscs. Vol. II. Academic Press, New York and London, pp. 309-351.

FOLCH, J.M. LEES AND G.H. SLOANE-STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

FOX, D.L. AND W.R. COE. 1943. Biology of the California Sea - mussel (Mytilus californianus). II. Nutrition, metabolism, growth and calcium deposition. J. Exp. Zool., 93: 205-249.

FRANC, A. 1960. Classe des bivalves. In: P.P. Grassé (ed.) Traité de Zoologie. Masson, Paris, 2: 1845-2133.

FRANZEN, A. 1955. Comparative morphological investigations into spermatogenesis among mollusca. Zool. Bidr. Uppsala, 30: 399-456.

FRETTER, V. AND A GRAHAM. 1964. Reproduction. In: K.M. Wilbur and C.M. Yonge (eds.) Physiology of Mollusca. Vol.I. Academic Press, New York, pp. 127-164.

GABBOTT, P.A. 1975. Storage cycles in marine bivalve molluscs. A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. Proc. Eur. Mar. Biol. Symp., 9th Oban, Scotland, pp. 191-211.

_____ 1976. Energy metabolism. In: B.L. Bayne (ed.) Marine mussels. International Biological Programme 10. Cambridge University Press, pp 293-355.

GABBOTT, P.A. AND B.L. BAYNE. 1973. Biochemical effects of temperature and nutritive stress on Mytilus edulis L. J. mar. biol. Ass. U.K., 53: 269-286.

GABE, M. 1955. Particularities histologiques de cellules neurosecretrices chez quelques lamellibranches. C.R. Acad. Sci., 240: 1810-1812.

_____1965. La neurosecretion chez les mollusques et ses rapports avec la reproduction. Arch. Anat. Microsc. Morphol. Exp., 54: 371-385.

_____ 1966. Neurosecretion. Pergamon, Oxford, 872 pp.

GALSTOFF, P.S. 1961. Physiology of reproduction in molluscs. Am. Zool., 1: 273-289.

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

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

_____ 1969. A new approach to the biochemical composition of the molluscan body. Oceanogr. mar. Biol. Ann. Rev., 7: 175-229.

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

GODDARD, C.K., AND A.W. MARTIN. 1966. Carbohydrate metabolism. In: Wilber K.M. and C.M. Yonge (eds.) Physiology of Mollusca Vol. II. Academic Press. New York, pp. 275-308.

GOMORI, G. 1939. A differential stain for cell types in the pancreatic islets. Am. J. Pathol., 15: 497-499.

_____ 1950. Aldehyde fuschin: A new stain for elastic tissue. Am. J. Clin. Pathol., 20: 665-666.

HANUMANTE, M.M., U.M. FAROOQUI, G.K. KULKARNI AND R. NAGABHUSHANAM. 1979. Inhibition of hypertonic saline stimulated neurosecretory changes in the fresh water bivalve. Indonaia caerulea (Prashad) by Chlorpromazine and reserpine. Malacologia. 18: 569-573.

HAUSCHKA, S.D. 1963. Purification and characterisation of Mytilus egg membrane lysin from sperm. Biol. Bull. (Woods Hole, Mass.), 125:363.

HIRAI, E. 1963. On the breeding seasons of invertebrates in the neighbourhood of the marine station of Asamushi. Science Reports Tohoku Univ., Ser. 4, Biology, 24: 369-375.

HORNELL, J. 1922. The common molluscs of south India. Madras Fish. Bull., 14: 97-210.

HOUTTEVILLE, P. 1974. Contribution a l'E'tude Cytologique et Experimentale du Cyde Annuel du Tissue de Reserve du Manteau de Mytilus edulis (L.). Doctor of Science Thesis, Univ. Caen.

HRS - BRENKO, M. 1971. Observations on the occurrence of planktonic larvae of several bivalves in the northern Adriatic Sea. In: D.J. Crisp (ed.) Fourth European marine biology symposium. Cambridge Univ. Press, London, pp. 45-53.

HUGHES, T.E. 1973. An examination of eggs challenged with cryopreserved spermatozoa of the American oyster, Crassostrea virginica. Cryobiology: 10: 342-344.

HUMASON, G.L. 1962. Animal Tissue Techniques. W.H. Freeman and Company, San Francisco, CA, 468 pp.

HUMPHRSYS, W.J. 1962 Electron microscope studies on eggs of Mytilus edulis. J. Ultrastruct. Res., 7: 467-487.

IDELMAN, S. 1967. Donn'ees recentes sur l'infrastructure du spermatozoide. Annn'e Biologique, Paris, 6: 113-190.

ILLANES, J. 1979. Recherches cytologiques et experimentales sur la neurosecretion de la moule, Mytilus edulis L. Bulletin Societe Ecologique de France, 105: 141-145.

ILLANES, J. AND P. LUBET. 1980. Stude de l'activite' Neurosecretrice au cours due cycle sexual annual de la moule, Mytilus edulis L. Bulletin Societe Ecologique de France, 105: 141-145.

IMAI, T. 1971. Aquaculture in shallow seas: Progress in shallow sea culture. Amerind Publishing Pvt. Ltd., New Delhi, pp. 115-614.

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

JAYASHANKAR, N.P. 1966. Studies on the aerobic microflora associated with the retting of coconut husks. Ph.D. Thesis, Indian Institute of Science, Bangalore.

JENSEN, A.S. AND F. SPARCK. 1934. Bloddyr 2. Saltvandsmuslinger. Danmarks Fauna, Copenhagen, 40: 1-208.

JOHNSTONE, J. 1898. The spawning of the mussel (Mytilus edulis). Proceedings and Transactions of the Liverpool Biological Society, 13: 104-121.

JONES, S. 1950. Observations on the bionomics and fishery of the brown mussel (Mytilus sp.) of the Cape Region of Peninsular India. J. Bombay nat. Hist. Soc., 49: 519-528.

JONES, S. AND K. ALAGARSWAMI. 1973. Mussel fishery resources of India. In: Proceedings of the Symposium on Living Resources in the Seas around India. Cochin, India, CMFRI, Special Publication, pp. 641-647.

KELLY, R.N., M.J. ASHWOOD-SMITH AND D.V. ELLIS. 1982. Duration and timing of spermatogenesis in a stock of the mussel Mytilus californianus. J. mar. biol. Ass. U.K., 62: 509-519.

KEMP, A. AND VAN KITS HEIJNINGEN. 1954. A colorimetric micromethod for the determination of glycogen in tissues. Biochem. J., 56: 646.

KORRINGA, P. 1976. Farming Marine Organism low in the food chain. Elsevier Scientific Publishing Company, Amsterdam, 264 pp.

KRISHNAMOORTHY, T.M. AND R. VISWANATHAN. 1968. Primary productivity studies in Bombay Harbour Bay using ^{14}C. Indian J. mar. biol., 2: 116-116.

KRISHNAN, S. 1968. Histochemical studies on reproductive and nutritional cycles of the holothurian, Holothuria scabra. Mar. Biol., 2: 54-65.

KURIAKOSE, P.S. 1973. Studies on Mytilids from India. Ph.D. Thesis. Univ. Kerala. pp. 347.

_____ 1980. Open sea raft culture of green mussel at Calicut. CMFRI Bulletin 29, Coastal Aquaculture: Mussel farming. Progress and Prospects. pp. 33-38.

KURIAKOSE, P.S. AND N.B. NAIR. 1976. The genus Perna along the coasts of India with the description of a new species Perna indica. Aqua. Biol., 1: 25-36.

LAMY, E. 1936. Revision des Mytilidae vivants due Museum National d' Histoire Naturelle de Paris. J. Conchyliol., 80: 66-102.

LANNAN, J.E. 1971. Experimental self-fertilization of the Pacific oyster, Crassostrea gigas, using cryopreserved sperm. Genetics, 68: 599-601.

LAWRENCE, J.M. 1976. Patterns of lipid storage in post-metamorphic marine invertebrates. Am. Zool., 16: 747-762.

LINNAEUS, C. 1758. Systema Naturae 10th (Ed.)

LOMTE, V.S. AND M.L. JADHAV. 1981. Neurohormonal impact on neurosecretion in the cerebral ganglia of Lamellidens corrianus. GEOBIOS, 8: 197-200.

LONGO, F.J. AND E. ANDERSON. 1969. Cytological aspects of fertilization in the lamellibranch, Mytilus edulis. 1. Polar body formation and development of the female pronucleus. Exp. Zool., 172: 69-96.

LONGWELL, A.C. AND S.S. STILES. 1968. Fertilization and completion of meiosis in spawned eggs of the American oyster, Crassostrea virginica Gmelin. Carnologia, 21: 65-73.

LOOSANOFF, V.L. 1937. Development of the primary gonad and sexual phases in Venus mercenaria Linnaeus. Biol. Bull. (Woods Hole, Mass.), 72: 389-405.

_____ 1965. Gonad development and discharge of spawn in oysters of Long Island Sound. Biol. Bull. (Woods Hole, Mass), 129: 546-561.

LOOSANOFF, V.L. AND H.C. DAVIS. 1950. Conditioning V. mercenaria for spawning in winter and breeding its larvae in the laboratory. Biol. Bull. (Woods Hole, Mass). 98: 60-65.

LOOSANOFF V.L. AND H.C. DAVIS. 1963. Rearing of bivalve molluscs. In: F.S. Russel (ed.) Advances in Marine Biology. Vol. I., Academic Press, pp. 1-130.

LOOSANOFF, V.L. AND C.A. NOMEJKO. 1951. Existence of physiologically different races of oysters, Crassostrea virginica. Biol. Bull., 101: 151-156.

LORENZEN, C.J. 1967. Determination of chlorophyll and pheopigments: Spectrophotometric equations. Limnol. Oceanogr., 12: 343-346.

LOUGH, R.G. AND J.J. GONOR. 1971. Early embryonic stages of Adula californiensis (Pelecypoda: Mytilidae) and the effect of temperature and salinity on developmental rate. Mar. Biol., 8: 118-125.

LOWE, D.M., M.N. MOORE AND B.L. BAYNE. 1982. Aspects of gametogenesis in the marine mussel Mytilus edulis L. J. Mar. Biol. Ass. U.K., 62: 133-145.

LOWRY, O.H., N.J. ROSE BROUGH, A.L. FARR AND L.J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

LUBET, P. 1955 a. Effects delablation des centres nerveux sur lemission des-gametes chez Mytilus edulis L. et Chlamys varia L. (Moll. Lamellibranches). Ann. Sci. Nat., Zool. Biol. Anim., 2: 175-183.

_____ 1955 b. Cycle neurosecretoire chez Chlamys varia L. (Moll. Lamellibr.). C.R. Acad. Sci., 235: 1680-1681.

_____ 1956. Effect de Lablation des centres nerveux sur lemission des gametes chez Mytilus edulis et Chlamys varia. Ann. Sci. Natur. Zool., 18: 175-183.

_____ 1957. Cycle sexuel de Mytilus edulis et de M. galloprovincialis dans le bassin d' Arcachon. Annls Biol., 33: 19-26.

_____ 1959. Recherches sur le cycle sexual et l'emission des gametes chez les Mytilides et les Pectinides. Rev. Trav. Inst. Pechas Marit., 23: 389-548.

_____ 1965. Incidences de l'ablation bilaterale des ganglions cerebroides sur la gametogenese et le developpement du tissue conjunctif chez la moule Mytilus galloprovincialis Lam. (Moll. Lamellibranches). C.R. Soc. Biol., 159: 397-399.

_____ 1966. Essai d'analyse experimentale des perturbations produites par les ablations des ganglions nerveux chez Mytilus edulis L. et Mytilus Lmk (Mollusques Lamellibranches) Ann. Endocrinol., 27: 383-385.

LUBET, P. 1973. Expose Synoptique des Donnees Biologique sur la Moule Mytilus galloprovincialis (Lamarck 1819). Synopsis FAO sur les Peches No. 88 (SAST - Moule, 3, 16 (10), 028, 08 pag. var). FAO, Rome.

_____ 1976. Ecophysiologie de la reproduction chez les Mollusques Lamellibranches. Haliotis, 7: 49-55.

_____ 1980-81. Action des facteurs internes sur la reproduction des Mollusques Lamellibranches. OCEANIS, 6: 491-510.

LUBET, P. AND P. LeGALL. 1967. Observations sur le cycle sexuel de Mytilus edulis L. a Luc-s-Mer Bulletin de la Societe Linneene de Normandie, 10: 303-307.

LUBET, P., P. MERLIN, M. MATHIEU AND P. COLLIN. 1976-78. Tissu de reserve cycle sexuel chez les lamellibranches. Haliotis 7: 59-62.

LUBET, P., J.P. GIMAZANA, AND G. PRUNUS. 1981. Etude du cycle reproduction de Mytilus galloprovincialis (Lmk) (Moll. Lamellibranche) A la limite meridinale de son aire de repartition. Comparison ave le les autres sectors de cette aire. Haliotis, 11: 157-170.

LUBET, P. AND M. MATHIEU. 1982. The action of internal factors on gametogenesis in Pelecypod molluscs. Malacologia, 22: 131-136.

LUCAS, A. 1965. Recherche sur la sexualite des mollusques bivalves. Ph.D. Thesis, Fac. Sci. Univ. de Rennes.

LUNETTA, J.E. 1969. Fisiologia da reprodugao dos mexilhoes (Mytilus perna L. Mollusca Lamellibranchia). Zool. Biol. Mar. (Sao Paulo) Nova Ser., 26: 33-111.

MALLORY, F. 1944. Physiological Technicues. S.W. Saunders Co., Philadelphia.

MANE, V.M. 1974. Growth and breeding habits of the clam, Katelysia opima in the Kalbadevi estuary at Ratnagiri. Indian J. Fish., 21: 386-398.

_____ 1981. The edible clam, Katelysia opima from the Ratnagiri coast. Mahasagar. 14: 91-94.

MANE, U.M. AND R. NAGABHUSHANAM. 1975. Body distribution and seasonal changes in the biochemical composition of the estuarine mussel, Mytilus viridis at Ratnagiri. Riv. Idrol., XIV: 163-175.

MANE, V.M. AND R. NAGABHUSHANAM. 1980. Seasonal changes in the gonad and the associated neurosecretion in the green mussel Perna viridis from Bhatia creek at Ratnagiri on the west coast. Symposium on coastal Aquaculture. 1980. MBAI, Cochin. Abstract No. 57.

MANN, R. 1979. Some biochemical and physiological aspects of growth and gametogenesis in Crassostrea gigas and Ostrea edulis growth at sustained elevated temperatures. J. mar. biol. Ass. U.K., 59: 95-110.

MARTOJA, M. 1972. Endocrinology of Molluscs. In: M. Florkin and S.T. Scheer (eds) Chemical Zoology. Academic Press, New York, pp. 349-392.

MATHIEU, M. 1979. Etude Experimentale du Centrale Neuroendocrinien de Cycles de Developpement de la Gonade et du Tissue de Reserve Chez la Moule Adulte Mytilus edulis L (Mollusque Lamellibranches). Doctor of Science Thesis. Univ. of Caen.

MENON, M.A.S. 1945. Observations on the seasonal distribution of the plankton of Trivandrum coast. Proc. Indian Acad. Sci., 22: 31-62.

MILSIKOVSKY, S.A. 1970. Seasonal and daily dynamics in pelagic larvae of marine shelf bottom invertebrates in nearshore waters of Kandalasha Bay (White sea). Mar. Biol., 5: 180-194.

MOORE, D.R. AND D.J. REISH. 1969. Studies on the Mytilus edulis community in Alamitos Bay, California. IV. Seasonal variation in gametes from different regions of the bay. Veliger, 11: 250-255.

MOORE, M.N. 1976. Cytochemical demonstration of latency of lysosomal hydrolases in digestive cells of common mussel Mytilus edulis, and changes induced by thermal stress. Cell and Tissue Research, 175: 279-287.

MORSE, D.E., H. DUNCAN, N. HOOKER AND A. MORSE. 1976. An inexpensive chemical method for the control and synchronous induction of spawning and reproduction in molluscan species important as protein-food sources. FAO Fish. Rep. No. 200, FIR - R 200 (E/ES), pp. 291-300.

MURALEEDHARAN, V., K. GEORGE JOSEPH AND K. DEVADASAN. 1982. Pickled products from Green mussel. Fishery Technology, 19: 41-43.

NAGABHUSHANAM, R. 1962 a. Neurosecretion in the oyster Crassostrea virginica. Amer. Zool., 2: 433.

_____ 1962 b. The histology of neurosecretory system of the clam, Spisula solidissima. Amer. Zool., 2: 543.

_____ 1962 c. On the neurosecretory system in the wood boring mollusc, Bankia (Bankiella) gouldi. Proc. 2nd All India Cong. Zool. (Varanasi), 17 Abst.

_____ 1963 a. Neurosecretion in the oyster, Crassostrea virginica. J. Zool. Soc. India, 15: 100-111.

_____ 1963 b. Neurosecretory cycle and reproduction in the bivalve Crassostrea virginica. Indian J. Exp. Biol., 1: 161-162.

NAGABHUSHANAM, R. 1963 c. Neurosecretory cells of the bivalve, Yoldia limatula J. mar. biol. Ass. India, 5: 1-2.

_____ 1964 a. Note on the neurosecretory cells in the nervous system of the mussel, Modiolus demissus. Curr. Sci., 33: 20-21.

_____ 1964 b. Studies on the neurosecretion in the mollusc, Mulinia lateralis (Say). J. Anat. Soc. India, XII: 12-14.

_____ 1964 c. Neurosecretory changes in the nervous system of the oyster, Crassostrea virginica, induced by various experimental conditions. Indian J. Exp. Biol., 2: 1-4.

_____ 1968. Observations on neurosecretion in the Central nervous system of Molluscs. Bull. Natl. Inst. Sci. India, No. 38: 1-16.

_____ 1969. A cytological and cytochemical study of the neurosecretory cells in the bivalve, Meretrix casta. Proc. Symp. Mollusca, Cochin, 1968, Mar. Biol. Assoic. India., Symp, Part 2, 633-634.

_____ 1970. The Neurosecretory system of the Pholad, Diplothyra smithii (Mollusca: Lamellibranchiata). Records of the Zoological Survey of India, 62: 101-107.

_____ 1972. Neurosecretory phenomena in the clam, Parreysia corrugata (Mollusca: Pelecypoda). J. Zool. Soc. India, 24: 117-123.

NAGABHUSHANAM R. AND V.S. LOMTE. 1972. Some histochemical studies on the neurosecretory cells of the freshwater mussel, Parreysia corrugata under stressed condition. Marath. Univ. J. Sci., 11: 393-395.

NAGABHUSHANAM, R. AND V.S. LOMTE. 1973. Effect of ganglion ablation on spawning and water filtration in Parreysia corrugata. Marath. Univ. J. Sci., 12: 57-59.

NAGABHUSHANAM, R. AND U.H. MANE. 1973. Neurosecretion in the clam, Katelysia opima. Marathwada Univ. J. Sci., 12: 193-203.

_____ 1975 a. Reproduction in the mussel, Mytilus viridis, at Ratnagiri. Bull. Dept. Mar. Sci., Univ. Cochin, 7: 377-378.

_____ 1975 b. Reproduction and breeding of the clam, Katelysia opima, in Kalledevi estuary at Ratnagiri, west coast of India. Indian J. Mar. Sci., 4: 86-92.

NAGABHUSHANAM, R. AND D.S. BIDARKAR. 1975. Studies on neurosecretion in Indian rock oyster. Crassostrea cucullata. Marath. Univ. J. Sci., XIV: 259-270.

NAGABHUSHANAM, R. AND P.M. TALIKHEDKAR. 1975. Neurosecretory cells in the cerebral and visceral ganglia of Donax cuneatus. Bull. Dept. Mar. Sci. Univ. Cochin, 7: 447-453.

NAGABHUSHANAM, R., B.M. MANTALE, U.H. MANE AND R.S. DESHMUKH. 1975. On the neurosecretory cells in the central ganglia of mussel, Mytilus viridis. In: R. Natarajan (ed.) Recent Researches in Estuarine Biology, pp. 105-108.

NAGABHUSHANAM, R. AND U.H. MANE. 1978. Seasonal variation in the biochemical composition of Mytilus viridis at Ratnagiri on the west coast of India. Hydrobiologia, 57: 69-72.

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

MAIDU, K.S. 1970. Reproduction and breeding cycle of the giant scallop, Placopecten magellanicus (Gmelin) in Port are Port Bay, Newfoundland. Can. J. Zool., 48: 1003-1012.

NAIR, P.V.R. 1974. Studies on the Primary Production in the Indian Seas. Ph.D. Thesis. Univ. Cochin.

NARASIMHAM, K.A. 1980. Fishery and biology of the green mussel, Perna viridis (Linnaeus). CMFRI bulletin 29. Coastal Aquaculture; Mussel Farming Progress and Prospects, pp. 10-16.

NELSON, T.C. 1928. Relation of spawning of the oyster to temperature. Ecology, 9: 145-154.

NIIJIMA, L. 1963. Acrosome reaction and sperm entry in Mytilus edulis. Bull. Mar. Biol. Sta. Asamushi, 11: 217-221.

NIIJIMA, L. AND J. DAN. 1965. The acrosome reaction in Mytilus edulis L. 1. Fine structure of the intact acrosome. 2. Stages in the reaction, observed in supernumerary and calcium-treated spermatozoa. J. Cell. Biol., 25: 243-259.

ORTON, J.H. 1920. Sea temperature, breeding and distribution of marine animals. J. mar. biol. Ass. U.K., 12: 339-366.

PANIKKAR, M.K. AND R.G. AIYAR, 1939. Observations on breeding in brackish-water animals in Madras. Proc. Indian Acad. Sci., 9 B: 343-364.

PARULEKAR, A.H. AND S.G. DALAL. 1980. Some aspects of environmental physiology of raft grown mussels. CMFRI, Workshop on Mussel Farming. 25-27 September, 1980. Madras, India.

PAUL, M.D. 1942. Studies on the growth and breeding of certain sedentary organisms in the Madras harbour. Proc. Indian Acad. Sci., 15 B: 1-42.

PEARSE, A.G.E. 1968. Histochemistry theoretical and applied. Vol. I. (3rd Edn.) J. & A. Churchill Ltd., London, 759 pp.

PEARSE, J.S. AND M.J. BARKSDALE. 1984. Temporal patterns of reproduction by shallow water marine invertebrates in the Indian Ocean. International Conference on Biology of Marine Benthic Organisms, Aurangabad, 20-24 January 1984, Abstract I-1. (Marathwada Univ., Univ. Grants Commission and Indian Soc. Comp. Endocrinology of India and American Inst. Biol. Sciences and Office of Naval Research of U.S.A.).

PIETERS, H., J.H. KLUYTMANS, D.I. ZANDEE AND G.C. CADEE. 1980. Tissue composition and reproduction of Mytilus edulis dependent on food availability. Netherlands Journal of Sea Research, 14: 349-361.

PIKE, R.B. 1971. Report on mussel farming and mussel biology for the fishing industry board. Technical Report. New Zealand Fisheries Industry Board, 71: 1-7.

PILLAY, T.V.R. AND Wm. A. DILL. 1979. Advances in Aquaculture. Fishing News Books Ltd.

QASIM, S.Z., S. WELLERSHAUS, P.M.A. BHATTATHIRI AND S.A.H. ABIDI. 1969. Organic production in a tropical estuary. Proc. Indian Acad. Sci., 69 B: 51-74.

QASIM, S.Z., A.H. PARULEKAR, S.N. HARKANTRA, Z.A. ANSARI AND A. NAIR. 1977. Aquaculture of green mussel Mytilus viridis L. Cultivation on ropes from floating rafts. Indian J. Mar. Sci., 6: 15-25.

RAMAMIRTHAM, C.P. AND R. JAYARAMAN. 1960. Hydrographic features of the continental shelf waters off Cochin during the years 1958 and 1959. J. mar. biol. Ass. India, 2: 199-207.

RAO, SURYANARAYANA, S.V. AND P.C. GEORGE. 1959. Hydrology of the Korapusha Estuary Malabar, Kerala State. J. mar. biol. Ass. India, 1: 212-223.

RAO, VIRABHADRA, K. 1951. Observations on the probable effects of salinity on the spawning, development and setting of the Indian backwater oyster, C. madrasensis Preston. Proc. Indian Acad. Sci., 33 B: 231-256.

_____ 1953. Sex change in the oviparous Indian backwater oyster, Ostrea madrasensis Preston, Curr. Sci., 22: 377-378.

_____ 1956. Seasonal gonadal changes in the adult backwater oyster, Ostrea (crassostrea) madrasensis Preston from Ennur near Madras. Proc. Indian Acad. Sci., XLIV: 332-356.

RAO, VIRABHADRA, K; L. KRISHNA KUMARI AND S.N. DIWEDI. 1975. Biology of the Green Mussel, Mytilus viridis. Indian J. Mar. Sci., 4: 189-197.

REDDY, C.V.G. AND V.N. SANKARANARAYANAN. 1968. Distribution of nutrients in the shelf waters of the Arabian sea along the West coast of India. Bull. Natn. Inst. Sci. India, 38: 103-122.

RENZONI, A. AND F. GIUSTI. 1972. Further observations on the reproductive cycle of Mytilus galloprovincialis Lam. In: B. Battaglia (ed.) Proc. Europ. Mar. Biol. Symp. 5th Venice. Piccin Editore Padua, Italy, pp. 295-300.

REVERBERI, G. 1971. Mytilus. In: G. Reverberi (ed.) Experimental embryology of marine and fresh water invertebrates. North-Holland Publishing Co., Amsterdam. pp. 175-87.

SASTRY, A.N. 1963. Reproduction of the bay scallop Aequipecten irradians Lamarck. Influence of temperature on maturation and spawning. Biol. Bull. (Woods Hole, Mass), 125: 146-153.

_____ 1966. Temperature effects in reproduction of the bay scallop, Aequipecten irradians Lamarck. Biol. Bull. (Woods Hole, Mass.), 130: 118-134.

_____ 1968. Relationships among food, temperature and gonad development of the bay scallop, Aequipecten irradians Lamarck. Physiol. Zool., 41: 44-53.

_____ 1970. Reproductive physiological variation in latitudinally separated populations of the bay scallop, Aequipecten irradians Lamarck. Biol. Bull. (Woods Hole, Mass), 138: 56-65.

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

SASTRY, A.N. AND N.J. BLAKE. 1971. Regulation of gonad development in the bay scallop Aequipecten irradians Lamarck. Biol. Bull. (Woods Hole, Mass.), 140: 274-283.

SAWYA, P. 1965. Physiological aspects of the ecology of the mussel Mytilus perna L. Anales da la Academia Brasileira de Ciencias, 37 (Supplement), 176-178.

SCHARRER, B. 1935. Uber das Nanstromanche Organ Xhei Ophistlobranchiern. Publ. Stat. Zool. Napoli, 15: 132-142.

SEED, R. 1969. The ecology of Mytilus edulis L. (Lamellibranchiata) on exposed rocky shores. 2. Growth and mortality. Oecologia, 3: 277-316.

_____ 1971. A physiological and biochemical approach to the taxonomy of Mytilus edulis L. and M. galloprovincialis Lmk. from south-west England. Cahier de Biologie Marine, Roscoff, 12: 291-322.

_____ 1975. Reproduction in Mytilus (Mollusca: Bivalvia) in European waters. Pubblicazioni della Stazione Zoologica di Napoli, Milan.

_____ 1976. Ecology. In: B.L. Bayne (ed.) Marine Mussels, their ecology and physiology IBP. 10. Cambridge University Press, London. pp. 13-65.

SEED, R. AND R.A. BROWN. 1975. The influence of reproductive cycle, growth and mortality on population structure in Modiolus modiolus (L.), Cerastoderma edule (L.) and Mytilus edulis L., (Mollusca: Bivalvia). In: Harold Barnes (ed.) Proc. 9th Europ. Mar. Biol. Symp. Aberdeen Univ. Press. pp. 257-274.

SHARMA, G.S. 1968. Seasonal variation of some hydrographic properties of the shelf waters off the west coast of India. Bull. Nat. Inst. Sci., India, 38: 263-276.

SILAS, E.G. 1980. Mussel culture in India - Constraints and Prospects. CMFRI bulletin 29. Coastal Aquaculture: Mussel Farming. Progress and Prospects. pp. 51-56.

SILAS, E.G. AND K. ALAGARSWAMI. 1967. On an instance of parasitisation by the Pea crab (Pinnotheres sp.) on the backwater clam (Meretrix casta) (Chemnitz) from India with a review of the work on the Systematics, Ecology, Biology and Ethology of the pea-crabs of the genus Pinnotheres latreille. Proceedings of the Symposium on Crustacea, Part III, pp. 1161-1227.

SILAS, E.G., K. ALAGARSWAMI, K.A. NARASIMHAM, K.K. APPUKUTTAN AND P. MUTHIAH. 1982. In: F. Brian Davy and Michael Graham (eds.) Bivalve Culture in Asia and the Pacific: Proceeding of a Workshop held in Singapore 16-19 February 1982., pp. 34-43.

SNEDECOR, G.W. AND W.G. COCHRAN. 1967. Statistical Methods. (6th Ed.). Oxford IBH Publishing Co., 593 pp.

SOOT-RYEN, T. 1955. A report on the family mytilidae (Pelecypoda). Allan Hancock Pacific Expeditions, 20: 1-174.

STOSS, J. AND E.M. DONALDSON. 1982. Preservation of fish gametes. Prog. Internat. Symp. Regul. Physiol. Fish. (C.J.J. Richter and H.J. Th. Goos, eds.). pp. 114-122. Centre for Agricultural Publishing and Documentation, Wageningen.

STRICKLAND, J.D.H. AND T.R. PARSONS. 1968. A practical handbook of water analysis. Bull. Fish. Res. Bd. Canada, 167: 311 pp.

SUBRAHMANYAN, R. 1959 a. Studies on the phytoplankton of the west coast of India. Part I. Proc. Indian Acad. Sci., 50 B: 113-187.

_____ 1959 b. Studies on the phytoplankton of the west coast of India. Part II. Proc. Indian Acad. Sci., 50 B: 189-252.

SUBRAMONIAM, T., (ed.). 1982. Manual of Research Methods for Marine Invertebrate Reproduction. C.M.F.R.I. Special Publication No .9, 21-40.

SUGIURA, Y. 1959. Seasonal changes in sexual maturity and sexuality of Mytilus edulis. Nippon Suisan Gakkaishi, 25: 1-6.

TAYLOR, A.C. AND T.J. VENN. 1979. Seasonal variation in weight and biochemical composition of the tissues of the Queen Scallop, Chlamys opercularis, from the Clyde Sea area. J. mar. biol. Ass. U.K., 59: 605-621.

THOMPSON, R.J. 1972. Feeding and metabolism in the mussel Mytilus edulis L. Ph.D. thesis Univ. Leicester.

_____ 1977. Blood chemistry, bio-chemical composition of tissues and the annual reproduction cycle on the gland scallop, Placopecten magellanicus, (Gmelin) from south-east Newfoundland. J. Fish. Res. Board. Canada, 34: 2104-2116.

THOMPSON, R.J., N.A. RATCLIFFE AND B.L. BAYNE. 1974. Effects of starvation on structure and function in the digestive gland of the mussel (Mytilus edulis L.) J. mar. biol. Ass. U.K., 54: 699-712.

THORSON, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates. Meddelelser fra Danmarks Fiskeri-og Havundersøgelser, Serie Plankton, 4: 1-523.

TRANTER, D.J. 1958 a. Reproduction in Australian pearl oysters (Lamellibranchia). I. Pinctada albina (Lamarck) Primary gonad development. Aust. J. Mar. Freshwater Res., 9: 135-143.

_____ 1958 b. Reproduction in Australian pearl oysters (Lamellibranchia) III. Pinctada albina (Lamarck): Breeding season and sexuality. Aust. J. Mar. Freshwater Res., 9: 191-216.

_____ 1958 c. Reproduction in Australian pearl oysters (Lamellibranchia) IV. Pinctada margaritifera (Linnaeus) Aust. J. Mar. Freshwater Res., 9: 509-525.

TRANTER, D.J. 1959. Reproduction in Australian pearl oysters (Lamellibranchia). V. Pinctada fucata (Gould). Aust. J. Mar. Freshwater Res., 10: 45-66.

UMIJI, S. 1969. Neurosecrecao em Mytilus perna (Molusco - Lamellibranquio). Zool. Biol. Mar. (Sao Paulo) Nova Ser., 26: 181-254.

VELEZ, A. 1977. Crecimiento, edad y madurez sexual del ostion Crassostrea rhizophorae. Bol. Inst. Oceanogr. Univ. Oriente, Venezuela, 16: 27-34.

VELEZ, A. AND C.E. EPIFANIO. 1981. Effects of temperature and ration on gametogenesis and growth in the tropical mussel Perna perna (L.) Aquaculture, 22: 21-36.

WADA, K.T. 1976. Temperature requirement for maturation of gonads in Japanese pearl oyster. Bull. Natl. Pearl Res. Lab., 20: 2244-2253.

WAFAR, M.V.M., S. VIJAYARAGHAVAN AND L. KRISHNA KUMARI. 1976 Seasonal changes in the nutritive value of the green mussel, Mytilus viridis Linne. Indian J. Mar. Sci., 5: 252-254.

WELSH, J.H. 1961. Neurohormones of molluscs. Am. Zool., 1: 267-272.

WHEDON, W.F. 1936. Spawning habits of the mussel Mytilus californianus with notes on the possible relation to mussel poison. Univ. Calif. Publ. Zool. 41: 33-44.

WHITE, K.M. 1937. Mytilus. Mem. Liverpool Mar. Biol. Comm., 37: 1-117.

WIDDOWS, J. 1972. Thermal acclimation by Mytilus edulis L. Ph.D. thesis, Univ. Leicester, England.

WILLIAMS, C.S. 1969. The effect of Mytilicola intestinalis on the biochemical composition of mussels. J. Mar. biol. Ass. U.K., 49: 161-173.

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

WILSON B.R. AND E.P. HODGKIN. 1967. A comparative account of the reproductive cycle of five species of marine mussels (Bivalvia: Mytilidae) in the vicinity of Fremantle, Western Australia. Aust. J. Mar. Freshwater Res., 18: 175-203.

WILSON, J.H. AND R. SEED. 1975. Reproduction in Mytilus edulis L. (Mollusca: Bivalvia) in Carlingford Lough, Northern Ireland. Irish Fisheries Investigations, Dublin, Ser. B. Marine.

YOUNG, C.M. 1960. Oysters. Collins, St. games, Place, London pp. 1-118.

YOUNG, R.T. 1942. Spawning season of the California mussel, Mytilus californianus. Ecology, 23: 490-492.

_____ 1945. Stimulation of spawning in the mussel (Mytilus californianus). Ecology, 27: 354-363.

_____ 1946. Stimulation of spawning in the mussel, Mytilus californianus. Ecology, 26: 58-69.

ZANDEE, D.I., J.M. KLUYTMANS, W. ZURBURG AND H. PIETERS. 1980. Seasonal variations in biochemical composition of Mytilus edulis with reference to energy metabolism and gametogenesis. Netherlands Journal of Sea Research, 14: 1-29.

ZWAAN, A. De AND D.I. ZANDEE. 1972. Body distribution and seasonal changes in the glycogen content of the common sea mussel Mytilus edulis. Comp. Biochem. Physiol., 43 A: 53-58.