TO
MY LOVING PARENTS

STUDIES ON THE BIOLOGY OF THE BIVALVE *MUSCULISTA SENHAUSIA* (BENSON) FROM COCHIN WATERS

THESIS

SUBMITTED TO

THE COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

MARINE BIOLOGY

UNDER THE FACULTY OF MARINE SCIENCES

By

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1991

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried out by Miss. SHINY SREEDHAR, K., under my supervision and guidance in the Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology in partial fulfilment for the degree of DOCTOR OF PHILOSOPHY of the Cochin University of Science and Technology under the Faculty of Marine Sciences, and no part there of has been presented for the award of any other degree, diploma or associateship in any University.

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DECLARATION

I, Shiny Sreedhar, K., do hereby declare that this thesis entitled "STUDIES ON THE BIOLOGY OF THE BIVALVE MUSCULISTA SENHAUSIA (BENSON) FROM COCHIN WATERS" is a genuine record of the research work done by me under the supervision of Dr. C.K. Radhakrishnan, Senior Lecturer, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, and has not previously formed the basis for the award of any degree, diploma, or associateship in any University.

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ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to Dr.C.K. Radhakrishnan, Senior Lecturer, School of Marine Sciences, Cochin University of Science and Technology for suggesting this topic, constant guidance, and encouragement throughout the period of my research work.

I am indebted to Prof. (Dr.) N. Ravindranatha Menon, Director, School of Marine Sciences and Head, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology for providing me all the facilities and encouragement during the tenure of my work.

I am greatly thankful to the late Prof. (Dr.) P.N.K. Nambisan, Former Director, School of Marine Sciences for his invaluable help and encouragement during the period of my research.

My sincere thanks are due to Shri. H. Krishna Iyer, Principal Scientist, C.I.F.T., Cochin, Dr. M.V. Mohan, Assistant Professor, Rice Research Station, Vyttila, Shri. T.M. Sankaran, Associate Professor, Fisheries College, Panangad, Shri. T. Prabhakaran Nair, Scientist - SG and Shri. Narayana Kurup, Scientist - SG., C.M.F.R.I., Cochin and Dr. Mohammed Salih, K.Y.M., Reader, School of Marine Sciences, Cochin University of Science and Technology, Cochin for their valuable help and support.

Thanks are also due to British museum and also to Dr. Akimichi Hosomi, Japan for their help in the identification of the test organism.

I thank the authorities of Cochin University of Science and Technology for awarding me a fellowship and for providing all the necessary facilities for the successful completion of my research work.

I would like to express my sincere thanks to Miss. Reethamma, O.V., Dr. Suresh, K., Mr. Maqbool, T.K., Dr. Suresh, P.G., Dr. Asok Kumar, K., Dr. Philip Mathew, Mr. Padmasenan, R. and all others who have been helpful in one way or the other during the period of research work.

SHINY SREEDHAR, K.

CONTENTS

C H A P T E R - I

GENERAL INTRODUCTION

Mollusca, a word meaning 'soft', includes a variety of invertebrate animals, with soft unsegmented body having a slippery skin and commonly sheltered in a hard calcareous shell of their own secretion. The molluscs are the second largest animal phylum, outnumbered only by the arthropods. They are remarkably diverse with regard to their morphology, anatomy, habits and habitats.

The bivalves (class Bivalvia) are the largest and the best known among the various classes of shelled molluscs, and comprise a large group of highly specialised, laterally compressed molluscs. Bivalves with the shell having two valves, the right and the left do not undergo torsion during ontogeny. Body is bilaterally symmetrical with no distinct head, and is completely enveloped by the mantle, and with a distinct foot adapted for burrowing in sand or mud. Bivalves are considerably less diverse than the gastropods. This class includes clams, mussels, oysters, scallops and some less familiar groups. Many are edible, and from prehistoric times they have formed an important part of the human food. India has extensive bivalve resources in the coastal waters, brackish waters and estuaries which have been utilised either as food or as a source of lime and cement, or sometimes as decorative shellcraft articles.

Bivalves are by far the most important group compared to gastropods and cephalopods for commercial exploitation and utilisation as food. Mussels

and clams are two important sources of protein. They have got the advantage of easy digestibility and are a good and cheap source of minerals and vitamins. Their sedentary habit, easy accessibility, profuse proliferation within a short time, hardy nature etc. make them easily reached and cheaply gathered. Improvement in the production of bivalves requires detailed studies of the habits, growth, reproduction, diseases and predators. During the past half a century a great deal of research on the physiology, behaviour and development of bivalves have been carried out in many of the marine laboratories.

Edible mussels of the Family Mytilidae are not fully exploited as a food resource in spite of their high productivity, natural abundance and world-wide distribution. The mussels live mostly in the intertidal zone but occur to depths of a few fathoms. They remain attached by their byssus threads to rocks and piers, within sheltered harbours and estuaries, and on rocky shores of the open coast, sometimes forming dense beds in the muddy bottom sediments. The mussel landings of the west coast fluctuate between 2500 to 4000 tonnes annually (Mahadevan, 1988). Kuriakose (1973) reported the presence of 17 species of mytilid fauna belonging to 7 genera. In India extensive studies have been made in various aspects like, fishery biology, culture, etc. of the green mussel (Perna viridis) and the brown mussel (P. indica) as these species occupy a top position among edible bivalves. Except these two species, studies on other related genera and species of the mytilidae are comparatively few. Thus large areas of the biology and bionomics of many mussels remain to be covered, without which complete exploitation of the existing mussel resources in the country is impossible.

The mussel Musculista senhausia occurs in large quantities in Cochin backwaters. This is found to form thick beds in bottom sediments in varying densities. It is fished in large quantities from different parts of the backwater and extensively used as poultry feed and manure. Though M. senhausia enjoys a wide distribution in the backwaters of Kerala, it is quite surprising to note that, it is not considered as a food mussel, and hence not ranked in the fishery list. Several hundred kilograms of this species are fished from backwaters and merely used as poultry feed. Prospects are amble there to develop this mussel as a highly commercial commodity. Besides poultry feed, several products like fish feed, protein concentrate etc. can be developed from this cheap food source. Though it is seasonal, this huge resource should not be wasted. In Cochin backwater, especially in the harbour area there are possibilities for water contamination by oil spills, but it is interesting to note that the beds of M. senhausia are not much affected by the effluents or oil spill. The population is affected only by the freshwater inflow during monsoon. There should be a proper research programme aimed at developing the resource for better utilisation. Its distribution and abundance with regard to different hydrological factors, growth, feeding, biochemical changes, reproduction etc. are the areas which require detailed study. The main objective of the present investigation is to provide sufficient data on these aspects so as to facilitate an economic exploitation of the resources.

The present study is presented here in eight chapters. The general introduction forms the first chapter. The second chapter describes the systematic position of the species. The third chapter deals with the description

of the study area and distribution of the species according to the salinity variation and sediment texture.

The fourth chapter deals with the age and growth of M. senhausia. The data obtained from the methodic observation extending for a period of seventeen months serve as valuable information on the growth rate of this species. Here the growth is correlated with salinity fluctuation.

The important physical factor affecting the organisms is the salinity of the medium. It affects the organisms in different ways. The salinity tolerance of M. senhausia and filtration rate in different salinities encountered in its habitat are presented in the fifth chapter.

The sixth chapter deals with the reproductive cycle of the organism. Histochemical aspects of different organic constituents are also studied.

The biochemical composition of M. senhausia forms the seventh chapter. Variation in different organic components like protein, glycogen and lipid are correlated with reproductive cycle of the species.

Summary of the work is presented in the eighth chapter followed by the list of references.

C H A P T E R - II

TAXONOMY

2.1 INTRODUCTION

The genus Musculista belongs to the bivalve family Mytilidae of the phylum Mollusca, one of the largest and most successful phyla within the animal kingdom. It constitute a group of animals of extraordinary diverse form which have become adapted to different types of habitat.

Among the six classes of the phylum Mollusca, class Bivalvia, also known as Lamellibranchia and Pelecypoda, with approximately 31,000 living species (Russel-Hunter, 1979) form the second largest class and in some way the most highly modified of all the molluscs. The bivalves are untorted, bilaterally symmetrical, laterally compressed molluscs with a rudimentary cephalic region bearing two pairs of labial palpes, with a ventral muscular foot and with a single enveloping mantle in the form of two lateral lobes that secrete a two valved shell arranged in parallel plates.

The family Mytilidae includes representatives living in a wide variety of estuarine and marine habitats. They are usually found attached in great numbers together by slender byssus threads to firm substrates like ships, piers, jetties and rocks, while some live in sand or mud or bore into the rocks. Evolution into such a wide variety of habitats has produced considerable morphological and anatomical diversity. The members of the family Mytilidae are easily recognised from other bivalves by the following characters: "Shell equivalve, generally very inequilateral, with prosogyre umbones near the anterior end; ligament elongate, deep seated generally in nymphae, the inner resilial part typically connected with the nymphae by a calcareous white ridge, mantle

lobes united below the anal siphonal opening, branchial opening confluent with the pedal opening; posterior part of mantle edges often pigmented and furnished with papillae; anterior adductor muscle smaller than the posterior one, sometimes obsolete in adult shells. Anterior byssal retractors small fastened behind the umbones; posterior retractor generally confluent with the posterior adductor; foot finger-shaped with a posterior furrow; byssal gland behind the foot and highly functional; gills filibranch, and ventricle embracing the rectum" (Soot-Ryen, 1955).

2.2 REVIEW

Several attempts have been made to explain the different genera and species of the family Mytilidae from different parts of the world. Of these von Ihering (1900) was the first to describe the subgeneric groups of the genera Mytilus and Modiolus inhabiting the eastern coast of South America. Here the superficial characters has only been taken as the basis of classification. Jukes-Browne (1905) explained several genera coming under the family Mytilidae. Prashad (1932) described the taxonomic position of sixteen species under four genera obtained during Siboga expedition. Soot-Ryen (1955) has given an excellent account on the Mytilidae collected from the Pacific coast of America. Several works have been attempted in India also. Preston (1909) described a new species Modiola cochinensis from south India. Preston (1914) reported another species Modiola undulata var. crassicostata from Chilka lake on the west coast of India. Form Chilka lake four species of the genera Mytilus and Modiolus have also been listed by Annandale (1916). Cherian (1968) has described different species of the genera Mytilus and Modiolus

of Cochin Harbour area. Kuriakose (1973) made an extensive survey of mytilid fauna of Indian waters. He described 17 species belonging to 7 genera. Among the different species, he reported the presence of two species of Musculista, M. senhausia and M. arcuatula.

The genus Musculista (Yamamoto and Habe 1958) has been wrongly identified as Modiolus Lamarck earlier. Much of the confusion existing in the systematics of this species has arisen because of its modioliform shells. But Musculista lack a hirsute periostracum which is an important character of Modiolus. Musculista is characterised by the absence of hinge teeth, the presence of teeth-like crenulations in the lunular margin and behind the ligament in the dorsal shell margin. But teeth like crenulations are absent in Modiolus. In Musculista smooth shell surface is provided with 18-22 radial lines, whereas in Modiolus radial lines are absent (Kuriakose, 1973).

2.3 DIAGNOSTIC FEATURES

Musculista senhausia is found in abundance in Cochin backwaters, covering the bottom, attached by byssus threads. The animals burrow into the mud exposing only the posterior siphonal area outside. Its distribution is controlled mainly by the salinity change of the medium. The synonymy of this species are.

1842.	Modiola senhausi	Benson, Ann. and Mag. Nat. Hist., IX: 489.
1856.	Modiola undulata	Dunker, Zool. Soc. London Proc., XXIV: 358-366.
1874.	Modiola ballaridiana	Tapparone, Zool. Viag. Int. Globo. Rgia. Fpeg. Magenta, : 144.

1911.	Modiola chilkaensis	Preston, Rec. Ind. Mus., VI: 39.
1914.	Modiola undulata & variety crassicostata	Preston, Rec. Ind. Mus., X: 304.
1916.	Modiola undulata	Annandale, Mem. Indi. Mus., V: 358.
1932.	Branchyodontes senhausia	Nomuraet, Hatai. Saito. Hoon Kai Mus. Notes, I: 4.

Musculista senhausia is characterised by elongate and oblong shell with sub-terminal umbos. Umbos conspicuous, edentulous, located somewhat close to the anterior end; anterior margin markedly extending beyond the umbos. Anterior end bluntly rounded; dorsal ligamental margin almost straight, posterior margin arcuate and ventral margin slightly concave. Lunule well developed with radial striae which makes the margin crenulated. Teeth-like crenulations present on the dorsal shell margin behind the ligament. Periostracum thin, smooth, shiny, brownish - purple in colour. Eighteen to twenty two radial lines extend from umbo to the posterior border of the shell. Interior of the shell valves white or purplish-white, iridescence, limited to the area of muscle scars. Anterior adductor long, thin, arcuate and located near the anterior ventral margin; anterior byssal retractors nearly circular and inserting into the umbonal cavity. Posterior adductor slightly oval-shaped and located above the midway between dorso-ventral margins in the posterior quarter of the shell. Posterior byssal-pedal retractors partially or entirely split into two main bundles and inserted to the dorsal shell margin along with the posterior adductor; anterior bundle inserted to the shell a little anterior to the posterior bundle. Pedal retractor is well developed. Mid gut reaches only upto the posterior end of ventricle. Mantle lobes bordering

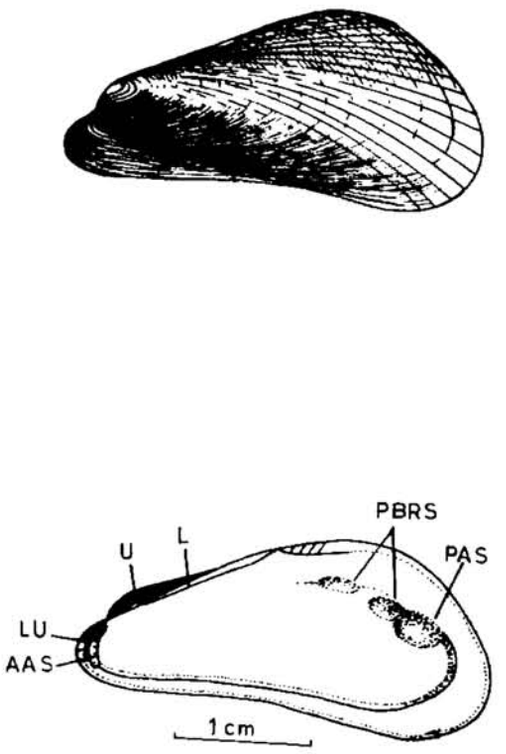


Figure 1. External and internal view of shell of Musculista senhausia showing the muscle impressions (Anterior and posterior adductor scars (AAS & PAS), posterior byssal retractor scar (PBRS)), ligament (L), lunule (Lu) and Umbo (U).

the incurrent aperture extensible and excurrent siphon tubular and extensible. Foot digitiform, highly extensible and byssal apparatus located close to the base of foot. Byssus threads many, long, very thin and silky in nature (Kuriakose, 1973).

Of the two species M. senhausia builds nests and forms extensive beds in the muddy bottom of the marine and brackish water habitat while M. arcuatula is often found attached firmly to the hard substrates in marine, brackish water and almost freshwater habitat. The shell of M. senhausia is much larger than M. arcuatula and provided with 18 to 22 radial cords arising from the summit of the umbo to the posterior border of the shell, whereas in M. arcuatula the radial striae are absent or very feebly marked (Kuriakose, 1973).

C H A P T E R - III

STUDY AREA AND ITS ENVIRONS

3.1 COCHIN BACKWATER

The Cochin backwater is a part of the largest lake in Kerala, the Vembanad lake (Lat 9°28' and 10°10' N and Long 76°13' and 76°30'E). It is a shallow tropical estuarine system of about 270 square kilometres located in the southwest coast of India. It has a narrow perennial connection with the Arabian Sea, and on the northern and southern sides it receives two major rivers, the Periyar and the Pampa respectively. Four small rivers, viz. the Achancoil, the Manimala, the Meenachil and the Muvattupuzha empty into the lake. Among these the Muvattupuzha river and the Meenachil river join it around the middle. The hydrographical conditions depend on the influence of the sea and rivers. The depth of the backwater varies from 1 to 5 m except in the dredged channels, namely the Cochin Approach Channel, the Ernakulam Channel and the Mattanchery channel for the passage of ships. The location of the study area in the Cochin backwater is shown in Fig.2.

Hydrographical structure is subjected to great changes depending on the seasons. Climatologically, the annual cycle consists of the pre-monsoon, monsoon and the post-monsoon seasons. It is influenced by the southwest monsoon from about the middle of May to August and by some precipitation from the northeast monsoon during October-December. The river discharge large quantities of freshwater into the estuary during these periods. Owing to these factors, the salinity of the water gets lowered during the peak periods of monsoon. The hydrography of Cochin backwater has been investigated

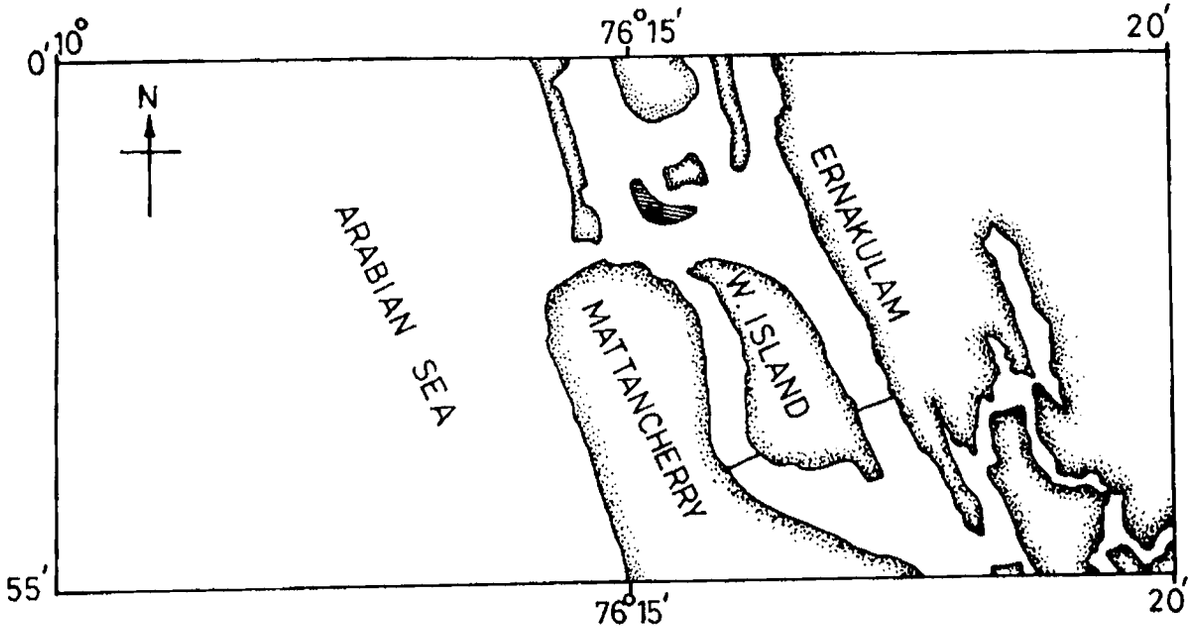


Figure 2. Map showing location of mussel bed (M)

by several workers (Ramamirtham and Jayaraman, 1963; Cheriyan, 1967; Wellershaus, 1971; Haridas et al., 1973; Balakrishnan and Shynamma, 1976; Anirudhan and Nambisan, 1990).

3.2 DETERMINATION OF SALINITY AND SEDIMENT TEXTURE

In an estuarine habitat, salinity of the water has been recognised as an important factor controlling the fauna and flora. It is well known that in tropics temperature does not show considerable fluctuations between the maxima and minima (Paul, 1942).

A thorough knowledge of the hydrographical conditions of an area is essential to understand the distribution, growth pattern, breeding and abundance of the fauna of a region during the different seasons of the year. Some attempts have been made to relate the distribution of the organisms with hydrology of Cochin backwaters. Desai and Kutty (1967) studied the distribution and abundance of benthic fauna. Madhupratap (1978) has made a study on the ecology of zooplankton resources.

Among the various physical factors of the environment, the study of sediment is a principal factor in understanding the complexity of ecological relationship with bottom fauna (Josanto, 1971a). Veerayya and Murthy (1974) have studied the distribution of sediments in the Vembanad Lake. Josanto (1971b) studied the grain size distribution of the Cochin backwater sediments. Similarly, Vizakat et al. (1991) gave an account of the community structure of benthos of Konkan, west coast of India, in relation to sediment texture, organic carbon content of sediment and bottom water salinity.

This section of the study deals with the description of the salinity and sediment texture of the mussel bed of M. senhausia.

3.3 MATERIALS AND METHODS

Collections of the specimens and sediment samples were made using an ordinary two jaw van Veen grab. Sediment samples taken along with the mussels during pre-monsoon period and monsoon period were removed and dried for grain size analysis. Bottom water was collected by means of bottom water sampler and salinity was estimated by titration against silver nitrate using potassium chromate as indicator.

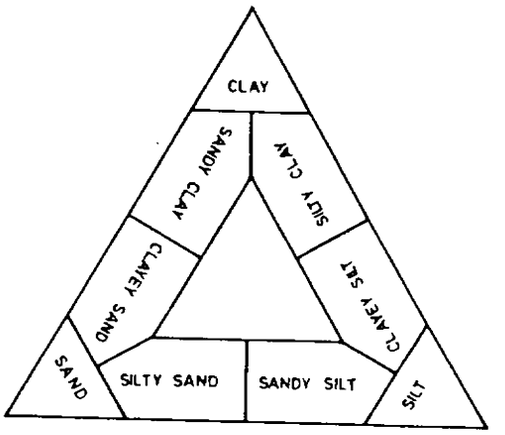
A known quantity of sediment was dispersed overnight in 0.025 N solution of sodium hexa metaphosphate. The coarse fraction was separated using 230 mesh seive, dried and weighed. The finer fractions like silt and clay were analysed by pipette analysis (Krumbein and Pettijohn, 1938). The sand, silt and clay percentage of the sediment sample were calculated and plotted in a triangular graph paper (Shepard, 1954).

3.4 RESULTS

Salinity variation in the mussel bed during the period from February 1987 to December 1988 are given in Table 1. Based on this observation, the year can be divided into pre-monsoon (January-May), monsoon (June-September) and post-monsoon (October-December) periods. During the pre-monsoon period gradual increase in salinity could be noticed. The highest salinity (30.9 ppt) observed was in May. From July onwards the salinity showed a decreasing trend with the advancement of southwest monsoon. During

Table 1. Salinity variation (in ppt) at the study area during the period 1987-88

Month	Year	
	1987	1988
February	28.4	28.0
March	28.9	26.7
April	30.2	28.9
May	30.9	29.2
June	21.8	18.6
July	8.9	6.7
August	1.4	0.8
September	6.5	4.2
October	14.4	16.2
November	14.8	14.8
December	18.6	15.8
January	24.2	–



Sand - silt - clay diagram
(after Shepard, 1954)

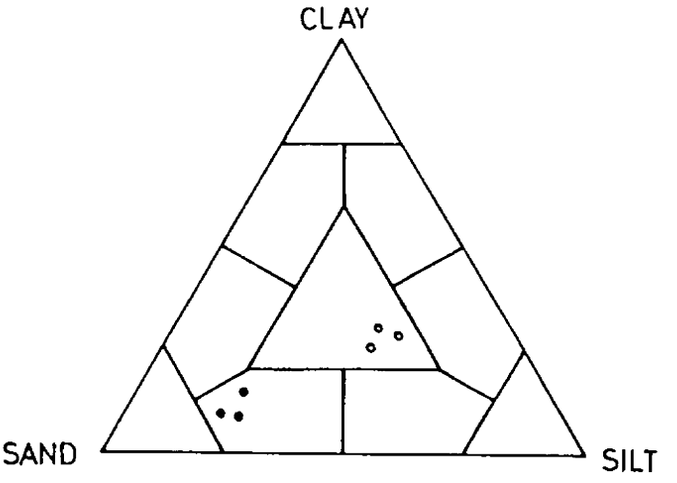


Figure 3. Sand-silt-clay contents in the sediment of the study area during pre-monsoon (o) and monsoon (•) period.

this period the salinity marked a fall almost to a near-freshwater condition. Lowest salinity (1.4 & 0.8 ppt) could be noticed during August. Again in September a rise in salinity value was noticed. Salinity distribution showed only minor variation till November due to the small amount of precipitation during the northeast monsoon period. From December onwards again a steady increase in salinity was noticed.

The results of grain size analysis of the sediments obtained during the pre-monsoon and monsoon periods are shown in Figure 3. The average percentage of sediment fractions of the pre-monsoon period was 28.1% (sand), 48.4% (silt) and 23.5% (clay) and was of sand silt clay in nature. During the monsoon period sediment was of silty sand type with 69.68% of sand.

3.5 DISCUSSION

An understanding of the seasonal variation of important physico-chemical factors of estuaries is an essential pre-requisite for the interpretation of the distribution, abundance, settlement and various behavioural responses of an animal population. Temperature, salinity and the nature of the bottom deposit are the significant factors that may affect the distribution of bottom fauna (Jones, 1950). Among these factors, salinity is the major factor affecting the distribution and abundance of Musculista senhausia in the study area. A marked difference in salinity values between pre-monsoon and monsoon periods was observed. The river discharge during the period of southwest monsoon results in drastic decrease in the salinity. During the peak period of the southwest monsoon, it is almost close to freshwater condition. The quantity of freshwater discharged into the backwater through the rivers and

land run off is so much that the tidal influences become almost negligible. During this period remarkable change in the distribution of M. senhausia could be encountered. The population was totally absent in this area. The settlement of spat could also not be encountered during this season which may be due to the prevalence of very low saline condition in the backwater.

Those animals which are acclimated to changing salinities through several years of physiological adjustments survive wide fluctuations in salinity, while less tolerant forms are completely eliminated or they migrate to the adjacent suitable area and the area left bare by the organisms is subsequently recolonised by the larvae of these organisms brought in by tidal currents when optimum condition is reestablished (Batcha, 1984). Extremely low salinity of the ambient medium results in the reduction in the number of individuals. Thus diversity of species was low during the southwest monsoon period. Desai and Kutty (1967) observed that the salinity of the water governs the abundance of bottom fauna of the Cochin backwater. Parulekar et al. (1973) suggested that a decrease in the population of Meretrix casta in Benastrim clam bed in Mandovi-Cumbarjua canal and Zuari estuarine system of Goa was associated with the unfavourable natural condition of high temperature and high salinity. Madhupratap (1978) found higher numerical counts and biomass of zooplankton in Cochin backwaters during high saline pre-monsoon period and a decrease with the onset of monsoon. Ajithakumar (1984) found difference in the distribution of Perna indica and Perna viridis according to salinity fluctuation.

Presence of M. senhausia again observed in November-December months as a result of the gradual rise in salinity of the backwater and its intensity

attains a peak during the pre-monsoon months of March-May when the salinity become high. During these periods the mussel is found to form thick beds in bottom sediment in varying densities. Besides, these are found to invade the inner part of the whole estuary with the salinity increase. Thus it is found aggregated in different regions of the estuary.

Nature of substratum can be another important factor restricting the occurrence of the mussel M. senhausia. Here the sediment of the mussel bed is a mixture of sand, silt and clay. This is found to be the suitable substratum for the vigorous growth of the species. During late-monsoon period the sediment is found to contain more sand and less silt with fragments of shells in it. In this sediment M. senhausia could not be encountered. Thus silty sand and thick clay are found to be not suitable for the existence of the species. Particle size of the sediment is a function of the mixing process and dilution of sea water with freshwater (Batcha, 1984). Desai and Kutty (1967) have stressed the importance of sediment texture in the distribution of benthic fauna. Vizakat et al. (1991) also obtained similar results in the study on the distribution of benthic community in the sub-tidal region of Konkan coast. Thus it can be suggested that sediment texture is also an important factor in controlling the formation of the mussel bed, eventhough the salinity is regarded as the major limiting factor in the occurrence and distribution of M. senhausia.

During pre-monsoon period the abundance and exploitation of this mussel are quite high. The mussel divers reach the location of bed by canoes and collect the mussel by hand. Using a net bag they separate the mussels

Figure 4. Mussel divers collecting the mussels from bottom sediments

Figure 5. A load of mussels collected

from mud. The fishing is generally done at low tides for 3-4 hours till the canoe is full with mussels or the high tide begins. A canoe with two persons collects about 200-300 kg of mussels per day. The season for the mussel fishery in this zone starts from October/November to June. There will be no mussel fishery during monsoon months.

C H A P T E R - IV

AGE AND GROWTH STUDIES

4.1 INTRODUCTION

The growth rate of the mussel forms an important aspect of the study of mussel biology owing to their high potential for culture. Growth of an organism can be divided into two phases: that before and that after sexual maturity. The growth characteristics of each phase differ because an increasing proportion of energy consumed by the organism is devoted to the formation of sex products. Thus in a constant environment the responses of an aquatic organism vary with the 'physiological state' of the organism. This can be adequately represented by size and age (Slobodkin, 1953; Sinko and Streifer, 1967). Besides, growth is influenced by several environmental factors such as water temperature, salinity, food supply, nature of substratum etc. Thus the study of growth is of fundamental importance in theoretical and applied ecology.

4.2 REVIEW

The growth of bivalves is determined by using many methods such as size-frequency distribution, the measurements of marked animals and the use of annual growth rings on the shell. Allometric relationships have also been used extensively for determining the growth of bivalves. For all growth studies shell length is considered to be the most appropriate parameter (Seed, 1976).

Growth studies of bivalves are extensively carried out in many parts of the world. Abraham (1953) made a detailed study of the growth of Meretrix casta. He used measurement of marked clams, analysis of natural populations

and laboratory-reared clams, and the use of coaxial rings on the shell, and found that in the laboratory conditions growth rate was very low due to the inadequate food supplies but in natural conditions it was found to be adversely affected by crowding. Nayar (1955) studied the growth of Donax (Latona) cuneatus from length - frequency distribution, different allometric relationships and growth rings, and suggested that it was not uniform throughout the year. He observed high mortality in the high saline condition and during the period followed by highest temperature. Durve and Raja (1965) reported differences in the dimensional relationships of the Meretrix casta from two localities and opined that this could be attributed in widely differing environmental conditions prevailing there. Gilbert (1973) studied the growth and longevity of a population of Macoma baithica from Massachusetts, using marked-recapture technique and concluded that temperature influences the maximum size, growth rate and longevity. Salih (1973) explained growth of Meretrix casta from Cochin barmouth based on length-frequency distribution. Mane (1974a) used length-frequency method to study the growth pattern of Katelysia opima. He also tried to study the allometric relationships, discussed the significance of disturbance rings and reported a retardation of growth during monsoon season (low salinity). According to Harkantra (1975), the higher salinity and temperature accelerated growth in Meretrix casta in Kali estuary, but no growth could be recorded in low temperature-saline conditions. His observation was based on the length-frequency distribution and the appearance of regular annual rings on the shell. Salih (1977) estimated the growth of Meretrix casta by applying the von Bertalanffy equation,

and studied relationships between different body dimensions, in relation to changing environmental parameters and reported a minimum monthly average growth in length during monsoon season. Hughes (1977) determined the growth rate of the Japanese oyster, Crassostrea gigas. Ansell and Bodoy (1978) studied the rate of shell growth of Donax vittatus and D. trunculus from European waters, and found that the growth was affected by temperature and food supply. Ansell and Parulekar (1978) tried to give a new interpretation of the age structure of the population of Nuculana minuta occurring in Clyde sea area, Scotland and its growth based on the presence of clear shell growth rings. Conan and Shafee (1978) reported an absolute annual increase in shell height of Chlamys varia using von Bertalanffy growth models. The allometric relationships between a variety of shell and soft body characters of Patella vulgata from the east coast of Scotland were studied by Jones et al. (1978). Growth of various meristic characters of Donax incarnatus in relation to ecological parameters has been investigated by Nair et al. (1978). Jones (1979) described the population structure of Cerastoderma edule using a combination of length-frequency and growth ring analyses. He also examined the effect of season upon the relationships between a number of shell and soft body characters. Shafee (1980) made an attempt to explain the seasonal growth rates of Chlamys varia by applying several other mathematical models and finally concluded that temperature and food together form a decisive factor for growth processes. Mohan and Damodaran (1981) presented a brief account of the allometric relationship of Sunetta scripta in Cochin waters. The effects of different substrata on growth rates of natural populations of the unionid bivalve Elliptio complanata have been studied by Kat (1982).

He reported a significant effect of bivalve density and type of substratum on growth rate of this species. Chattergi et al. (1984) determined monthly growth increment in shell length of Villorita cyprinoides and its relationship with other growth indices like shell width, shell breadth, total weight and total volume. He also used von Bertalanffy growth equation to represent growth of this clam in terms of length. The allometry of Donax incarnatus has been studied by Mohan et al. (1986) and found that it is the same for the entire size range of animals unlike in many other bivalves. Nair and Nair (1986) studied height-length relation of shells in the Indian backwater oyster, Crassostrea madrasensis. Age and growth of the blood clam Anadara granosa kept in cage were determined by the length-frequency study and various morphometric and length-weight relationships (Narasimham, 1988a). He also estimated the parameters of the von Bertalanffy growth equation and found a comparatively faster growth rate in the species. Balasubramanian and Natarajan (1988) by employing length-frequency method and probability plot method observed a slower growth rate in Meretrix casta. Rao (1988) worked out the length-weight relationship of Meretrix casta and Paphia malabarica and other dimensional relationships of M. casta. He used von Bertalanffy equation and growth rings on the shells also for the study of growth and indicated that analysis of annual rings of Meretrix casta was not useful in age determination. He discussed these studies in relation to ecological conditions. Narasimham (1988b) estimated the parameters of the von Bertalanffy equation for growth in length in Anadara granosa from the Kakinada Bay. Blay Jr. (1989) worked out the shell morphometrics, length-weight relationships, and length distributions of some lotic and lentic populations of the mutelid bivalve, Aspatharia sinuata occurring in Nigeria. He stated

that variations observed in size distributions of the population were probably due to environmental influences resulting in different growth rates. Growth of Meretrix casta was studied by plotting the size-frequency histograms and from the length-weight relationship in relation to some physico-chemical parameters (sand excavation and deposition of mining waste in the estuarine belt, salinity etc.) (Modassir, 1990). Thippeswamy and Joseph (1991) estimated growth rate of Donax incarnatus by the analysis of the size-frequency data by Pauly's integrated method and calculated theoretical pattern of growth using von Bertalanffy equation.

The literature on the growth of mytilids is extensive. George (1973) studied the growth of Musculista arcuatula using length frequency distribution. Kuriakose (1973) estimated the age and growth of Perna indica by following the progression of the monthly modal values in the frequency distribution and again by von Bertalanffy equation and found considerable agreement in the length at ages determined by the two methods. Growth rate of Modiolus metcalfei in the natural bed and on the raft were estimated by Parulekar et al. (1978) from the size frequency distribution and the allometric relationships, and there were higher growth in the natural bed than on the raft. Hickman (1979) studied allometry and growth of the greenlipped mussel, Perna canaliculus in New Zealand. A study of the growth rates of cultured mussel Perna indica in the farm at Vizhingam and in the open sea revealed a faster growth rate in the former habitat condition (Appukuttan and Nair, 1980). Narasimham (1980) estimated the age and growth of Perna viridis using length frequency distribution and reported an accelerative effect of higher salinity and temperature. Mohan (1980) determined the allometric relationship of Perna viridis. Chatterji et al. (1984) compared the growth of Perna viridis from

three different environments (floating raft, circulating sea water and a natural bed) and found that the average annual growth in natural environment was less when compared to the other two. They tried von Bertalanffy equation and the allometric relationship between length and weight and concluded that the growth rate was chiefly influenced by the availability of food than the other hydrological parameters. Hosomi (1985) reported several fundamental allometries of Mytilus galloprovincialis based on shell length. Morton (1988) estimated age and growth of Brachidontes variabilis in a Hong Kong mangrove by employing length-frequency analysis. Growth rate of Perna indica was studied by tracing the peak modes by Appukuttan et al. (1989) and it was found to be influenced by various hydrological factors like temperature and rainfall.

In the present investigation the methods adopted are the length frequency analysis, allometric relationship between different dimensions and von Bertalanffy growth equation. Information on the age and growth of M. senhausia is important in understanding the nature of stock and the role played by various year classes in the fishery constituted by the animal and the condition under which optimum growth is possible and the influence of various environmental factors on growth.

4.3 MATERIALS AND METHODS

The study on the growth rate of M. senhausia was based on fortnightly random samples collected from Cochin backwaters during the period from February 1987 to June 1988. The mussel samples were collected using a van Veen grab of 0.05 m^2 and kept it in water of habitat salinity. Length,

height and depth of each mussel were measured with sliding calipers correct to one-tenth of a millimetre. The greatest anterior-posterior measurement was taken as length, the maximum distance between the hinge and ventral margin of the valves as height and the greatest distance between the outer surface of the two valves measured in a direction perpendicular to the anterio-posterior axis as depth. In order to determine the total weight, flesh weight and shell weight, the mussels were kept in aerated water of habitat salinity for 24 hours to defecate, and weights were determined to the nearest 0.1 mg in electric balance.

Shell length was taken as the standard measurement for determining age and growth of the mussel M. senhausia. The frequency distribution of length was assessed by taking the class interval as 1 mm. The monthly size frequencies were then converted into percentages of total number of animals present in the sample, which formed the basis for the interpretation of the population structure. By using modal progression analysis (Pauly, 1983) progression of the modal value was noted and determined growth of the mussel.

The relation of height, depth and weights on length was studied by fitting the regression equation of the type

$$Y = a + bX,$$

where required logarithmic transformation was applied. Here Y is the dependant variable, X the independent variable, a and b (coefficients of allometry) the constants which were estimated by the least square regression analysis. Length was used as an independent variable in all studies. The allometric relationships between length and height, length and depth, length and total weight, length and flesh weight and length and shell weight were studied.

The coefficient of correlation between length and height, length and depth, length and total weight, length and flesh weight and length and shell weight were calculated using Pearson's formula. Covariance analysis of the different allometric relationships of different months were determined (Zar, 1974).

The theoretical pattern of growth was calculated from length data obtained during the period of the experiment using the following von Bertalanffy equation (Bertalanffy, 1938, 1957).

$l_t = L_\infty [1 - e^{-K (t - t_0)}]$, where

l_t is the length at age t,

L_∞ is the theoretical maximum length,

K is the growth coefficient,

t_0 is the age at which the animal has zero length.

Parameters of the von Bertalanffy growth equation L_∞ and K were determined separately using the least square method.

4.4 RESULTS

4.4.1 Population structure and growth by length-frequency analysis

The length-frequency distribution of the mussel M. senhausia during the period from February '87 to June '88, when the specimens are available are presented in the frequency Table 2 and by percentage frequency histogram in Figure 6. The distribution shows the distinct modes in most of the months.

In the histogram of February '87, the mode was at 13 and 14 mm, with more number of organisms having a length more than the mode on the right side. Here the curve is positively skewed. Few smaller specimens

Table 2. Percentage frequencies of M. senhausia of different size groups for different months

LENGTH (mm)	MONTH FEB. '87	MAR.	APR.	MAY	JUNE	JULY	DEC.	JAN.'88	FEB.	MAR.	APR.	MAY	JUNE
5 - 6	0.3922					0.9346							0.7299
6 - 7					4.2308	3.7383		1.0417				0.3731	1.7032
7 - 8	0.3922				5.7692	10.2804		4.1667				0.3731	6.8127
8 - 9	0.3922	0.9259			9.2308	12.1495	5.0847	6.2550				2.2388	9.9757
9 - 10	1.9608				7.3077	19.6262	3.3898	7.2917				5.9701	22.3844
10 - 11	2.7451	0.9259			10.7692	13.0841	1.6949	7.8125				7.8358	29.4404
11 - 12	5.0980	1.8519	2.2222		6.9231	14.0187	5.0847	13.0208			1.1429	16.4179	20.1946
12 - 13	10.9804	2.7778			5.7692	8.4112	1.6949	11.4583	1.3793		4.0000	14.5522	6.3260
13 - 14	13.3333	4.6296			1.1538	8.4112	13.5593	11.4583	7.5862		0.5714	17.1642	0.9732
14 - 15	13.3333	4.6296	4.4444		0.7692	2.8037	20.3390	14.5833	14.4828	0.8621	8.0000	10.8209	0.7299
15 - 16	12.2569	5.5556			1.1538	3.7383	20.3390	6.2500	21.3793	8.6207	11.4286	8.5821	0.7299
16 - 17	12.1569	8.3333	2.2222	3.9216	0.7692	1.8692	10.1695	3.1250	29.6552	8.6207	14.2857	5.9701	
17 - 18	6.6667	10.1852			2.6923		8.4746	1.5625	14.4828	16.3793	16.0000	6.3433	
18 - 19	9.4118	19.4444	8.8889	55.8824	3.0769		8.4746	1.5625	6.8966	18.1034	19.4286	3.3582	
19 - 20	6.2745	13.8889	2.2222	7.8431	5.7692		1.6949	2.0833	2.7586	19.8276	12.0000		
20 - 21	3.5294	16.6667	4.4444	9.8039	6.9231			3.1250	0.6897	11.2069	7.4286		
21 - 22	1.1765	6.4815	4.4444	13.7255	11.5385	0.9346		1.5625	0.6897	9.4828	4.5714		
22 - 23		3.7037	15.5556	17.6471	8.4615			2.0833		5.1724			
23 - 24			22.2222	17.6471	5.0000			1.0417		1.7241	0.5714		
24 - 25			11.1111	7.8431	1.9231						0.5714		
25 - 26			15.5556	9.8039	0.3846								
26 - 27			4.4444	5.8824	0.3846								
27 - 28													
28 - 29													
29 - 30			2.2222										

could be seen along with the larger ones. Mode at 13 and 14 mm of February were traced to 18 mm in March resulting a growth of 4 mm in one month and another mode at 20 mm also could be seen. Here the curve is negatively skewed. In April the mode is at 23 mm which could be traced back to the mode at 18 mm of March, representing a growth of 5 mm. The figure shows a skewness towards left. During May the mode at 23 mm remained stationary. A mode at 22 mm also could be seen. The number of organisms having a length less than 22 mm were more in the sample. So the curve shows negative skewness. In June the mode again shifted to 21 mm which is indicated by the fewer number of large specimens. Here the graph is polymodal having another prominent mode at 10 mm. The population is said to be heterogeneous in nature in June. During July more addition of smaller ones took place and this resulted in the shifting of the mode towards left, to be at 9 mm. Here the younger mussels (2-10 mm) were more (46.73%) when compared to the number in previous months. But in July as a whole mussels having a length greater than 9 mm were more in the sample. The graph is positively skewed. One smaller mode at 21 mm could also be seen.

After the monsoon break of samples, all organisms obtained in December were below 20 mm. Modal length was found to be between 14 and 15 mm. Majority of specimens were small having a length less than the mode. During January 1988 again the population showed the addition of more younger ones and the mode remained stationary at 14 mm. The graph is negatively skewed. In February the distribution become more compact and unimodal with a mode at 16 mm. The graph shows skewness towards left. The mussels having a length less than 16 mm were more in the sample. In March the mode

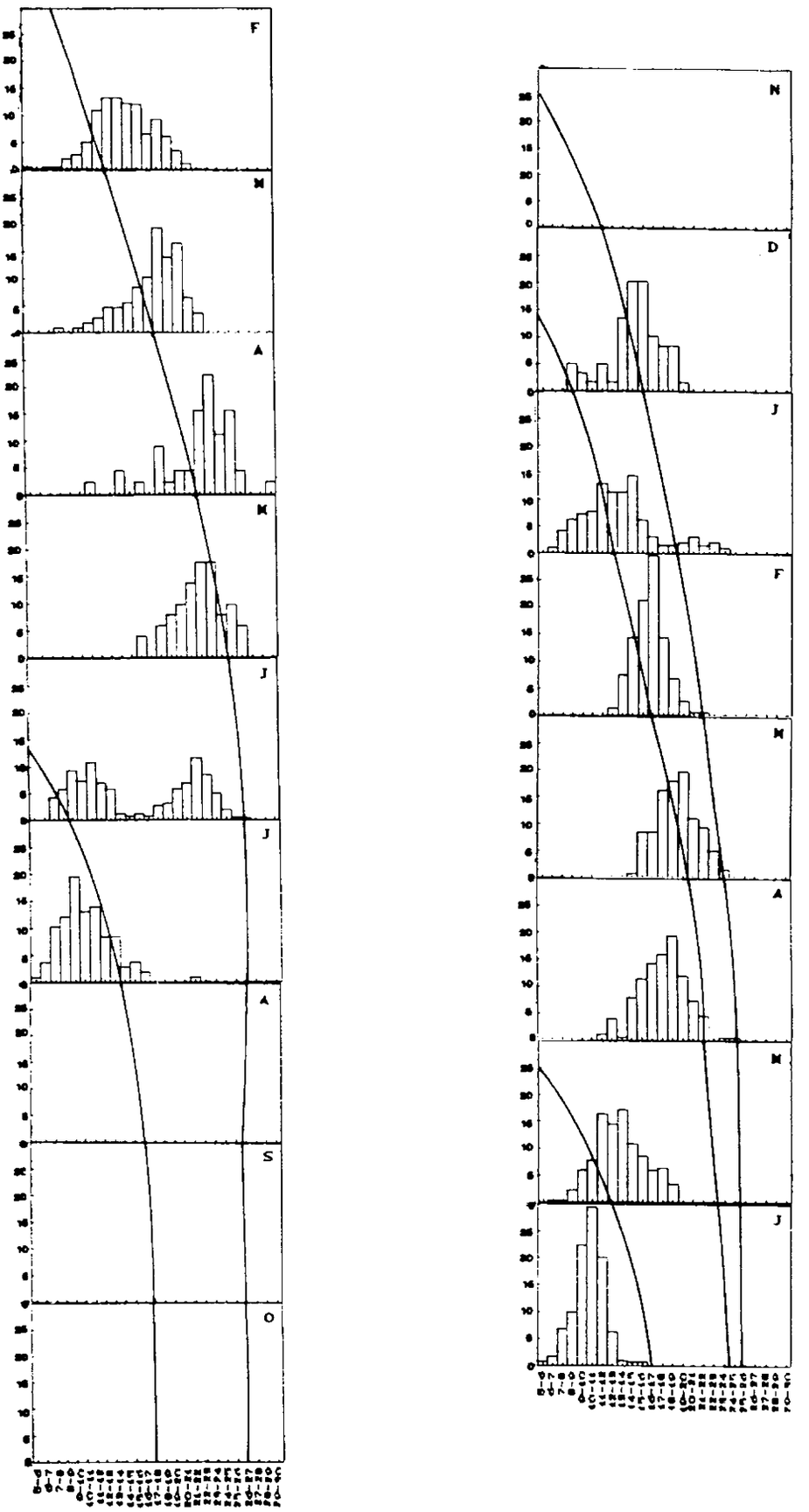


Figure 6. Length frequency histogram on M. senhausia plotted using modal progression method. The X axis represents shell length in mm and the Y axis represents the number of animals as percentage of the total sample.

was at 19 mm which could be traced back to 16 mm of February registering an increase of 3 mm. During April the mode was found to be at 18 mm and more younger ones were found in the sample. In May the mode again was found to have shifted to 13 mm. Another smaller mode at 11 mm also could be seen. More specimens having length less than the main modal size were present in the sample. All mussels were below 20 mm. In June the mode shifted to 10 mm indicating that new recruitment of small individuals has taken place. The mussels having a length less than 10 mm were more (41.61%) in the sample. In May and June the curve is found to be negatively skewed. Maximum size of the mussel observed during the period of study was 29 mm which was obtained in April 1987.

From the modal progression analysis it is observed that growth was more vigorous during the first few months after the settlement. During February - July, 1987 the mussels showed a growth rate of 5 mm in the first month and 4 mm in the second month. The growth rates during the three subsequent months were 3, 3, and 2 mm respectively. The growth was found to be decreasing as it reached sexual maturity. The recruitment of young mussels took place in June and July which was evident from the population structure as explained earlier. During December 1987 - June 1988 also almost the same trend could be noticed. Here the growth rates were 4.5 mm in the first month and 3.5, 3, 2, and 2 mm during the second, third, fourth and fifth months respectively. Beyond the fifth month the growth rate was poor. From these observations it is evident that in the case of the mussels collected, growth was masked by the post-spawning mortality

of older specimens and new spat settlement. Recruitment of young mussels to the population took place during several months as shown by the occurrence of small mussels in December, January, February, June and July indicating a protracted breeding period for the population.

4.4.2 **Dimensional relationships**

The relationships between different parameters were worked out by regression analysis (least square method). In all cases, the shell length was considered as basic index to establish the relationship. The shell length-shell height, shell length-shell depth, shell length-total weight, shell length-flesh weigth and shell length-shell weight relationship showed a linear growth pattern. The a, b and r values calculated are given in Tables 3-7. Inter-relationship between different dimensions are given in Figure 7-13.

Covariance analysis of the various relationships between different dimensions by month is given in Tables (8-12). Covariance analysis of the linear regression of logarithm of height on logarithm of length during various months resulted in non-significant 'F' value of 0.34. The ratio between these two parameters shows that there was no seasonal variation. But analysis of covariance between length and depth showed a significance at 0.1% level ('F' value 6.44). Here the growth in depth showed variation with length. Here growth rate was high during March, June and December in the first year and January, April, May and June in the second year. Lowest growth rate was observed in May and July.

Table 3. Allometric relationship between length and height in M. senhausia

Month	log a	b	r	N
February 1987	- 0.2180	0.9030	0.9710	255
March	- 0.2691	0.9510	0.9711	108
April	- 0.2020	0.8960	0.9510	45
May	- 0.2690	0.9399	0.9377	51
June	- 0.2236	0.9108	0.9840	50
July	- 0.0970	0.7956	0.9206	50
December	- 0.2022	0.9037	0.9727	59
January 1988	- 0.2541	0.9293	0.9851	192
February	- 0.2508	0.9295	0.9069	145
March	- 0.2781	0.9460	0.7298	116
April	- 0.2901	0.9545	0.9540	175
May	- 0.2689	0.9410	0.9690	268
June	- 0.2013	0.8913	0.9191	411

Table 4. Allometric relationship between length and depth

Month	log a	b	r	N
February 1987	- 0.4050	0.9630	0.9560	255
March	- 0.4934	1.0124	0.9315	108
April	- 0.3960	0.9510	0.9380	45
May	- 0.3092	0.8788	0.8078	51
June	- 0.5731	1.0995	0.9827	50
July	- 0.2952	0.8595	0.8928	50
December	- 0.5867	1.1037	0.9484	59
January 1988	- 0.6809	1.1555	0.9705	192
February	- 0.3884	0.9334	0.8567	145
March	- 0.3920	0.9266	0.8901	116
April	- 0.5052	1.0168	0.9000	175
May	- 0.5194	1.0296	0.9390	268
June	- 0.4796	1.0146	0.8857	411

Table 5. Allometric relationship between length and total weight

Month	log a	b	r	N
February 1987	- 0.1650	2.8860	0.9830	255
March	- 1.1928	2.8401	0.9791	108
April	- 0.8720	2.6500	0.9640	45
May	- 0.7901	2.5858	0.9315	51
June	- 1.3805	3.0362	0.9878	50
July	- 0.7633	2.5422	0.9651	50
December	- 0.9376	2.7403	0.9804	59
January 1988	- 1.1634	2.8819	0.9921	192
February	- 1.0426	2.8214	0.9304	145
March	- 0.5641	2.4213	0.9406	116
April	- 1.0768	2.7780	0.9430	175
May	- 0.9908	2.7332	0.9690	268
June	- 0.9920	2.7776	0.9606	411

Table 6. Allometric relationship between length and flesh weight

Month	log a	b	r	N
February 1987	- 1.5860	3.0640	0.9710	255
March	- 1.5001	2.9197	0.9614	108
April	- 0.8870	2.5385	0.9400	45
May	- 0.9015	2.5492	0.8982	51
June	- 1.7729	3.1941	0.9833	50
July	- 1.1544	2.7180	0.9538	50
December	- 1.2154	2.8397	0.9741	59
January 1988	- 1.6362	3.1213	0.9878	192
February	- 1.6233	3.1327	0.8965	145
March	- 0.7450	2.4058	0.8994	116
April	- 1.1936	2.7183	0.9170	175
May	- 1.2638	2.7972	0.9551	268
June	- 1.4326	3.0341	0.9522	411

Table 7. Allometric relationship between length and shell weight

Month	log a	b	r	N
February 1987	- 0.2600	2.6130	0.9820	255
March	- 1.4148	2.6790	0.9722	108
April	- 1.7120	2.8980	0.9730	45
May	- 1.3824	2.6457	0.9482	51
June	- 1.5228	2.7901	0.9842	50
July	- 0.8747	2.2411	0.9246	50
December	- 1.2060	2.5333	0.9771	59
January 1988	- 1.1774	2.5088	0.9900	192
February	- 0.8848	2.3352	0.9017	145
March	- 0.9757	2.4049	0.9333	116
April	- 1.6238	2.8569	0.9400	175
May	- 1.3944	2.7095	0.9661	268
June	- 1.0209	2.3398	0.9401	411

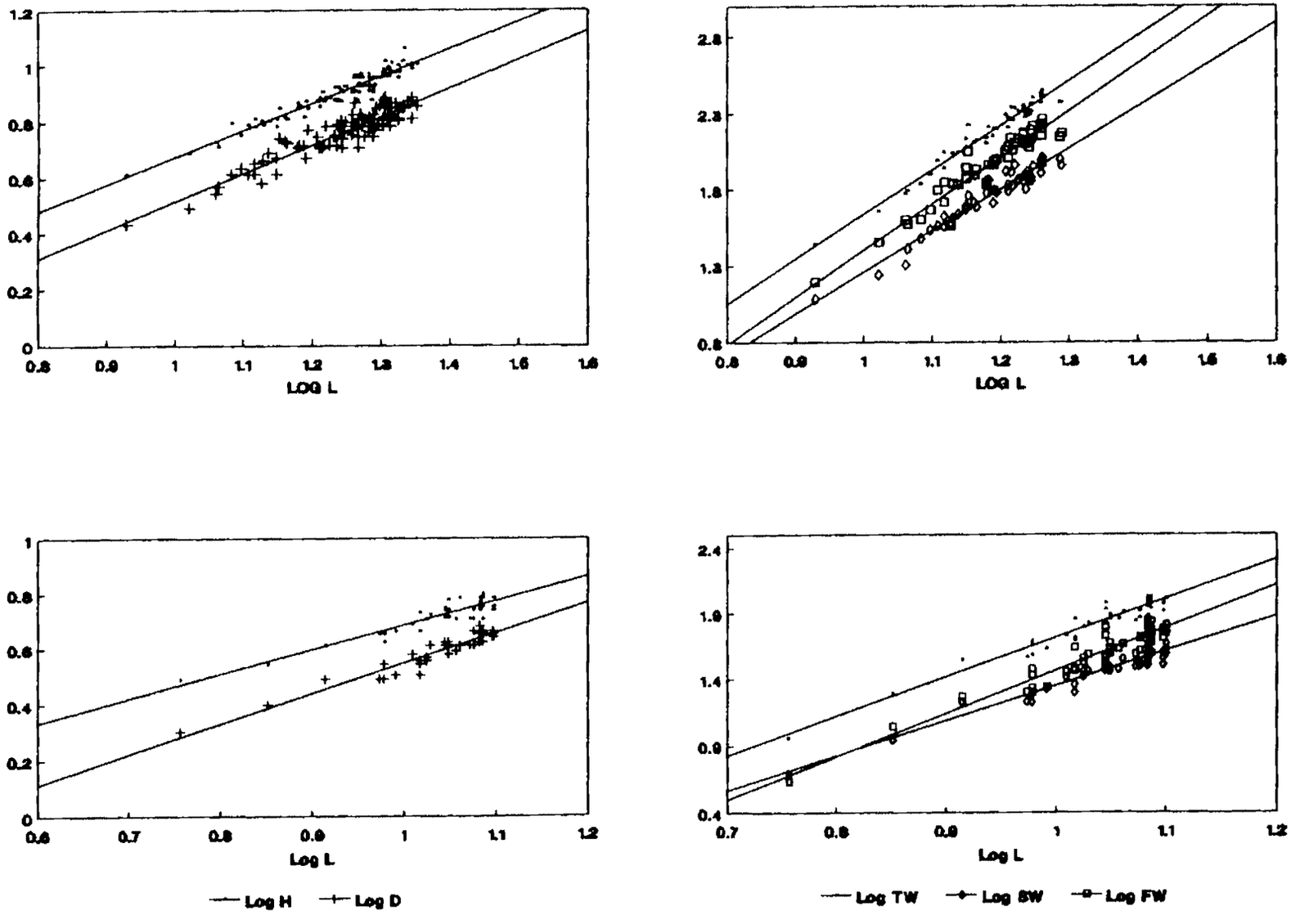


Figure 7. Interrelationship between different dimensions (L-Length, H-Height, D-Depth, TW-Total weight, SW-Shell weight and FW-Flesh weight) of M. senhausia during February and March 1987.

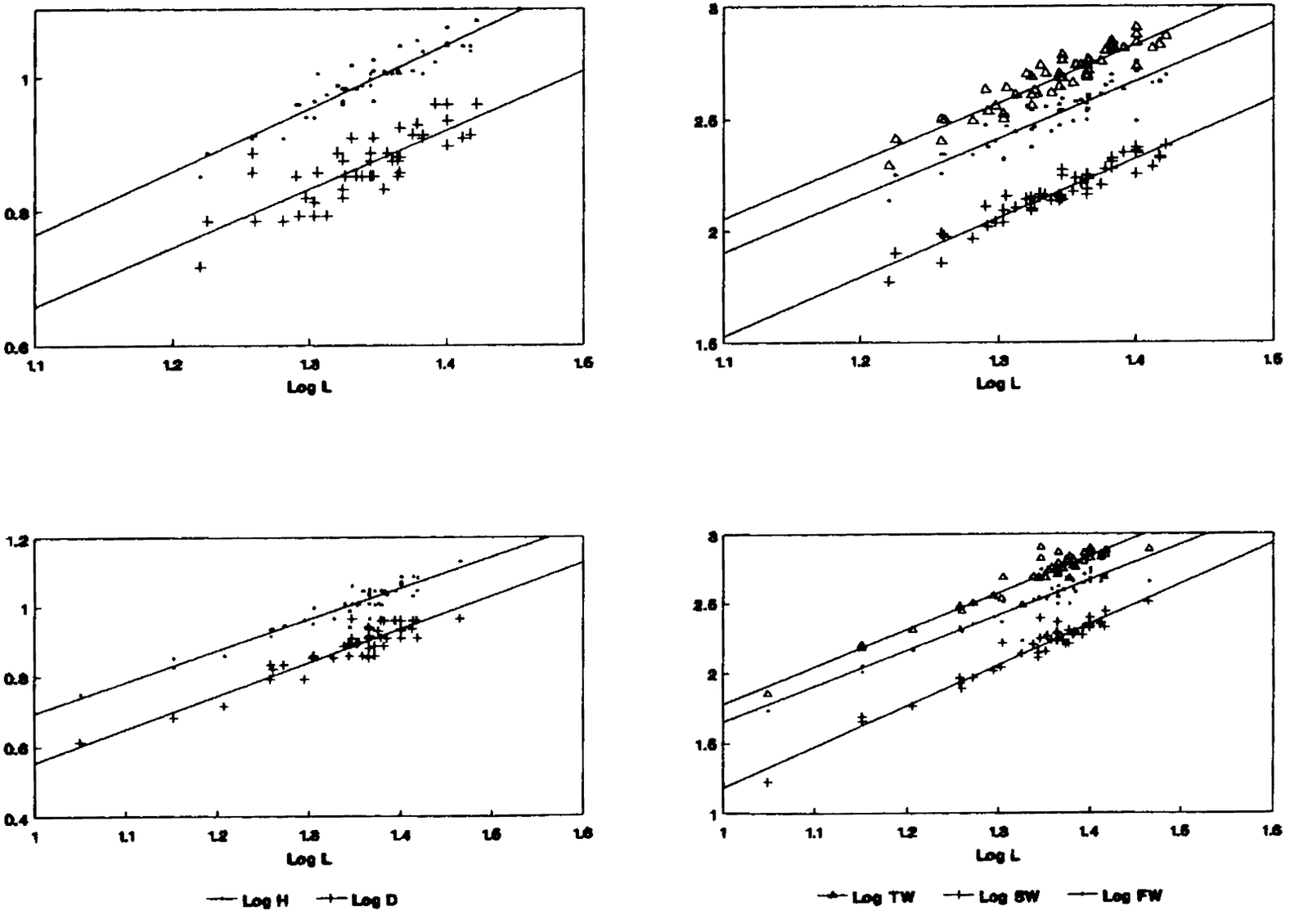


Figure 8. Interrelationship between different dimensions of M. senhausia during April and May 1987.

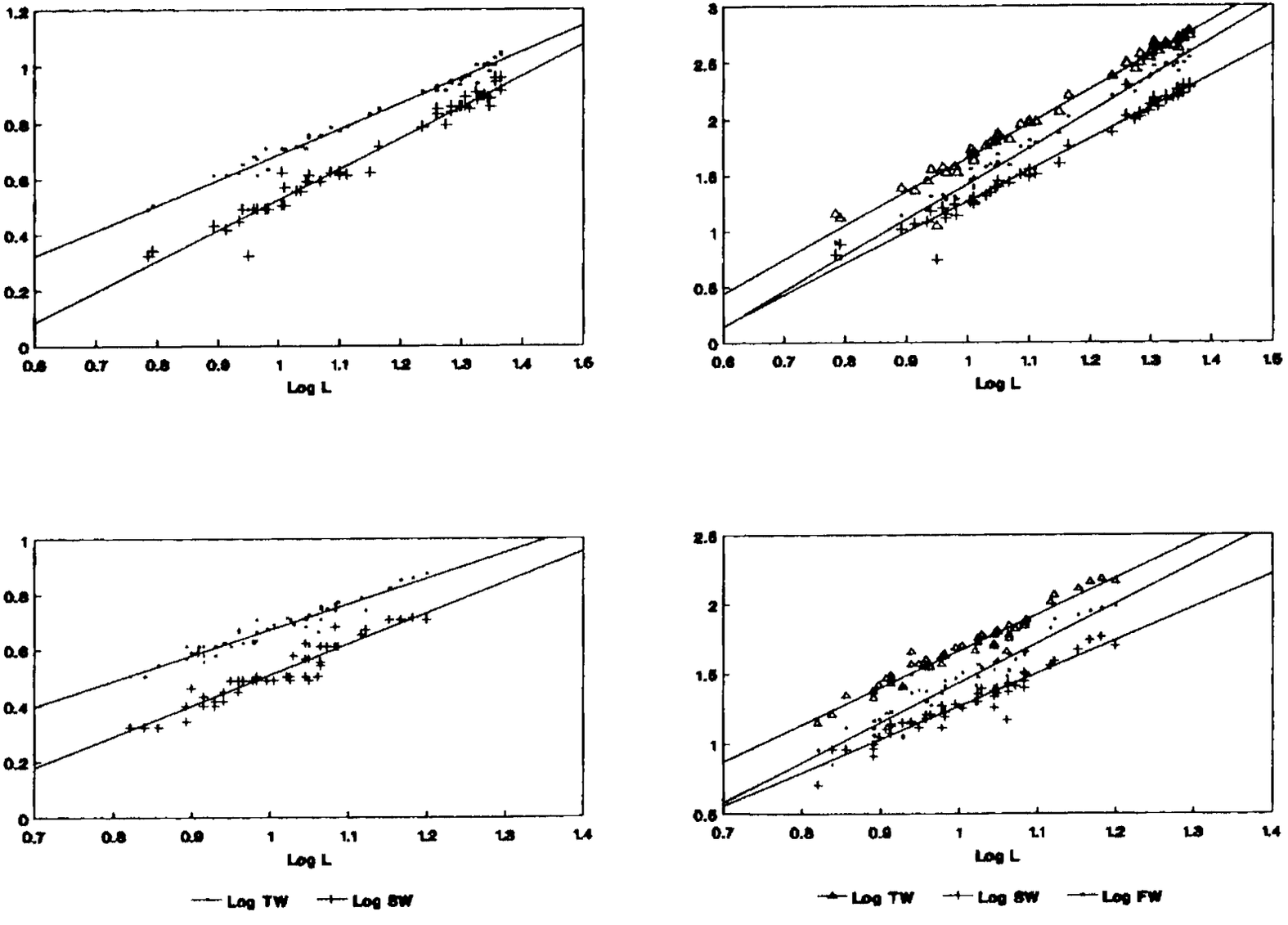


Figure 9. Interrelationship between different dimensions of M. senhausia during June and July 1987.

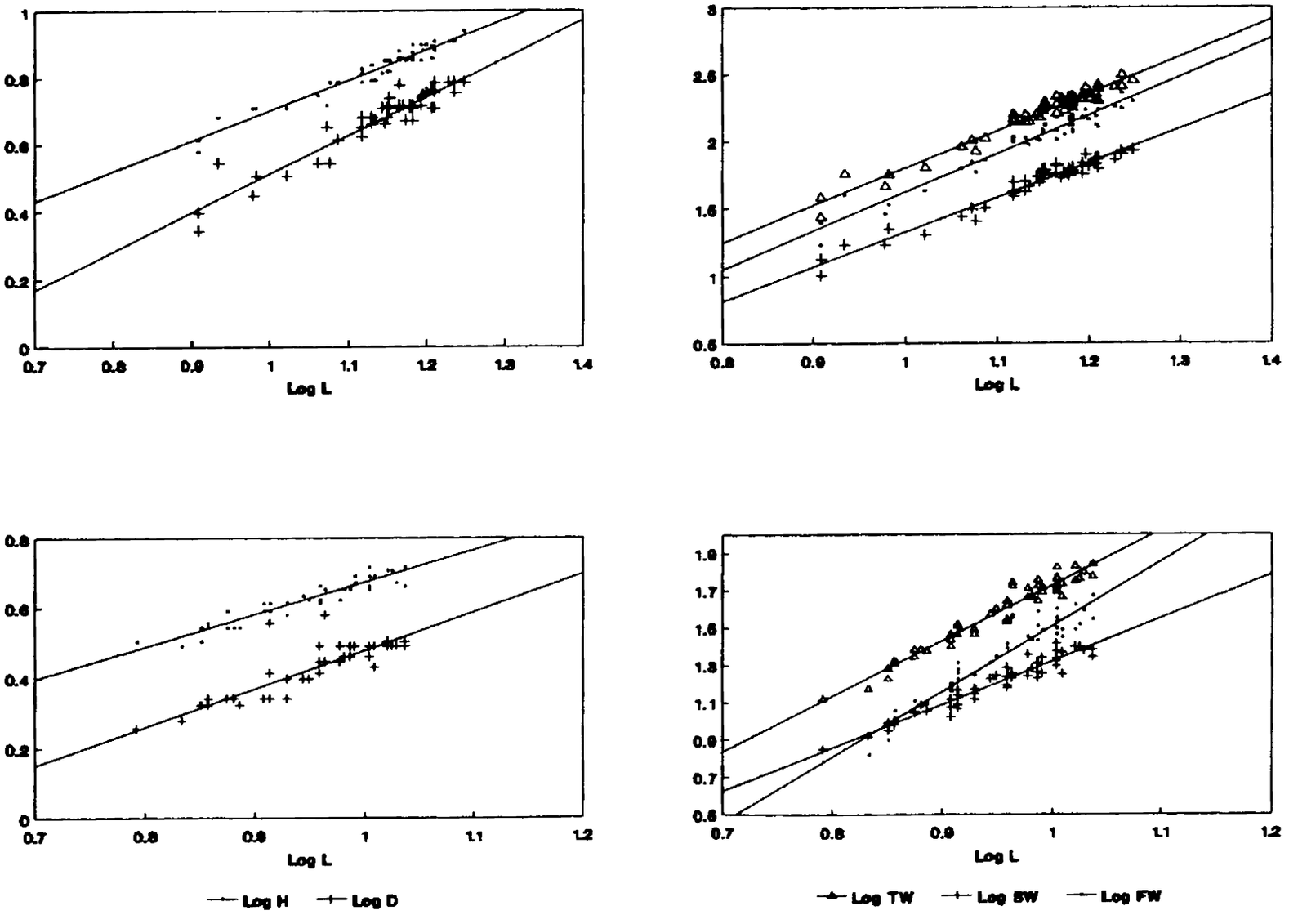


Figure 10. Interrelationship between different dimensions of M. senhausia during December 1987 and January 1988.

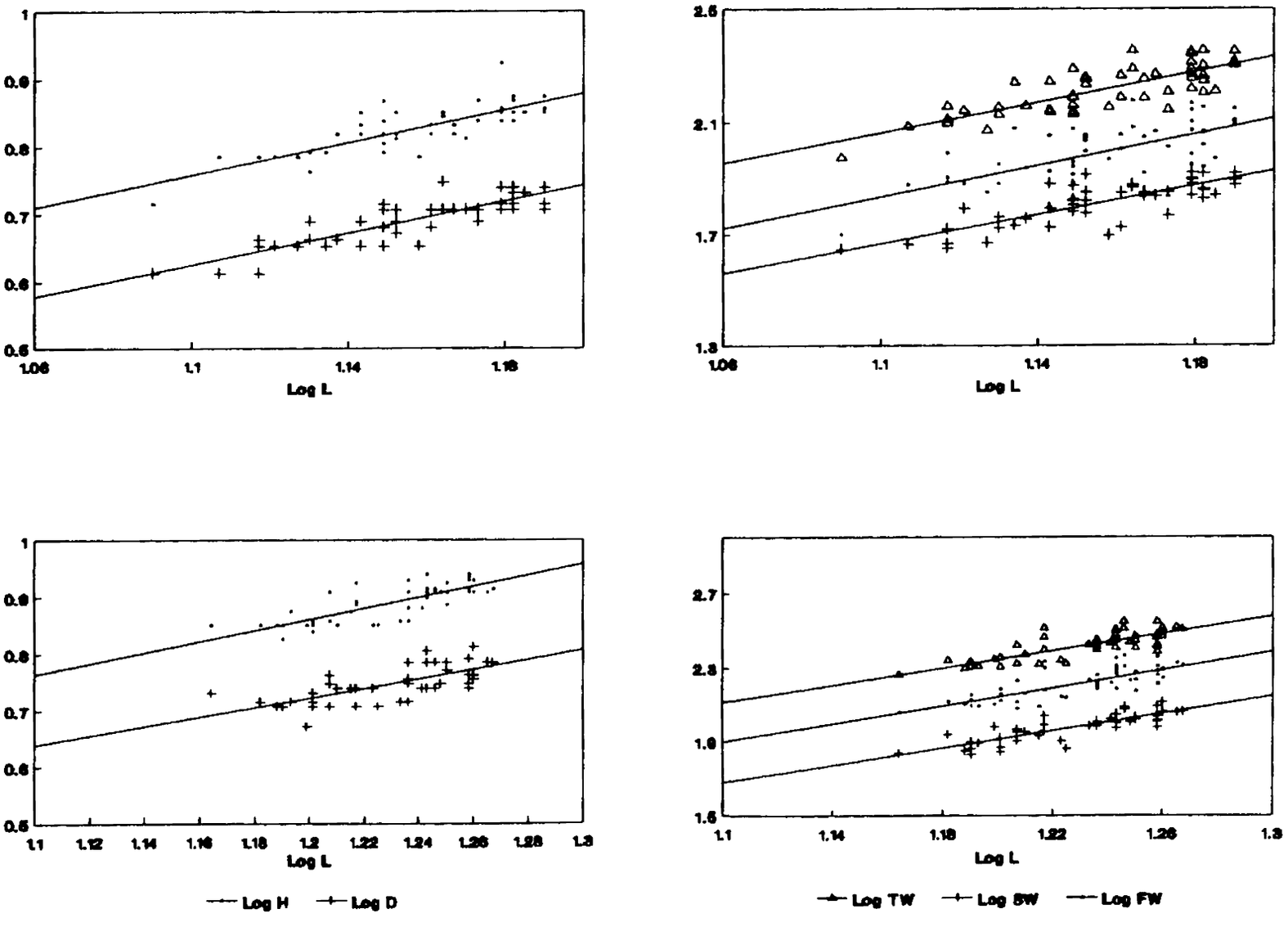


Figure 11. Interrelationship between different dimensions of M. senhausia during February and March 1988.

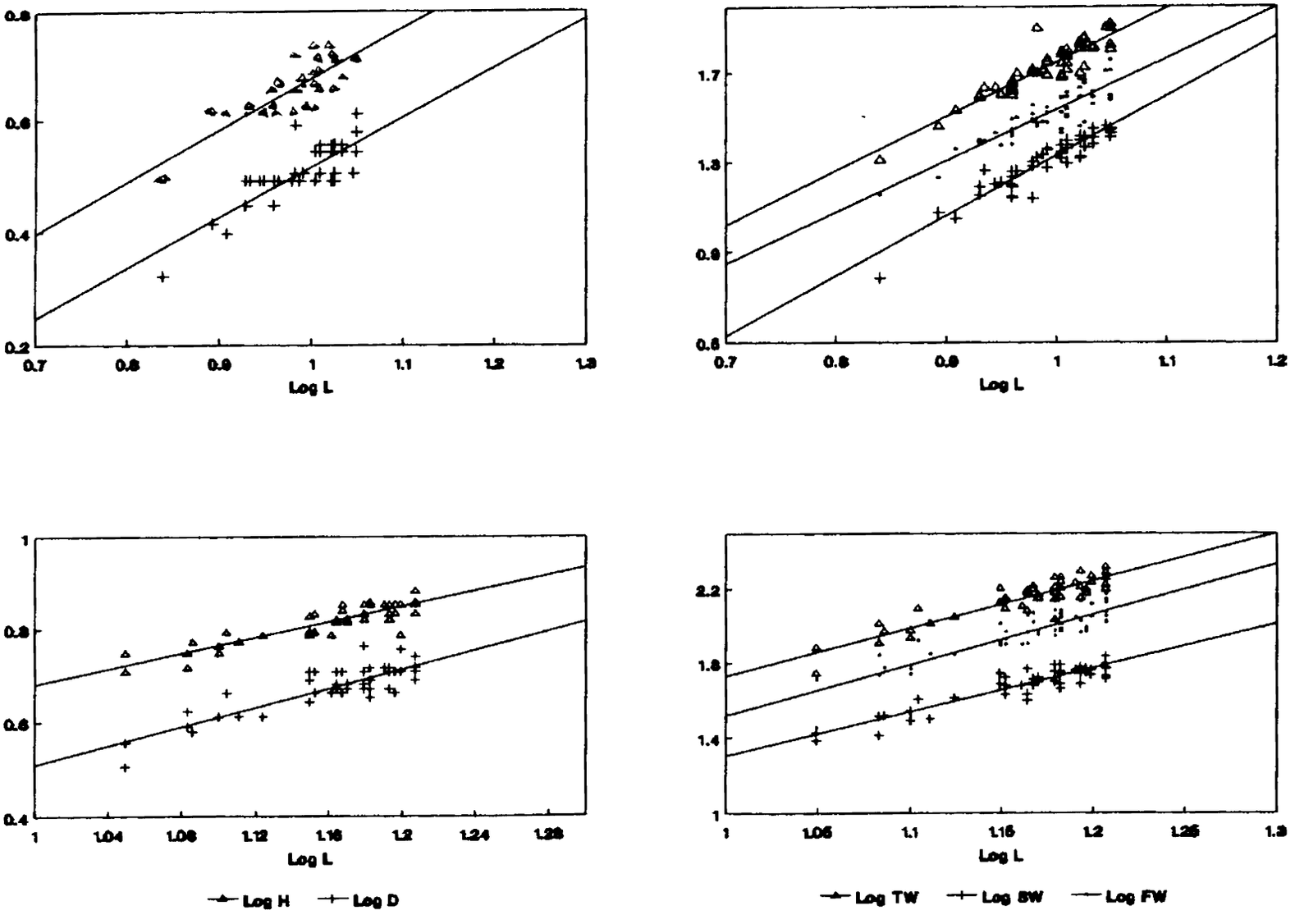


Figure 12. Interrelationship between different dimensions of M. senhausia during April and May 1988.

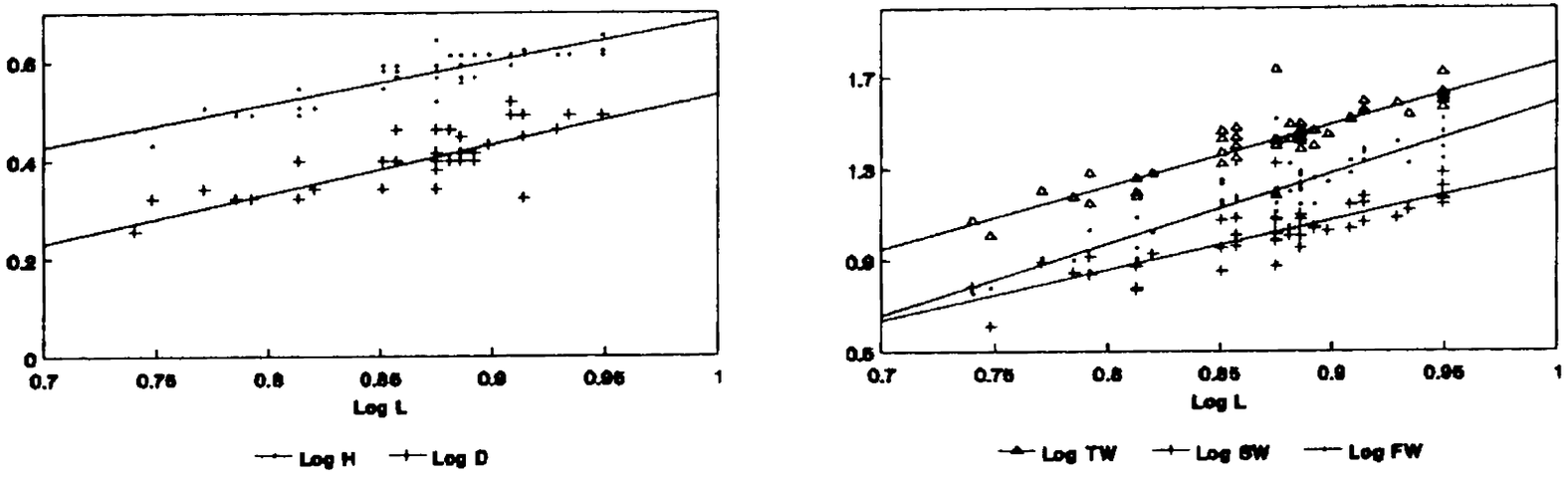


Figure 13. Interrelationship between different dimensions of M. senhausia during June 1988.

Table 8. Analysis of covariance of linear regression of logarithm of shell height on logarithm of shell length

Month	Σx^2	Σy^2	Σxy	N	df	Reg.coef.	Deviation from regression df	ss	ms
February 1987	1.9130	1.6541	1.7282	255	254	0.9034	253	0.0985	0.0004
March	0.6239	0.5983	0.5933	108	107	0.9510	106	0.0341	0.0003
April	0.2795	0.2483	0.2504	45	44	0.8959	43	0.0240	0.0006
May	0.1171	0.1176	0.1100	51	50	0.9394	49	0.0143	0.0003
June	1.4290	1.2241	1.3014	50	49	0.9107	48	0.0389	0.0008
July	0.1538	0.1149	0.1224	50	49	0.7955	48	0.0175	0.0004
Dec.	0.4266	0.3682	0.3855	59	58	0.9037	57	0.0198	0.0004
January 1988	2.8822	2.5649	2.6785	192	191	0.9293	190	0.0757	0.0005
February	0.2576	0.2706	0.2394	145	144	0.9294	143	0.0481	0.0003
March	0.2376	0.3992	0.2248	116	115	0.9461	114	0.1865	0.0016
April	0.6287	0.6293	0.6001	175	174	0.9545	173	0.0565	0.0003
May	1.8323	1.7277	1.7242	268	267	0.9410	266	0.1052	0.0004
June	1.9538	1.7231	1.7415	411	410	0.8913	409	0.1708	0.0004
Pooled	12.7351	11.6403	11.6997	1925	1912	0.9187	1911	0.8918	0.0005
							1899	0.8899	0.0005
			Difference between slopes				12	0.0019	0.0002

F (12,1899) = 0.3377

Table 9. Analysis on covariance of linear regression of logarithm of shell depth on logarithm of shell length

Month	Σx^2	Σy^2	Σxy	N	df	Reg.coef.	Deviation from regression df	ss	ms
February 1987	1.9130	1.9400	1.8422	255	254	0.9630	253	0.1660	0.0007
March	0.6239	0.7368	0.6316	108	107	1.0123	106	0.0974	0.0009
April	0.2795	0.2872	0.2657	45	44	0.9506	43	0.0346	0.0008
May	0.1171	0.1386	0.1029	51	50	0.8787	49	0.0482	0.0010
June	1.4290	1.7886	1.5710	50	49	1.0994	48	0.0615	0.0013
July	0.1538	0.1426	0.1323	50	49	0.8599	48	0.0289	0.0006
December	0.4266	0.5778	0.4708	59	58	1.1036	57	0.0582	0.0010
January 1988	2.8822	4.0858	3.3304	192	191	1.1555	190	0.2375	0.0013
February	0.2576	0.3058	0.2404	145	144	0.9332	143	0.0815	0.0006
March	0.2376	0.2575	0.2202	116	115	0.9268	114	0.0534	0.0005
April	0.6287	0.8028	0.6392	175	174	1.0167	173	0.1529	0.0009
May	1.8323	2.2028	1.8866	268	267	1.0296	266	0.2603	0.0010
June	1.9538	2.5635	1.9823	411	410	1.0146	409	0.5523	0.0014
Pooled	12.7351	15.8298	13.3156	1925	1912	1.0456	1911	1.9072	0.0010
							1899	1.8327	0.0010
			Difference between slopes				12	0.0746	0.0062

F(12,1899) = 6.4408

Table 10. Analysis of covariance of linear regression of logarithm of total weight on logarithm of shell length

Month	Σx^2	Σy^2	Σxy	N	df	Reg.coef.	Deviation from regression df	ss	ms
February 1987	1.9130	16.4990	5.5218	255	254	2.8865	253	0.5605	0.0022
March	0.6239	5.2496	1.7719	108	107	2.8400	106	0.2173	0.0021
April	0.2795	2.1126	0.7405	45	44	2.6494	43	0.1507	0.0035
May	0.1171	0.9022	0.3027	51	50	2.5850	49	0.1197	0.0024
June	1.4290	13.4986	4.3385	50	49	3.0361	48	0.3267	0.0068
July	0.1538	1.0677	0.3911	50	49	2.5422	48	0.0735	0.0015
December	0.4266	3.3327	1.1690	59	58	2.7403	57	0.1293	0.0023
January 1988	2.8822	24.3201	8.3063	192	191	2.8819	190	0.3819	0.0020
February	0.2576	2.3687	0.7268	145	144	2.8214	143	0.3181	0.0022
March	0.2376	1.5744	0.5753	116	115	2.4213	114	0.1814	0.0016
April	0.6287	5.4568	1.7465	175	174	2.7780	173	0.6051	0.0035
May	1.8323	14.5761	5.0079	268	267	2.7331	266	0.8889	0.0033
June	1.9538	16.3371	5.4270	411	410	2.777	409	1.2627	0.0031
Pooled	12.7351	107.2956	36.0253	1925	1912	2.8288	1911	5.3865	0.0028
							1899	5.2160	0.0027
						Difference between slopes	12	0.1705	0.0142

F (12,1899) = 5.1740

Table 11. Analysis of convariance of linear regression of logarithm of flesh weight on logarithm of shell iength

Month	Σx^2	Σy^2	Σxy	N	df	Reg.coef.	Deviation from regression df	ss	ms
February 1987	1.9130	19.0697	5.8622	255	254	3.0644	253	1.1056	0.0044
March	0.6239	5.7548	1.8216	108	107	2.9197	106	0.4363	0.0041
April	0.2795	2.0402	0.7095	45	44	2.5385	43	0.2392	0.0056
May	0.1171	0.9431	0.2984	51	50	2.5483	49	0.1827	0.0037
June	1.4290	15.0776	4.5640	50	49	3.1939	48	0.5007	0.0104
July	0.1538	1.2495	0.4182	50	49	2.7183	48	0.1128	0.0023
December	0.4266	3.6256	1.2114	59	58	2.8397	57	0.1856	0.0033
January 1988	2.8822	28.7800	8.9963	192	191	3.1213	190	0.6996	0.0037
February	0.2576	3.1457	0.8070	145	144	3.1328	143	0.6176	0.0043
March	0.2376	1.7001	0.5716	116	115	2.4057	114	0.3250	0.0029
April	0.6287	5.5267	1.7090	175	174	2.7183	173	0.8811	0.0051
May	1.8323	15.7158	5.1253	268	267	2.7972	266	1.3793	0.0052
June	1.9538	19.8367	5.9282	411	410	3.0342	409	1.8494	0.0045
Pooled	12.7351	122.4655	38.0227	1925	1912	2.9857	1911	8.9426	0.0047
							1899	8.5148	0.0045
			Difference between slopes				12	0.4278	0.0356

F(12,1899) = 7.9499

Table 12. Analysis of covariance of linear regression of logarithm of shell weight on logarithm of shell length

Month	Σx^2	Σy^2	Σxy	N	df	Reg.coef.	Deviation from regression df	ss	ms
February 1987	1.9130	13.5316	4.9987	255	254	2.6130	253	0.4699	0.0019
March	0.6239	4.7370	1.6713	108	107	2.6788	106	0.2599	0.0025
April	0.2795	2.4798	0.8099	45	44	2.8977	43	0.1330	0.0031
May	0.1171	0.9113	0.3097	51	50	2.6448	49	0.0922	0.0019
June	1.4290	11.4839	3.9868	50	49	2.7900	48	0.3608	0.0075
July	0.1538	0.9037	0.3446	50	49	2.2406	48	0.1317	0.0027
December	0.4266	2.8677	1.0807	59	58	2.5333	57	0.1300	0.0023
January 1988	2.8822	18.5101	7.2309	192	191	2.5088	190	0.3691	0.0019
February	0.2576	1.7277	0.6016	145	144	2.3354	143	0.3227	0.0023
March	0.2376	1.5776	0.5714	116	115	2.4049	114	0.2035	0.0018
April	0.6287	5.8107	1.7961	175	174	2.8569	173	0.6795	0.0039
May	1.8323	14.4124	4.9644	268	267	2.7094	266	0.9620	0.0036
June	1.9538	12.1031	4.5716	411	410	2.3399	409	1.4062	0.0034
Pooled	12.7351	91.0566	32.9377	1925	1912	2.5864	1911	5.8675	0.0031
							1899	5.5205	0.0029
			Difference between slopes				12	0.3470	0.0289

F (12,1899) = 9.9456

The analysis of covariance between length and total weight showed a significance at 0.1% level ('F' value 5.17). This indicate that the rate of growth varied between months. The maximum growth rate was observed in June. The analysis of covariance between length and flesh weight showed a significant difference in the regression coefficient between months at 0.1% level ('F' value 7.95). In January, February and June the mussels had a significantly high growth rate. Covariance analysis between length and shell weight also showed a significance at 0.1% level ('F' value 9.95) between the regression coefficients. Here the maximum growth was observed during April.

The correlation coefficients of different dimensions worked out were significant at 0.1% level, indicating that linear regression is a good fit for the data.

4.4.3 Fitting of von Bertalanffy growth equation

Growth parameters L_{∞}, K and t_o values were estimated by least square method. The L of the two stock (population before and after the monsoon) were found to be 32.37 (first stock) and 30.16 (second stock). The K value was observed to be 0.2347 and 0.2254. Thus the Bertalanffy growth equation for M. senhausia were written as

$$L_t = 32.37 \left(1-e^{-0.2347\ (t+0.2930)} \right) \text{ for first stock}$$

$$L_t = 30.16 \left(1-e^{-0.2254\ (t+0.3768)} \right) \text{ for second stock}$$

From these equation it could be revealed that the growth rates of the two stocks were almost equal.

4.5 DISCUSSION

The age and growth of M. senhausia has been estimated by length frequency analysis and by von Bertalanffy growth equation. Seasonal allometric relationships were also determined to find out the relative growth rate.

Spawning in this mussel is intermittent and prolonged. From the observations on the length frequency distribution it is evident that the most suitable season for growth is pre-monsoon period which is a period of almost constant environment without much fluctuation. Pre-monsoon conditions such as high salinity and abundant supply of rich planktonic food etc. support growth. The individuals observed in February 1987 continue their growth until April, thus showing a growth of 9 mm in three months. During these period the mussels were in the developing stage of the gonad. Major spawning was found to be during April-May which was evident from the appearance of large number of smaller mussels in the month of June. This is again indicated by the presence of smaller number of larger individuals in the population. This may be the cause of the shifting of the mode to left. During this period the population seems to be heterogeneous in nature. Specimens were not available during August - November due to the mortality of the most of the mussels caused by the extremely low saline condition of the monsoon season. In 1988 year class spawning and new recruitment was a little earlier than the previous year class. Here the new recruitment started in April and younger mussels increased in number, reaching the peak in June. The shifting of the mode to the left again represented by the post-spawning mortality of older specimens which is indicated by the presence of empty shells in the collection. These two processes result in a lot of masking effect

on the size distribution which renders interpretation of the growth of this year class rather difficult. Eventhough observations made during this period especially the commencement of new recruits in April reveals normal growth of the mussel as observed in the first year class. Similar length frequency graphs have been used to study the growth of Donax cuneatus (Nayar, 1955), Perna indica (Kuriakose, 1973) Musculista arcuatula (George, 1973) Modiolus Metcalfel (Parulekar et al., 1978), Perna viridis (Narasimham, 1980), Meretrix casta (Balasubramanian and Natarajan, 1988; Modassir, 1990) etc. Besides, in the present study maximum size observed during the period was 29 mm. Thus, based on the available data, and taking into consideration the loss of data during the few months in between the collection, it can be concluded that the life span of this specimen is about 10-12 months at the present study area.

According to Gould (1966) knowledge of allometry in shell and soft-body characters is essential to fully understand the growth of a species. Allometry in the length-height relationship showed no significant difference in different months which indicates that these parameters have a constant relative growth. In this case the general form of the body was more or less the same throughout its life from the time of settling. These parameters were unaffected by the physiological and ecological parameters. Jones (1979) observed a homogeneous length-height values in Cerastoderma edule and reported that these were unaffected by the season. The relationship between length and depth showed variation in different months. This could be correlated with the development and regression of internal organs in relation to reproductive cycle.

Difference in length-shell weight relationship was found to be due to the difference in shell thickness in different months. The decrease in shell weight during June may be the result of the stress due to low saline condition. Allometry in the length-total weight and length-flesh weight also showed variation in different months. Upto maturity the animals show rapid growth. The lowest value observed during April-May seems to be largely due to spawning and subsequent gonad regression. Again in June a high value was observed indicating the normal growth of new recruits. The increase in growth in the rest of the months may be largely due to somatic tissue growth and accumulation of food reserves before sexual maturity. Gonad development takes place at the expense of these reserves. Hancock and Franklin (1972) correlated seasonal variation in length - tissue weight relationship in Cardium edule with reproductive cycle and food availability. Jones et al. (1978) have got similar results in Patella vulgata. Jones (1979) also reported a variation in length - tissue weight relationship in Cerastoderma edule according to the breeding cycle and food availability.

The coefficient K, denoting the growth rate at which the animal size approaches the theoretical maximum, can be used to compare the growth of the species in the two year classes studied. The growth rate of the first stock, representing the population in the first year and the second stock representing the population after monsoon showed almost equal growth. Thus the estimated growth rate based on von Bertalanffy growth equation very closely agrees with that observed by the modal shift. It shows that in the length ranges studied, the theoretical growth equation adequately describes

actual growth. Kuriakose (1973) observed similar results in Perna indica. Several other attempts have been made to estimate the parameters of the von Bertalanffy equation for growth of many bivalves like Meretrix casta (Salih, 1977), Chlamys varia (Conan and Shafee, 1978), Villorita cyprinoides (Chattergi et al., 1984), and Anadara granosa (Narasimham, 1988a&b).

It appears that in tropical waters, salinity has been found to be the chief triggering factor influencing the growth in marine and estuarine bivalves as shown by Mane (1974a) who reported a retardation in growth rate of Katelysia opima due to low salinities. Parulekar et al. (1986) observed that the growth of Meretrix casta during monsoon was also affected by decreasing salinity. Further Narasimham (1988a) found a slower growth rate of Anadara granosa in low salinity. In the present study it was found that the growth is affected by salinity fluctuations. During highest saline period mortality of oldest specimens could be seen associated with spawning which was evident from the presence of large empty shells in the samples collected during these period. With the advancement of monsoon and subsequent lowering of salinity the breeding period was of shorter length when compared to that during pre-monsoon period. This might have contributed to the lower growth of the shell and the stress due to low salinity during the monsoon period. During late monsoon period the salinity of the study area approaches almost the freshwater condition. Mortality of the majority of specimens irrespective of size could be noticed in this extremely low saline period. It may be assumed that high degree of siltation and associated decline in the food supply during monsoon season may also be a factor responsible for the mortality of the specimens.

Already from the study on reproductive biology (vide chapter) it has been revealed that the spawning in this species is protracted with a major peak in April-May. Hence it makes difficult to trace a single mode throughout the year. Because of the spawning and subsequent mortality during the high saline period most of the older ones are removed from the population. At the same time appearance of large number of younger ones are masking the existence of the older individuals present. Growth studied by length frequency distribution and by using von Bertalanffy equation support this observation. The allometric relationship between different dimensions studied also presents difference in different months.

C H A P T E R - V

SALINITY TOLERANCE AND FILTRATION RATE

5.1 INTRODUCTION

Salinity is a major factor, affecting the activities and distribution of the organisms in the marine and estuarine habitats. The salinity variations in Cochin backwater are largely effected by the annual precipitation, evaporation and tidal oscillations. There have been many studies concerning the variation in salinity in Cochin backwaters (Ramamirtham and Jayaraman, 1963; Haridas et al., 1973; Balakrishnan and Shynamma, 1976). The constant change in the physical and chemical environment of the estuary necessitates physiological adjustment of its inhabitants (Theede, 1975). In adverse conditions organisms resort to avoidance or escape movements which help them to isolate its tissue from the extreme salinity fluctuation outside, resulting migration of species from one environment to another.

Musculista senhausia is found in abundance in Cochin backwater. Habitat of this mussel is fairly affected by seasonal monsoons. Increase in salinity during pre-monsoon season causes the establishment of new population in the inner region of the backwater. Heavy mortality of older specimens (measuring more than 20 mm) could be encountered during late pre-monsoon months when the water attains a salinity of about 31 ppt. Eventhough the animal shows remarkable physiological adaptation under a wide range of salinity fluctuation.

5.2 REVIEW

5.2.1 Salinity Tolerance Studies

A vast majority of bivalves are sedentary and any change in the environment has to be accomodated by the animal. Several studies on the effect

of salinity variations on bivalves have been carried out. Mane (1974b) conducted studies on the mode of tolerance of Katelysia opima. The survival and behaviour of Crassostrea cucullata in low salinities have been studied by Nagabhushanam and Bidarkar (1975). Akberali and Davenport (1981) have reported the effect of gradual changes in salinity on the behaviour of Scrobicularia plana. The effect of rapid changes in salinity on S. plana have been monitored by Trueman and Akberali (1981). Some behavioural avoidance mechanisms like siphonal closure, shell valve closure etc. developed to counter adverse environmental conditions have been reported in bivalves (Akberali and Trueman, 1985).

Salinity tolerance experiments have been conducted on different bivalves namely Rangia cuneata (Bedford and Anderson, 1972), Musculista arcuatula (George, 1973), Villorita cyprinoides var. cochinensis (Nair and Shynamma, 1975b), Pinctada fucata (Alagarswami and Victor, 1976), Donax cuneatus (Talikhedkar and Mane, 1976), Meretrix casta (Salih, 1977), Nausitora hedleyi (Mohan, 1979), Sunetta scripta (Thampuran et al., 1982), Meretrix meretrix, Crassostrea madrasensis and Perna viridis (Sundaram and Shafee, 1989).

5.2.2 **Filtration Rate**

The suspension feeding organisms in the sea obtain their food by filtering finely dispersed organic matter from the surrounding water. Filtration mechanism is a function of the amount of water transported across the feeding surfaces, the amount of food present in the surrounding water and the retention ability of the ctenidia (Owen, 1966). Lamellibranch bivalves subsist mainly on particle filtered from the ambient water. Ctenidia play a major role in feeding as well as in respiration.

Lamellibranchs have been extensively studied for their rate of water transport and filtration activity under different conditions. These attempts were initially concerned with the physiological concept of pumping water through the mantle cavity. Basically two types of methods have been adopted by different workers, the direct methods (Galtsoff, 1928; Loosanoff and Nomejko, 1946; Hamwi and Haskin, 1969; Winter, 1969; De Bruin and Davids, 1970; Hildreth, 1976) which measure the rate of flow of exhalant water by inserting a tube into the exhalant siphon and estimating the rate of filtered water, and the indirect methods (Cole and Hepper, 1954; Nagabhushanam, 1956; Durve, 1963; Bohle, 1972; Badman, 1975; Alagarswami and Victor, 1976; Holley and Foltz, 1987; Matthews et al., 1989) that estimate the filtration rate of particles removed from the water as measured by changes in optical density or particle counts.

Under experimental conditions different types of organic and inorganic particles result in different rate of filtration. Many kinds of particles, that is, inorganic like calcium carbonate (Fox et al., 1937), colloidal graphite (Jorgensen, 1949; Jorgensen and Goldberg, 1953; Morton, 1971; Mathers, 1974); neutral red (Cole and Hepper, 1954; Nagabhushanam, 1956; Durve, 1963; Ward and Aiello, 1973; Badman, 1975; Mane, 1975; Abel, 1976; Alagarswami and Victor, 1976; Talikhedkar and Mane, 1977); silt (Loosanoff and Tommers, 1948) etc. and organic particles like algal cells (Loosanoff and Engle, 1947; Jorgensen, 1949; Ballantine and Morton, 1956; Winter, 1969; Morton, 1971; Mathers, 1974; Epifanio and Ewart, 1977; Palmer, 1980; Holley and Foltz, 1987), bacterial cells (Galtsoff, 1928; Mathers et al., 1989), flagellates (Bohle,

1972), radioactively labelled microspheres (Morrison et al., 1977), radioactively labelled plankton cells (Chipman and Hopkins, 1954; Blake, 1961; Allen, 1962; Foster-smith, 1975) etc. have been used in various studies.

Several authors have studied the effect of varying concentration of either inert substances or active algal cells upon the rate of filtration. Blake (1961) found that filtration rate in Mya arenaria was independent of algal population densities from $3x10^6$ to $1.5x10^9$ cells per litre. Winter (1969, 1978) opined that filtration rate of Arctica islandica and Modiolus modiolus showed a decreasing trend towards increasing cell concentration. Palmer (1980) also obtained similar results in Argopecten irradians. But Epifanio and Ewart (1977) found that filtration rate of Crassostrea virginica was primarily dependent upon the density of the algal suspension. The actual amount of food removed increased with increasing food concentration upto a threshold limit, above which increase in concentration was not followed by an increase in the amount of particle ingested (Tenore and Dunstan, 1973).

The filtration rate is not only influenced by the suspension density but is also a function of particle size. Ballantine and Morton (1956) claimed that Lasaea rubra cleared suspension of particle size in the range from 1-50 μ with equal rates independent of size. According to Blake (1961) the same trend was maintained in Mya arenaria and Mytilus edulis. Epifanio and Ewart (1977) reported that filtration rate of Crassostrea virginica was high for small-celled algal suspension, but it was clearly less for larger algae. Similar result was observed by Rajaretnam et al., (1988) in the feeding behaviour of Perna indica in laboratory.

Filtration rate is influenced by a number of environmental factors like temperature, salinity, dissolved oxygen, flow rate etc. It is suggested that filtration rate is strongly affected by temperature changes (Ballantine and Morton, 1956; Winter, 1969; Widdows, 1973; Schulte, 1975; Bayne et al., 1976). Walne (1972) studied the influence of temperature on the filtration rate of five species of bivalves and found that Crassostrea and Mytilus were the least affected, Ostrea intermediately, while Venerupis decussata and Mercenaria mercenaria were strongly affected by temperature. Schulte (1975) found that filtration rate of Mytilus edulis was directly proportional to temperature upto an optimum level and decreased drastically with further increase in temperature. Mane (1975) observed similar results in Katelysia opima.

Several authors have investigated the effect of varying salinities on filtration rate. Nagabhushanam (1956) observed a decreasing effect of low salinity on the rate of filtration in Martesia striata. Blake (1961) found that in Mya arenaria filtration rate was independent of salinity. Durve (1963) reported a low filtration rate in Meretrix casta in extreme low and high salinities. Bohle (1972) also observed a similar trend in lower salinities in the case of Mytilus edulis. In Pinctada fucata Alagarswami and Victor (1976) demonstrated a higher filtration rate in higher concentrations than in dilutions. Holley and Foltz (1987) pointed out a decrease in filtration rate of Rangia cuneata upon transfer to higher salinities.

Walne (1972) found that filtration rate of five species of bivalves was positively affected by changes in the flow rate. In this study he observed that filtration rate increased with increasing flow rate upto a threshold range,

above which the flow rate had no further effect. Using a refined direct measurement apparatus, Hildreth (1976) studied the influence of flow rate on pumping rate in Mytilus edulis and claimed that within the range tested (2-42 l h^{-1}), the water flow rate over the mussel had no significant effect on pumping rate. Wildish et al., (1987) explained the relationship between ambient seawater flow velocity and filtration rate in Placopecten magellanicus.

In many studies the filtration rates have been found to be related to body dimensions like shell length (Winter, 1969, 1973), wet and dry weights (Blake, 1961; Winter, 1969; Palmer, 1980; Holley and Foltz, 1987), total weight (Ali, 1970) etc.

The basic objective of this part of the study is to acquire information on the range of salinity tolerance of M. senhausia and its filtration rate in relation to different salinity levels in the habitat. To evaluate the effect of varying salinities on filtration rate the method followed was dye clearance technique (Cole and Hepper, 1954) using a homogeneous solution of neutral red.

5.3 MATERIALS AND METHODS

5.3.1 Salinity Tolerance Studies

Mussels used for the experiment were collected from natural beds in the Cochin backwater. In the laboratory the animals were kept in water of habitat salinity for a period of one week for acclimation. Salinity tolerance experiments were carried out in beakers of one litre capacity containing one litre experimental solution of different salinities. Lower salinities were prepared by diluting seawater with distilled water and higher salinity by

evaporation of seawater. Salinity of the seawater was determined by titration with silver nitrate using potassium chromate as indicator. Mussels of three size groups, 9±1 mm (small), 17±1 mm (medium) and 25±1 mm (large) in length were selected for the study. Only those mussels which gave good response to touch were used for the experiments. Mussels were subjected to the salinities 0, 5, 10, 15, 20, 25, 30 and 35 ppt.

After the acclimation ten healthy mussels from each size group were directly transferred to the various gradations of experimental salinity. The animals were kept under observation for ten days. Mortality rate and behaviour of the mussels were recorded every 24 hours and the dead ones were removed from the experimental beakers. The animal was considered dead if it failed to close the valve or respond to external stimuli. The rate of mortality is generally considered as the criterion of tolerance. Those salinities which caused death of more than 50% of organisms during the period of the experiment, have been considered beyond the range of tolerance of the species (Salih, 1977).

Another set of experiment was conducted to find out the lethal salinity, that is, those salinity which causes the death of at least 50% of the animals during the period of the experiment. Ten mussels of small and medium size were exposed to 3, 4, 5, 6 and 7 ppt salinities and large mussels to 5, 6, 7, 8 and 9 ppt salinities.

Water was changed every 24 hours and kept under constant aeration. Food was not provided to the mussel during the experimental period. The temperature was found to be 29±1°C. Two sets of experiments have been conducted for each size group at a time.

5.3.2 Filtration Rate

For filtration rate experiments mussels were maintained in tanks containing medium of the habitat for two days under constant aeration. Later, mussels of different size (9-24 mm in length) were selected and acclimated in different experimental salinities (10, 15, 20, 25 and 30 ppt) for seven days. Filtered seawater was used to prepare the experimental solutions.

After the lapse of seven days, ten specimens of uniform size were transferred to a beaker of 1 litre capacity containing solution of the respective experimental salinities, having a concentration of 2 ppm of neutral red. At intervals of 30 minutes, 10 ml of the test solution was removed for two hours using a pipette and concentration of the dye after acidification was estimated using a Hitachi model (200-20) spectrophotometer. The experiment was repeated several times. Filtration rate was calculated using Quayle's equation (Quayle, 1948).

$$m = \frac{M}{nt} \log_e \frac{\text{Con } C_o}{\text{Con } C_t}$$, where

m = filtration rate ml h^{-1};

M = volume of solution in ml;

n = number of animals in the test vessel;

C_o = dye concentration in initial sample;

C_t = dye concentration in final sample;

t = time between dye sample

After the experiment, soft tissue were removed and dried at 70-80°C to estimate the dry weight.

Control beakers with empty shell in test solution and with only test solution were sampled in order to measure the possible difference due to absorption of the neutral red by shells and settling of the suspension.

The relationship between body weight and filtration rate in different salinities was studied by fitting the regression equation of the type

$$Y = aX^b$$

Here Y represents the filtration rate, X the body weight, and a and b are fitted parameters. The constants a and b were estimated by the method of least squares after logarithmic transformation. Analysis of covariance was used for the comparison of the regression values.

5.4 RESULTS

5.4.1 Salinity Tolerance Studies

In the first experiment the mussels (small, medium, and large) were subjected to the salinities 0, 5, 10, 15, 20, 25, 30 and 35 ppt. During the period of experiment the animals were kept under observation and the readings, as mortality rate, were taken. The results are presented in Tables 13-15. The three size groups studied showed difference in activity in different salinities.

When placed directly in freshwater the mussels closed their shell valves tightly and showed no activity till death. At the end of 48 hours all the specimens were found dead and gaping. In 5 ppt salinity the mussels showed no gaping, byssal attachment and faecal production. In small and medium sized mussels 100% mortality occurred within 72 hours and large mussels

Table 13. Rate of mortality of M. senhausia (small size) in salinities ranging from 0 ppt to 35 ppt

Salinity ppt	Number of days										
	1	2	3	4	5	6	7	8	9	10	Total
0	30	70	0	0	0	0	0	0	0	0	100
5	30	40	30	0	0	0	0	0	0	0	100
10	0	0	10	0	0	0	0	0	0	0	10
15	10	0	0	0	0	0	0	0	0	0	10
20	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	10	10
30	0	0	0	0	0	0	0	0	0	10	10
35	10	0	0	0	0	0	0	0	0	0	10

Table 14. Rate of mortality of M. senhausia (medium size) in salinities ranging from 0 ppt to 35 ppt

Salinity ppt	Number of days 1	2	3	4	5	6	7	8	9	10	Total
0	30	70	0	0	0	0	0	0	0	0	100
5	20	50	30	0	0	0	0	0	0	0	100
10	0	0	0	0	10	0	0	0	0	0	10
15	0	0	0	0	0	0	0	10	0	0	10
20	0	0	0	0	10	0	0	0	0	0	10
25	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	10	0	0	0	10	10
35	0	10	60	30	0	0	0	0	0	0	100

Table 15. Rate of mortality of M. senhausia (large size) in salinities ranging from 0 ppt to 35 ppt

Salinity ppt	Number of days 1	2	3	4	5	6	7	8	9	10	Total
0	70	30	0	0	0	0	0	0	0	0	100
5	50	50	0	0	0	0	0	0	0	0	100
10	10	30	0	0	0	0	0	0	0	0	40
15	0	0	0	0	0	0	0	0	0	0	0
20	0	20	0	0	0	10	0	0	10	0	40
25	0	0	10	20	0	10	10	0	0	0	50
30	0	20	20	20	20	0	0	0	0	0	80
35	20	70	10	0	0	0	0	0	0	0	100

did not survive the same salinity for not more than 48 hours. The animal resumed the activity in 10 ppt salinity, but the byssal attachment was very weak. Only a few mussels were seen attached to the walls of the beaker by byssus thread. Faecal production was less. The large mussels were less active when compared to the other two sizes. Small and medium mussels showed only 10% mortality during the period of experiment. In 15 ppt salinity all the mussels were active and started activity within few seconds after the transfer. The mussels were found attached to the wall of the container and continued to be active till the end of experiment. In this salinity 90% survival of the small and medium sized mussels could be seen. In the case of large sized mussels the survival was 100%. Like this, the grades above this, that is, 20, 25 and 30 ppt appeared to be the suitable salinities for the small and medium sized mussels and all the organisms showed normal activities without any indication of stress. In 20 ppt salinity 100% survival was found in small mussels. In other salinities the survival was 90%. In the case of medium sized mussels 100% survival was in 25 ppt salinity. Large mussels were active in 20 ppt and the survival was 60%. In 25 ppt mussels were less active and mortality was 50%. Here the faecal production and byssal attachment were very feeble. In 30 ppt salinity large mussels were not active and mortality was 80%. Above this, in 35 ppt salinity only small mussels were active. They showed appreciable tolerance to water of this salinity and the mortality was found to be 10%. But the other two sizes were not active and showed 100% mortality.

The specimens kept in the control beaker, containing lake water of habitat salinity showed all the activities till the end of the experiment. The

Table 16. Lower lethal limit of salinity of M. senhausia (small) in salinities ranging from 3 ppt to 7 ppt

Salinity ppt	Number of days 1	2	3	4	5	6	7	8	9	10	Total
3	0	80	20	0	0	0	0	0	0	0	100
4	0	70	30	0	0	0	0	0	0	0	100
5	0	40	40	0	20	0	0	0	0	0	100
6	0	20	50	0	0	0	0	0	0	0	70
7	0	0	20	0	10	0	0	0	0	0	30

Table 17. Lower lethal limit of salinity of M. senhausia (medium) in salinities ranging from 3 ppt to 7 ppt

Salinity ppt	Number of days 1	2	3	4	5	6	7	8	9	10	Total
3	10	80	10	0	0	0	0	0	0	0	100
4	0	90	10	0	0	0	0	0	0	0	100
5	0	70	30	0	0	0	0	0	0	0	100
6	0	50	20	0	0	0	0	0	0	0	70
7	0	20	0	0	0	0	0	0	0	0	20

Table 18. Lower lethal limit of salinity of M. senhausia (large) in salinities ranging from 5 ppt to 9 ppt

Salinity ppt	Number of days										
	1	2	3	4	5	6	7	8	9	10	Total
5	90	10	0	0	0	0	0	0	0	0	100
6	80	20	0	0	0	0	0	0	0	0	100
7	40	10	0	0	0	0	0	0	0	0	50
8	10	0	10	0	0	0	0	0	0	0	20
9	10	0	0	0	0	0	0	0	10	0	20

tolerance range of small mussels was found to be 10-35 ppt salinity. Medium sized mussels could tolerate 10-30 ppt salinity and in the case of large mussels tolerance salinities were 10-25 ppt.

Results of the determination of lethal salinity are presented in Tables 16-18. In 3, 4 and 5 ppt salinity all mussels showed 100% mortality. Here the mussels were not active and the valves tended to remain closed. All mussels were found dead at the end of 72 hours. In 6 ppt salinity, small and medium sized mussels showed low activity and the survival was only 30%. These two groups were more tolerant to 7 ppt salinity. The mortality rate in this salinity was found to be 30% for small mussels and 20% for medium sized mussels. Weak byssal attachment and faecal production could be noticed. Thus it is proved that lethal salinity for small and medium sized mussels is 7 ppt.

In the case of large mussels 100% mortality could be seen in 5 and 6 ppt salinity within 48 hours. In 7 ppt salinity the mortality was 50%. In 8 and 9 ppt the mussel exhibited only 20% mortality. Faecal production was noticed in 8 ppt salinity, but there was no byssal attachment. Weak byssal attachment could be seen in 9 ppt salinity. It revealed that the lethal salinity is 8 ppt.

5.4.2 **Filtration Rate**

In all the salinities and size groups, filtration rate (ml h^{-1}) was found to be increasing with body weight, and weight specific filtration rate (ml h^{-1} mg^{-1} dry weight) was decreasing. The log filtration rate when plotted

against log body weight showed a positive correlation (Fig.14a&c, 15a&c and 16a) and weight specific filtration rate showed a straight line with negative slope (Fig.14b&d, 15b&d and 16b).

In 10 ppt salinity, filtration rate varied from 7.94 ml h^{-1} to 56.33 ml h^{-1} and 0.15 ml $h^{-1}mg^{-1}$ to 2.32 ml $h^{-1}mg^{-1}$ dry weight. The b values were found to be 0.50 and -0.50 (Table 19). In 15 ppt, 23 mussels of 8-133 mg dry weight showed a filtration rate of 7.42 ml h^{-1} to 91.81 ml h^{-1} and 0.19 ml $h^{-1}mg^{-1}$ to 1.90 ml h^{-1} mg^{-1} dry weight. Here the b values were 0.76 and -0.27 respectively. The filtration rate in 20 ppt salinity varied from 9.47 ml h^{-1} to 98.19 ml h^{-1} and 0.23 ml $h^{-1}mg^{-1}$ to 1.33 ml $h^{-1}mg^{-1}$ dry weight. The b values calculated were 0.81 and -0.19 respectively. In 25 ppt, the filtration rate of 24 mussels of 11 to 161 mg dry weight varied from 4.23 ml h^{-1} to 71.31 ml h^{-1} and from 0.16 ml $h^{-1}mg^{-1}$ to 0.70 ml $h^{-1}mg^{-1}$ dry weight. Here the b values obtained were 0.76 and -0.24. In 30 ppt salinity, filtration rates varied from 7.03 ml h^{-1} to 108.85 ml h^{-1} and 0.13 ml $h^{-1}mg^{-1}$ to 0.78 ml $h^{-1}mg^{-1}$ dry weight. The estimate of b values were 0.77 and -0.23 respectively.

The analysis of covariance of the relationship between body weight (mg dry weight) and filtration rate is presented in Table 20. The comparison by covariance analysis of the linear regression of logarithm of filtration rate in different salinities on logarithm of body weight showed non-significant 'F' value of 1.68. That is, the filtration rate-body weight values are homogeneous. Filtration rate of standard weights in different experimental salinities were worked out and are given in Table 21. They also followed the same trend as obtained in the experiment.

Table 19. Statistical analysis of the data obtained for the filtration rate (FR) of M. senhausia in different experimental salinities

	Salinity ppt	log a	b	r	N
FR	10	0.5284	0.5009	0.7853	26
ml h^{-1}	15	0.0495	0.7590	0.7490	23
	20	0.0134	0.8076	0.8041	23
	25	0.0162	0.7578	0.8988	24
	30	0.0029	0.7670	0.8356	23
FR	10	0.5256	- 0.5014	0.7866	26
ml $h^{-1}mg^{-1}$	15	0.1076	- 0.2726	0.3659	23
dry weight	20	0.0151	- 0.1932	0.3078	23
	25	0.0162	- 0.2421	0.5479	24
	30	0.0029	- 0.2330	0.4194	23

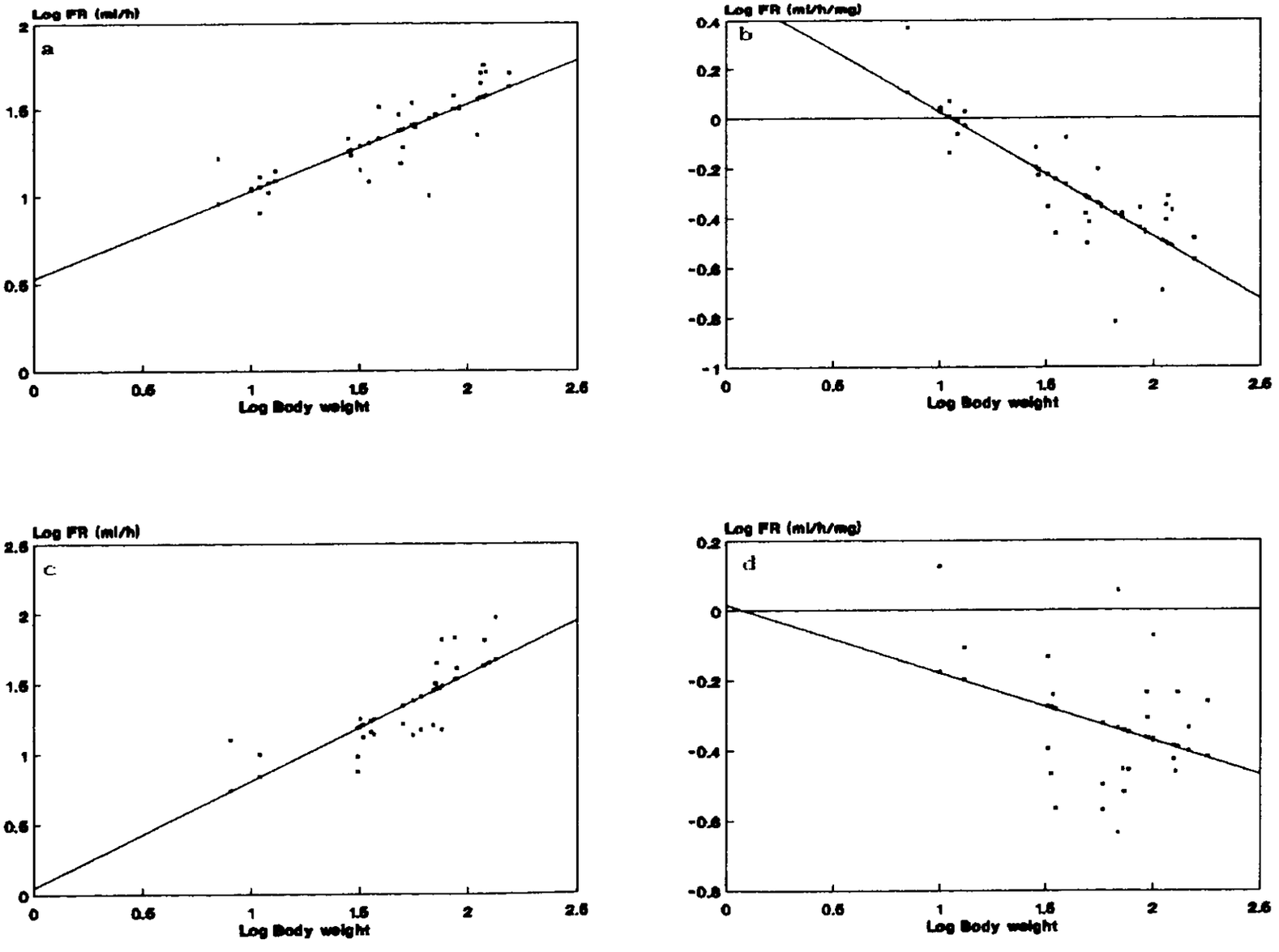


Figure 14. Relationship between filtration rate (FR ml/hr and FR ml/hr/mg dry weight) and dry body weight in 10 ppt (a&b) and 15 ppt (c&d) salinity.

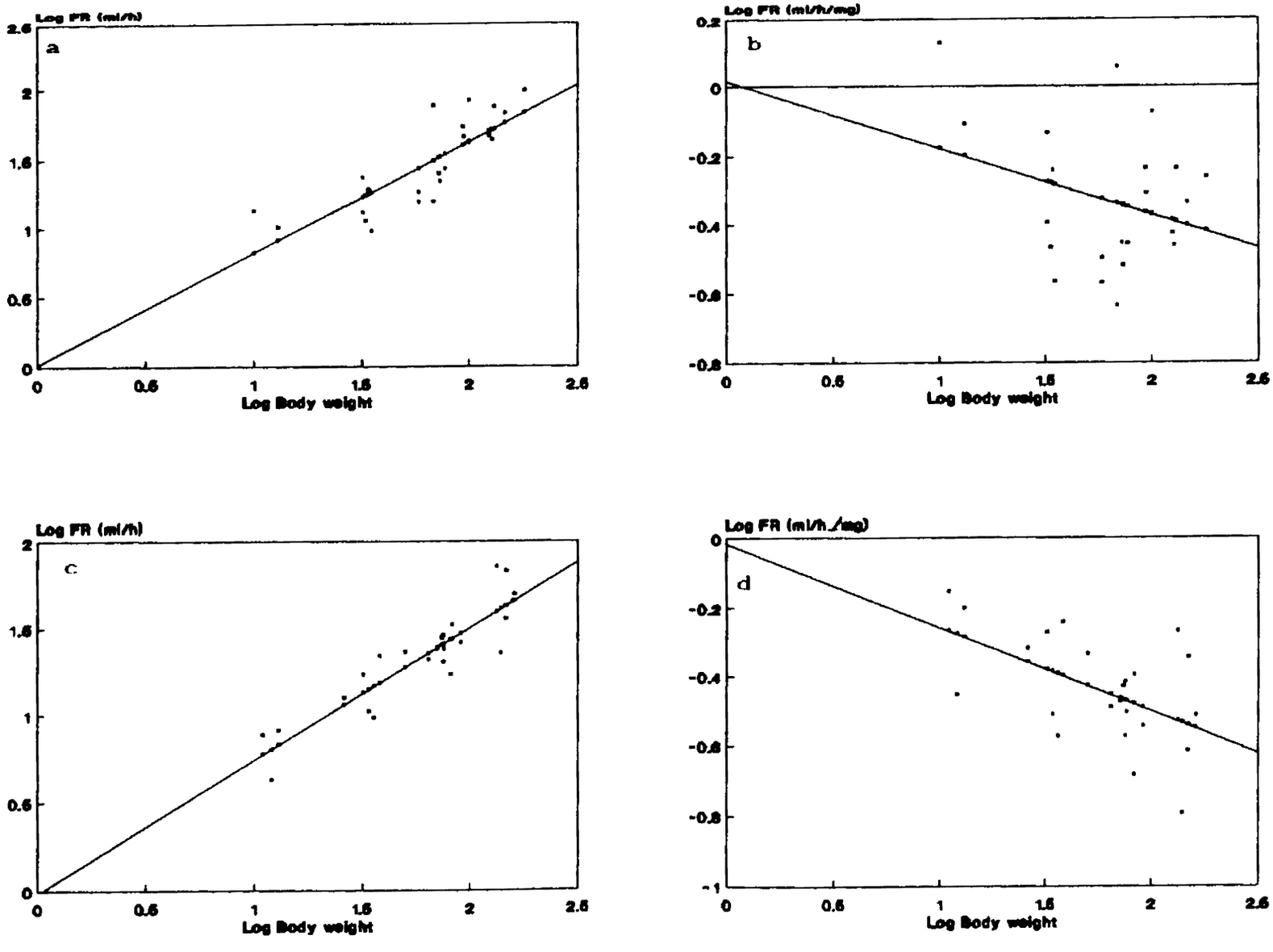


Figure 15. Relationship between filtration rate (FR ml/hr and FR ml/hr/mg dry weight) and dry body weight in 20 ppt (a&b) and 25 ppt (c&d) salinity.

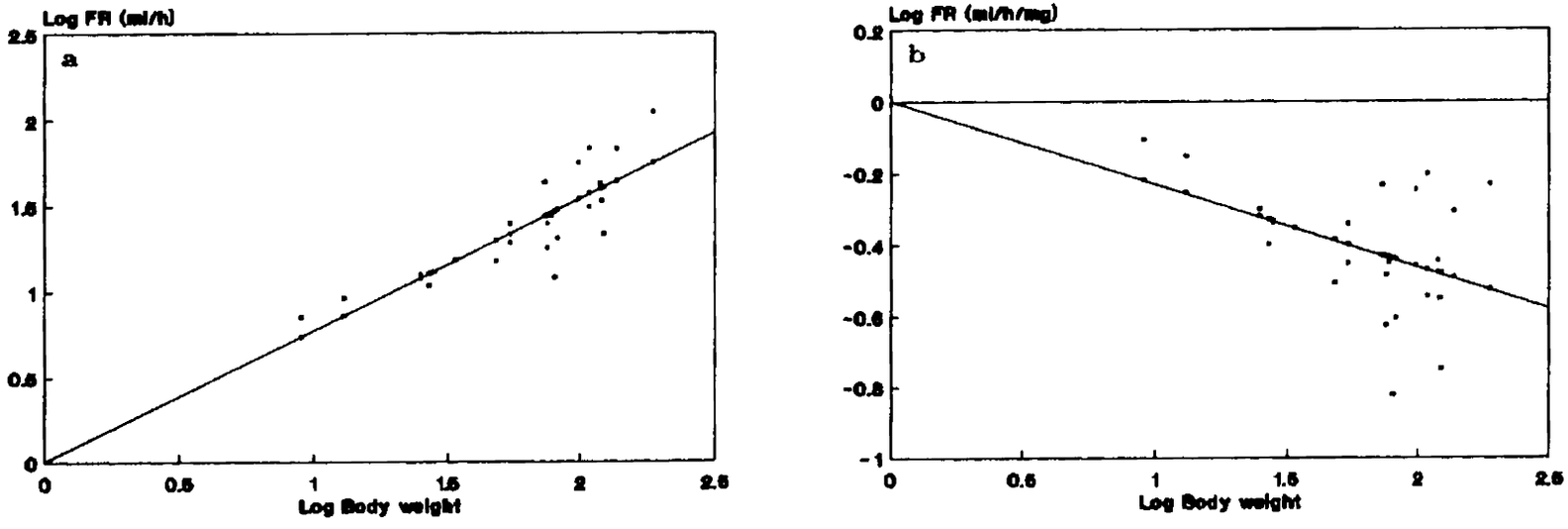


Figure 16. Relationship between filtration rate (FR ml/hr and FR ml/hr/mg dry weight) and dry body weight in 30 ppt salinity.

Table 20. Analysis of covariance of linear regression of logarithm of filtration rate (ml h^{-1}) in different salinities on logarithm of dry body weight (mg)

S ppt	Σx^2	Σy^2	Σxy	N	df	Reg.coef.	Deviation from regression df	ss	ms
10	3.9278	1.5976	1.9673	26	25	0.5009	24	0.6122	0.0255
15	2.1535	2.2093	1.6339	23	22	0.7588	21	0.9696	0.0461
20	2.2726	2.2935	1.8359	23	22	0.8079	21	0.8103	0.0386
25	2.8732	2.0427	2.1774	24	23	0.7578	22	0.3926	0.0178
30	2.4529	2.0669	1.8814	23	22	0.7670	21	0.6238	0.0297
Pooled	13.6800	10.2100	9.4960	119	114	0.6942	113	3.6183	0.0320
							109	3.4085	0.0313
					Difference between slopes		4	0.2098	0.0524

F (4,109) = 1.6771

Table 21. Filtration rate of M. senhausia in different experimental salinity

	Standard Weights mg dry weight	Salinity ppt 10	15	20	25	30
FR ml h^{-1}	25.00	13.00	11.00	16.00	13.00	13.75
	50.00	21.00	19.00	24.50	18.68	20.50
	75.00	28.00	27.00	33.00	25.00	27.50
	100.00	34.50	34.50	41.50	31.00	34.00
	125.00	41.50	42.50	50.50	36.75	40.50
	150.00	48.50	50.50	59.00	43.00	47.50
FR ml $h^{-1}mg^{-1}$	25.00	1.14	0.76	0.50	0.39	0.42
	50.00	0.97	0.66	0.48	0.37	0.40
	75.00	0.80	0.55	0.46	0.35	0.38
	100.00	0.63	0.44	0.43	0.33	0.35
	125.00	0.46	0.34	0.41	0.31	0.33
	150.00	0.29	0.23	0.39	0.28	0.31

5.5 DISCUSSION

The mussel, Musculista senhausia inhabits highly productive estuarine zone rich in organic detritus in suspended form. This area is subjected to wide range of salinity fluctuations, with a drastic fall during monsoon season.

It is evident that the bivalves exercise certain physiological mechanisms to withstand wide changes in the osmotic concentration of their environmental medium. Closure of shell valves prevents drastic changes in osmotic concentrations of their body fluids when exposed to short-term fluctuations in salinities (Shumway, 1977). But constant stress condition may ultimately lead to the death of the organisms. In lower salinities the oyster Crassostrea cucullata closed their valves and hence Nagabhushanam and Bidarkar (1975) concluded that the salinity plays an important role in opening and closing of the valves as well as the normal life of the individuals. Similar observation has been made by Davenport (1979) in Mytilus edulis in 25 ppt salinity. But in Scrobicularia plana the valve closure was triggered at 20 ppt salinity (Akberali and Davenport, 1981). In the present investigation in small and medium sized mussels complete closure of shell valve appears to occur at 6 ppt and 35 ppt salinities. In 7 ppt salinity mussels showed low activity. Here byssal attachment and faecal production were weak. In large organisms activity starts from 8 ppt salinity. Bohle (1972) observed very low byssus production by Mytilus edulis in 75% and 50% seawater. Bayne et al. (1975) stated that the byssus formation can be a sensitive index of the effects of salinity (and other environmental factors) on mussels.

The present study indicates that the small mussels can tolerate a wide range of salinity from a lower limit of 7 ppt to a higher salinity of 35 ppt. In the medium sized mussels the upper tolerance limit lowered to 30 ppt. But in the case of large mussels tolerance range was found to be between 8 ppt and 25 ppt. Thus it is assumed that the tolerance capacity of M. senhausia declines with increase in size. Similar results were reported in Villorita cyprinoides (Nair and Shynamma, 1975b), Meretrix casta (Salih, 1977), Nausitora hedleyi (Mohan, 1979), and Sunetta scripta (Thampuran et al., 1982).

Kinne (1967) reported that salinity plays a paramount role in limiting the distribution of animal populations in marine and brackish water environments. In the present study in M. senhausia the tolerance limit in natural environment is found to be much wider than that observed in the laboratory conditions. In the natural environment comparatively slow rate of fluctuation in salinity gives sufficient time for gradual acclimatisation, and the animal may get acclimatised particularly below the lethal level. During July when monsoon is at its peak resulting in almost freshwater condition of the lake water, the population of M. senhausia is adversely affected. Dead shells of different size groups could be encountered during this period. From December onwards, a gradual rise in salinity in the backwater marks the normal growth of the organisms, but during late pre-monsoon period the older animals fail to withstand and die as evidenced by the presence of bigger dead shells in the collection, while younger mussels particularly below 20 mm were found to survive. It has been assumed that the pre-monsoon factors

such as high salinity, rich planktonic food etc. may serve as a better condition for the growth of the mussel.

In the filtration rate experiments the method followed was an indirect method using a homogeneous solution of neutral red. According to Cole and Hepper (1954) the amount of neutral red removed from the test solution can be interpreted as the volume of water pumped through the gills during the period of observation. Feeding is a function of the efficiency of the filter, the food particles present in the ambient water and the pumping rate (Owen, 1966). Pumping rate or ventilation rate is the total water transport through the gills per unit time, and volume of water filtered completely free of particles per unit time is the filtration or clearance rate. When all the particles entering the mantle cavity are removed from the suspension (that is, filtration efficiency is 100%) the filtration rate is the same as the pumping rate (Bayne et al., 1976). In this study the duration of the experiment was two hours. The static system results in the accumulation of ammonia and other excreted compound and reduction of Po_2 in the surrounding water which may alter the normal filtration behaviour (Bayne et al., 1976). According to these authors the significance of these factors will depend on the duration of the experiment, the geometry of the vessel, the volume of the water in relation to the size of the animal, and the animal's metabolic rate.

As the water passes through the gills of bivalve, all the particles therein would be completely removed (Jorgensen, 1949). According to him, changes in the rate of clearing of the dye may be interpreted as resulting from changes in the amount of water filtered. Here, under all experimental

conditions filtration rate increased with increasing body weight. Filtration rate was less in small mussels, while it was high in larger mussels. That is, the rate of filtration and body weight showed a positive relationship. But the weight specific filtration rate showed an opposite trend. The higher filtration rate per body weight for smaller individuals indicates high rate of growth and active metabolism. Similar observations were noticed in Pecten irradians (Chipman and Hopkins, 1954) and Katelysia opima (Mane, 1975). Durve (1963) also observed rapid filtration rate per minute in the case of larger Meretrix casta. Talikhedkar and Mane (1977) stated that small sized Donax cuneatus filters at a faster rate than older ones when the filtration rate is expressed as the amount of neutral red removed per gram body weight.

Great variations exist in weight exponents of filtration rate. Winter (1969) estimated the value of b to be 0.74 for animals of size 0.43-3.95 g. Thompson and Bayne (1974) obtained a value of 0.38 for Mytilus edulis having a weight less than 1 gm dry weight, but it decreased in larger animals. Winter (1978) explained that the values lay between 0.66 and 0.82. In the present study the values were between 0.50 and 0.81 which is almost close to the observation of Winter (1978). The non-significant 'F' value obtained in the analysis of covariance of linear regression revealed that the filtration rate of the mussel, M. senhausia in different salinities showed no variation. That is, filtration activity of the organism was not changing significantly with body weight in different salinities.

Influence of salinity on filtration rate varies in different species of bivalves. Blake (1961) observed that in Mya arenaria filtration rate was

independent of salinity. Durve (1963) found that filtration rate of Meretrix casta decreased in extreme low and high salinities. Bohle (1972) observed a low activity in lower salinities. Alagarswami and Victor (1976) found that filtration rate of Pinctada fucata was low in dilutions, but in higher concentrations a higher rate than in dilutions was obtained. Mane (1975) observed an increase in the rate of water filtration of Katelysia opima with decreasing salinity. Likewise in Rangia cuneata a low filtration rate in higher salinities was observed by Holley and Foltz (1987). Influence of salinity on filtration rate may also be the result of acclimation in respective experimental salinities prior to the experiment. Theede (1975) suggested that the filtration rates are strongly reduced after direct transfer into sub or supranormal salinities, but after a period of acclimation to the changed osmotic conditions the filtering activities increase. The mechanisms allowing survival under irregular environmental conditions involve different types of responses like reactions, regulations and adaptations (Kinne, 1971; Lang, 1972; Theede, 1975). The time required for the adaptation to changed salinity depends on the species, on the salinity of the pre-acclimation and on the magnitude of salinity step (Theede, 1975). In the present study after the acclimation for a period of seven days the mussels showed no significant variation in filtration rate in different salinities. This indicates their wide tolerance to the drastically changing salinity condition of the backwater. The salinity tolerance studies also indicate that M. senhausia is euryhaline and hence well established in the Cochin backwater.

C H A P T E R - VI

REPRODUCTIVE BIOLOGY

6.1 INTRODUCTION

A thorough knowledge of the reproductive cycle of an animal is of vital importance in any biological investigation. The reproductive cycle of marine invertebrates may be continuous, annual, semi-annual or biennial (Giese and Pearse, 1974). In general, it is of continuous or extended type in most tropical marine invertebrates. Reproduction is a cyclic physiological process. The gametogenic cycle involves the initiation of gametogenesis, the growth of spermatocytes and oocytes including the process-vitellogenesis in the female, the physiological "ripening" of the full-grown gametes, and their release from the adult or spawning (Bayne, 1975). Intimately linked with this cycle, the nutrients in the form of lipid, protein and glycogen are stored and subsequently utilized in the production of gametes when the metabolic demand is high (Gabbot, 1975; Bayne, 1976). The sequence of different stages of gametogenic cycle varies between species. This difference can be attributed to the variations in hydrographic conditions. Environmental differences produce different physiological responses in respect to timing of development and developmental pattern.

6.2 REVIEW

There is extensive literature on the breeding cycle of mytilid species from Indian waters as well as from other waters. Patil and Bal (1967) studied the seasonal gonadal changes in adult freshwater mussel, Parreysia favidens var. marcens based on the macroscopic and microscopic changes in the structure of the gonad. Wilson and Hodkin (1967) compared the reproductive cycles of five species of Western Australian marine mussels, namely Mytilus edulis,

Xenostrobus pulex, Septifer bilocularis, Brachidontes cf variabilis and Amygdalum glaberrimum. Reproductive cycle of the estuarine bivalve, Musculista arcuatula have been studied by George and Nair (1973). Kuriakose (1973) studied the reproductive cycle of Perna indica and Nagabhushanam and Mane (1975b) that of Mytilus viridis. Seed (1976) has reviewed the literature published on reproduction and settlement of different species of Mytilus in European waters and other parts of the world together with an account of the factors controlling the reproductive cycle. Ajithakumar (1984) has investigated the reproductive physiology of two Indian species of mussels, Perna indica and P. viridis. The ultrastructural alterations taking place within the developing oocytes in Mytilus edulis have been studied by Pipe (1987). Reproductive cycle of Brachidontes variabilis in Hong Kong had been elucidated by Morton (1988). King et al. (1989) have investigated the reproduction and settlement of Mytilus edulis in Galway Bay, west coast of Ireland. Jasim and Brand (1989) have made observations on the reproduction of Modiolus modiolus in Isle of Man waters. Barkati and Ahmed (1989-1990) have given an account of the reproductive cycle of the mussel, Mytilus edulis from Western Germany.

A great deal of work has been done on the breeding cycle of other bivalves like Katelysia opima (Mane, 1974a), Cardium edule and C. glaucum (Kingston, 1974), Mercenaria mercenaria (Keck et al., 1975), Meretrix casta (Harkantra, 1975), Donax cuneatus (Nagabhushanam and Talikhedkar, 1977a), Paphia laterisulca (Nagabhushanam and Dhamne, 1977), Meretrix casta (Salih, 1977), Crassostrea madrasensis (Stephen, 1980a), Anadara senilis (Yankson, 1982), Argopecten irradians (Barber and Blake, 1983), Arctica islandica (Ropes et al., 1984), Crassostrea madrasensis (Joseph and Madhyastha, 1984), Meretrix

meretrix, M. casta, and Katelysia opima (Jayabal and Kalyani, 1986a), Pacten maximus (Wilson, 1987), Patinopecten caurinus (MacDonald and Bourne, 1987), Meretrix casta and Paphia malabarica (Rao, 1988), Anadara granosa (Narasimham, 1988a), Donax cuneatus (Victor and Subramoniam, 1988), Saccostrea cucullata (Sukumar and Joseph, 1988), Sunetta scripta (Katticaran, 1988), Crassostrea madrasensis (Sarvesan, 1990) etc.

The reproduction of bivalves is influenced by several factors of the environment such as water temperature, salinity, availability of the food etc. Wilson and Hodkin (1967) investigated the role of temperature as the chief determining factor controlling the reproductive cycles of the marine mussels. Newell et al. (1982) observed differences in the gametogenic cycle of Mytilus edulis in response to altered availability of food in different environments. The relation of water temperature and food sources with the major difference in reproductive characteristics of Argopecten irradians (Barber and Blake, 1983) and Patinopecten caurinus (MacDonald and Bourne, 1987) were discussed. Borrero (1987) suggested that the level of occurrence in the intertidal zone, the length of submersion and potential feeding time influence the timing of reproductive cycle in Geukensia demissa. MacDonald and Thompson (1988) correlated latitudinal variation in growth and fecundity of Placopecten magellanicus with local environmental factors.

Nagabhushanam and Mane (1975b) and Harkantra (1975) correlated seasonal variation of reproductive cycle of Mytilus edulis and Meretrix casta respectively with changes in temperature and salinity of the habitat. Nagabhushanam and Talikhedkar (1977a) found that increase in salinity and temperature soon after the monsoon appears to promote gametogenesis and initiate

spawning in Donax cuneatus. Nagabhushanam and Dhamne (1977) observed similar effect in Paphia laterisulca. Stephen (1980a) opined that increase and decrease in salinity during different seasons synchronize the gametogenic pattern in Crassostrea madrasensis. Joseph and Madhyastha (1984) related the onset of gametogenesis in C. madrasensis with the rapid increase in salinity. Besides, they also reported the influence of some other factors like turbidity, temperature and pH variations. In Donax cuneatus Victor and Subramoniam (1988) observed an influence of low salinity and temperature on active gametogenesis, and high salinity and temperature on spawning. A contrary picture has been presented by Sukumar and Joseph (1988) for Saccostrea cucullata in which an increased salinity triggered maturation while low saline condition initiated spawning.

Although extensive studies on breeding biology have been made, only scattered information is available on the histochemical localisation of different components like protein, lipid and carbohydrate in gonad. Lubet (1959) has carried out histochemical studies in relation to reproduction of the mussel, Mytilus edulis and M. californianus. Some histochemical aspects of the male and female gonads of Mytilus galloprovincialis have been elucidated by Costanzo (1966). Bayne et al. (1982) studied ultrastructural details of glycogen utilization and gametogenesis in Mytilus edulis. Ajithakumar (1984) has investigated the changes in the localisation of proteins, lipids and carbohydrate in gonadal tissues of Perna indica and P. viridis during developmental cycle.

The main objective of the present investigation is to elucidate the gametogenic pattern of Musculista senhausia and its relation to changing salinity in Cochin backwaters. An attempt was also made to analyse the histochemical localization of the storage substances in the gonad.

6.3 MATERIALS AND METHODS

Sampling was conducted at fortnightly intervals for a period from February 1988 to February 1989. Specimens falling into different size groups were used for the study. The mature females could be distinguished macroscopically by its yellow colour of the mantle, while in mature males it is creamy white. The sex and approximate stage of gonad development were ascertained by examining fresh smears of gonad under microscope.

Histological preparations were used to assess the annual reproductive cycle. Approximately 20 individuals, arbitrarily selected with respect to age and visible stage of gonad development, were excised, fixed in Bouin's fixative and prepared for sectioning by dehydration in ethanol and embedding in paraffin wax of melting point 60-62°C (Humason, 1972). Sections were cut at 8 μ thickness and stained with Ehrlich's hematoxylin and counterstained with eosin, and were examined under microscope and classified in to different developmental stages. Examination of the sections at regular intervals (throughout the year when specimens were available) furnished detailed information on the reproductive cycle including the actual period of spawning in this locality. Mantle thickness and oocyte diameter were measured with an ocular micrometer.

Studies were conducted to follow the histochemical localisation of the organic components like glycogen, protein and lipid of the gonad during gametogenesis of Musculista senhausia. Main stages studied for this purpose were the developing, mature and spawning stages. The methods adopted for this study were Periodic Acid-Schiff technique for glycogen (Humason, 1972). Mercury Bromophenol Blue for protein (Humason, 1972) and Sudan

Black B for lipid (Pearse, 1968). Cryostat (5030 microtome) was also used for sectioning.

6.4 OBSERVATIONS

6.4.1 Histology

Description of gonad development

The reproductive system of M. senhausia consists of numerous ducts which ramify throughout most of the body. During active phase of reproduction the mantle is occupied by reproductive tissue of different developmental stages. From histological preparation of the gonad four main types of gonad stages were recognised: developing, ripe, spawning and spent.

Indifferent or Inactive Stage

Gonads are in a state of quiescence. No gametogenesis is discernible and the sex is indistinguishable. Most of the gonad consists of numerous follicles with interfollicular connective tissues like adipogranular tissue and vesicular connective tissue in between (Fig.27). The follicle is usually expanded and the basal membrane and follicle wall are dominant. During this non-reproductive phase the mantle is generally thin and translucent.

Male: Developing or Active Stage

Each gonadal cycle begins with the proliferation and differentiation of the small earliest cells from stem cells which are distributed around the follicular wall. The proliferation of follicles become more apparent and the testicular follicles can be easily distinguished in the mantle tissue. In early stages large number of spermatogonia are present in early stages near the periphery of the lumen which may be found attached to the follicular wall,

but are more often free. Cell division proceeds and give rise to spermatocytes which are free in the follicular lumen in concentric band centripetal to the spermatogonia (Fig. 17). In more advanced stages, spermatocytes and spermatids are predominant, making it difficult to see follicular cells or spermatogonia. Interfollicular connective tissue disintegrates with the progress of spermatogenesis.

Male: Ripe or Mature Stage

In the ripe or mature male gonad (Fig.19) the follicles are densely packed and contain mainly spermatozoa. Spermatozoa aggregate in bands projecting into the lumen with their basophilic heads directed towards the periphery and eosinophilic sperm tails directed away from the follicular wall towards the centre (Fig.21). But spermatogonia, spermatocytes and spermatids appear as lightly stained band around the periphery of the follicle. Because of the rapid increase of the germ cells in the follicle, the walls of the adjacent follicle are apposed to each other. In this stage the mantle is found to be flabby and creamy white in colour.

Male: Spawning Stage

Spawning (Fig.23) takes place from the centre of the follicles where the oldest cells could be seen. In partially spawned individuals gonads are characterised by the shrinkage of the gonadal follicles. In some follicles the lumen is often seen empty due to the discharge of sperms while in other follicles gametogenesis continues and the central part of the follicle is still filled with spermatozoa.

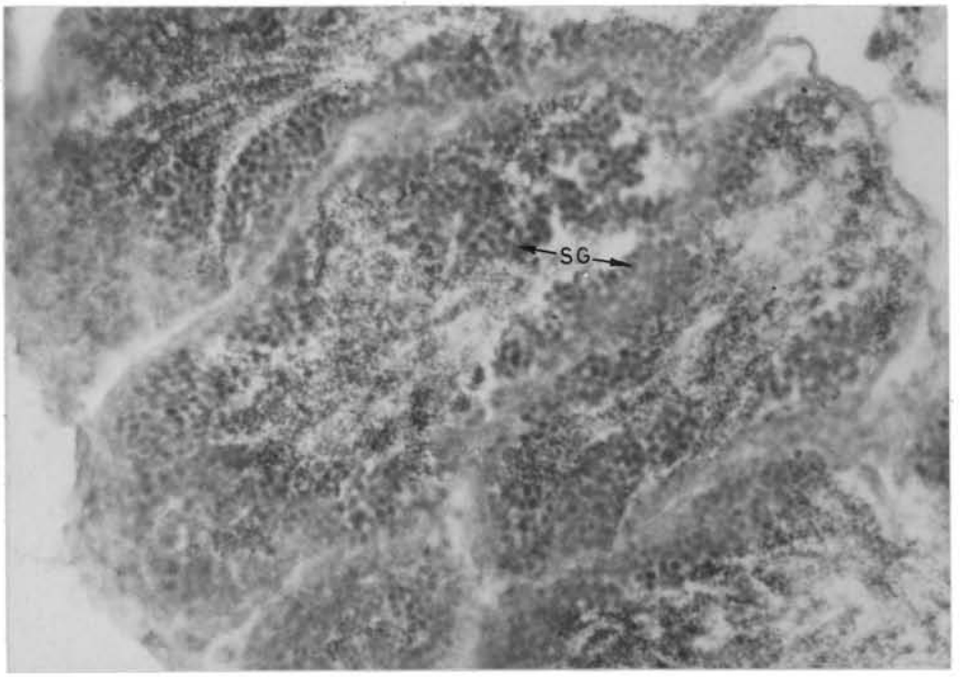


Figure 17. Section of developing male showing a large number of spermatogonia (SG).

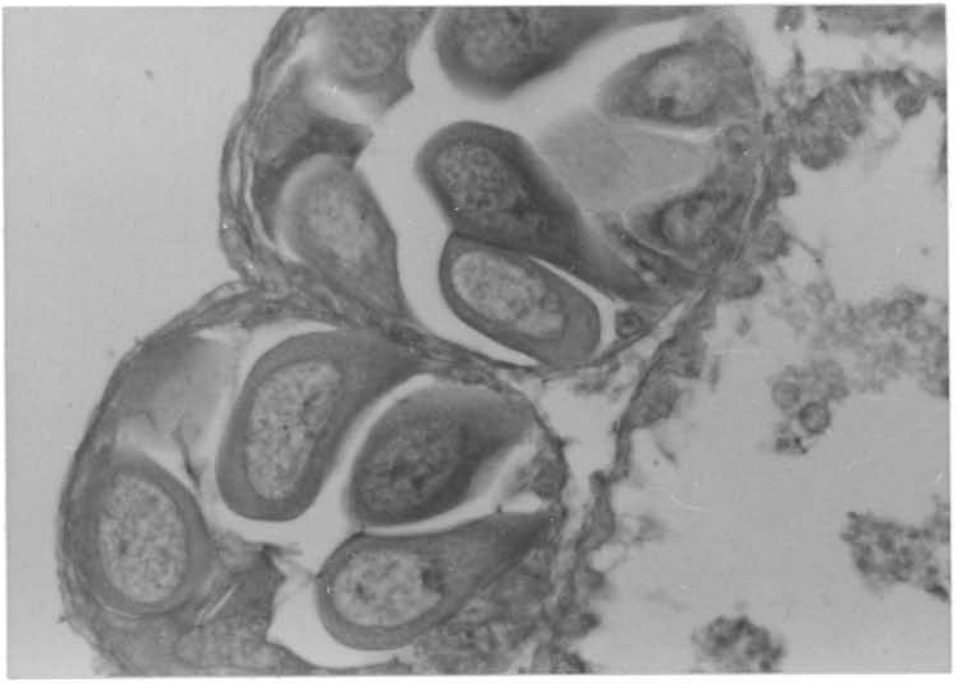


Figure 18. Section of developing female with stalked oocytes.

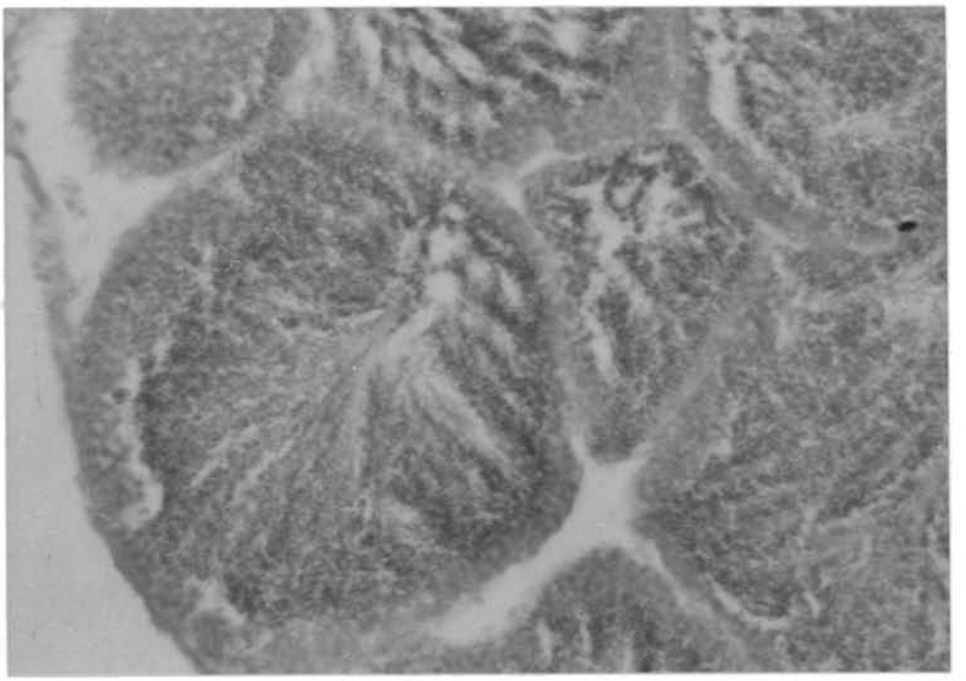


Figure 19. Section of ripe male gonad showing follicles densely packed with spermatozoa.

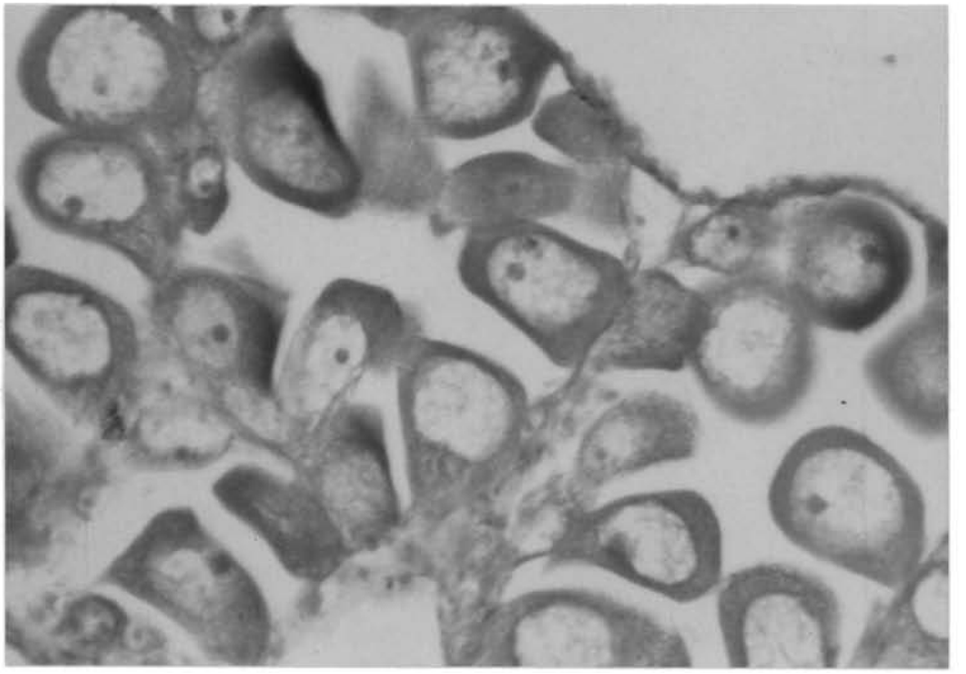


Figure 20. Section of female gonad with densely packed ova.

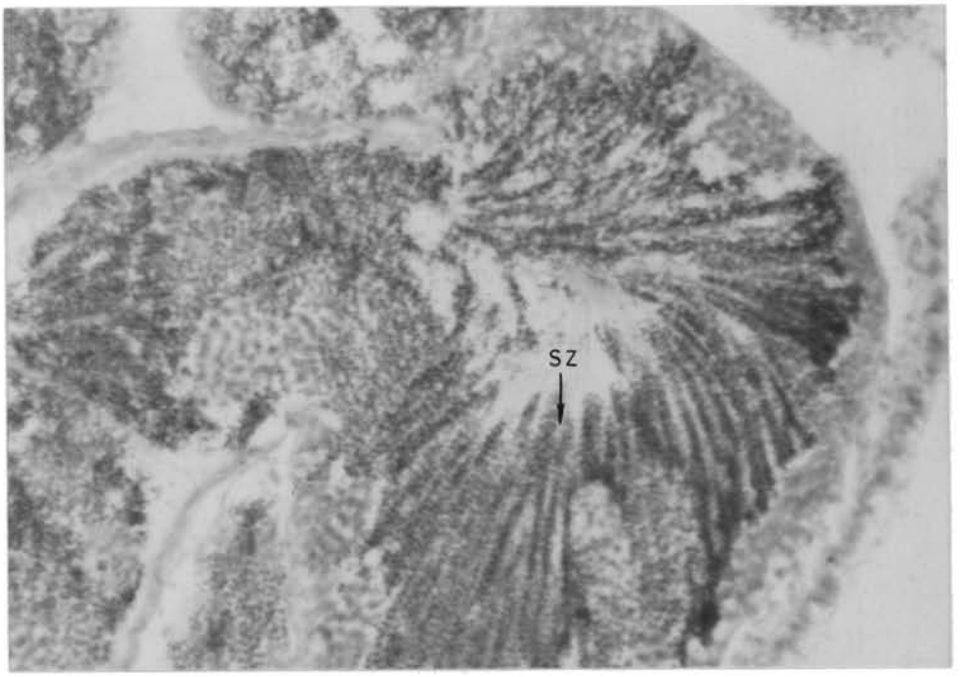


Figure 21. Ripe follicles at higher magnification showing densely packed spermatozoa (SZ).

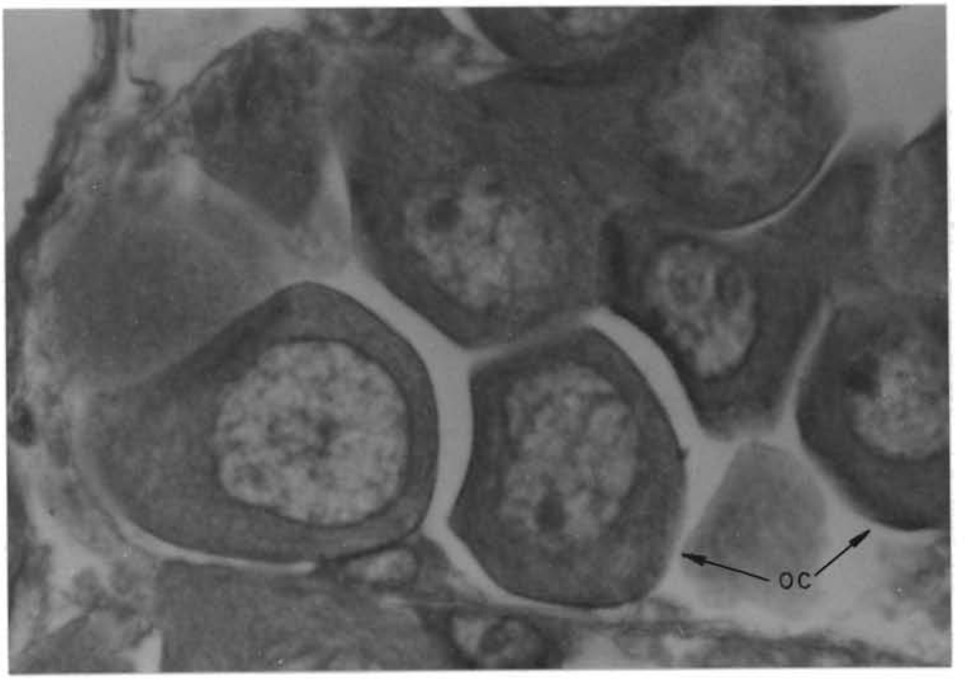


Figure 22. Female follicle at higher magnification showing the presence of oocytes (OC).

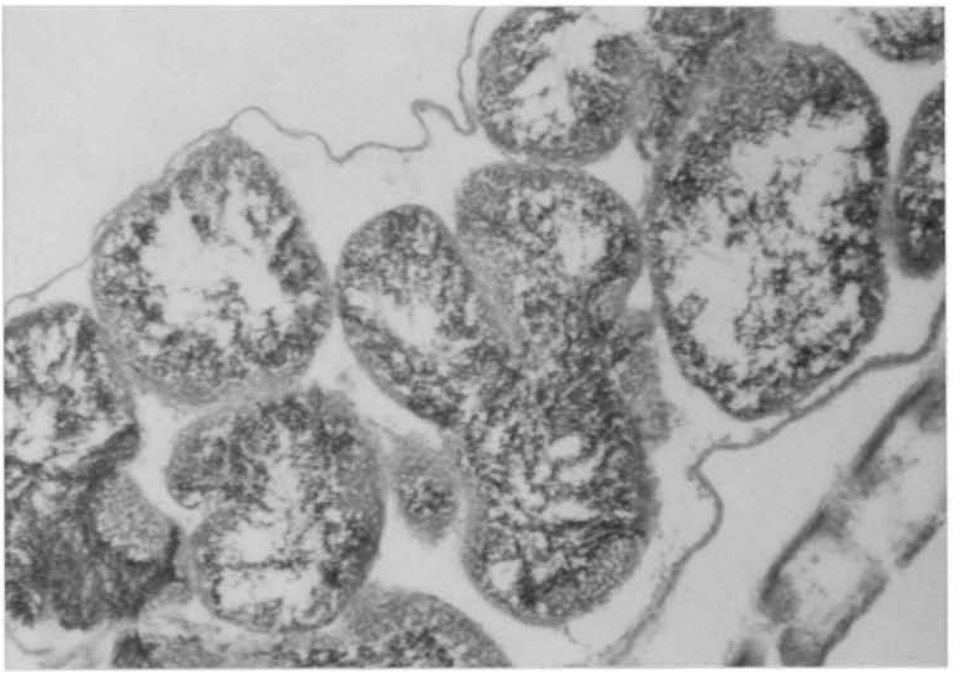


Figure 23. Section of partially spawned male gonad with moderate quantity of spermatozoa.

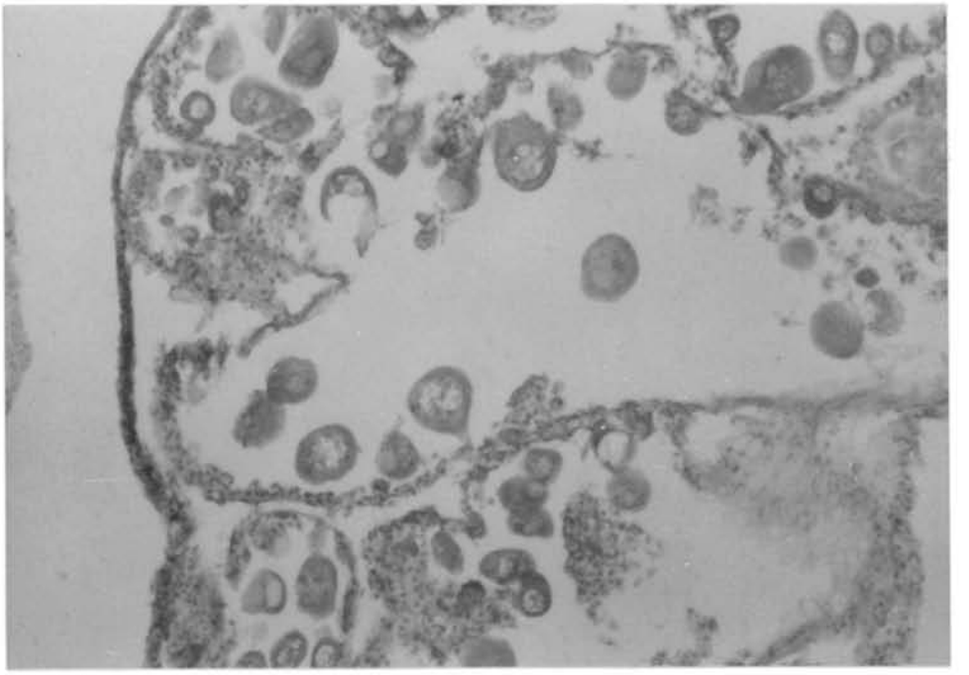


Figure 24. Partially spawned female gonad with free ripe oocytes.

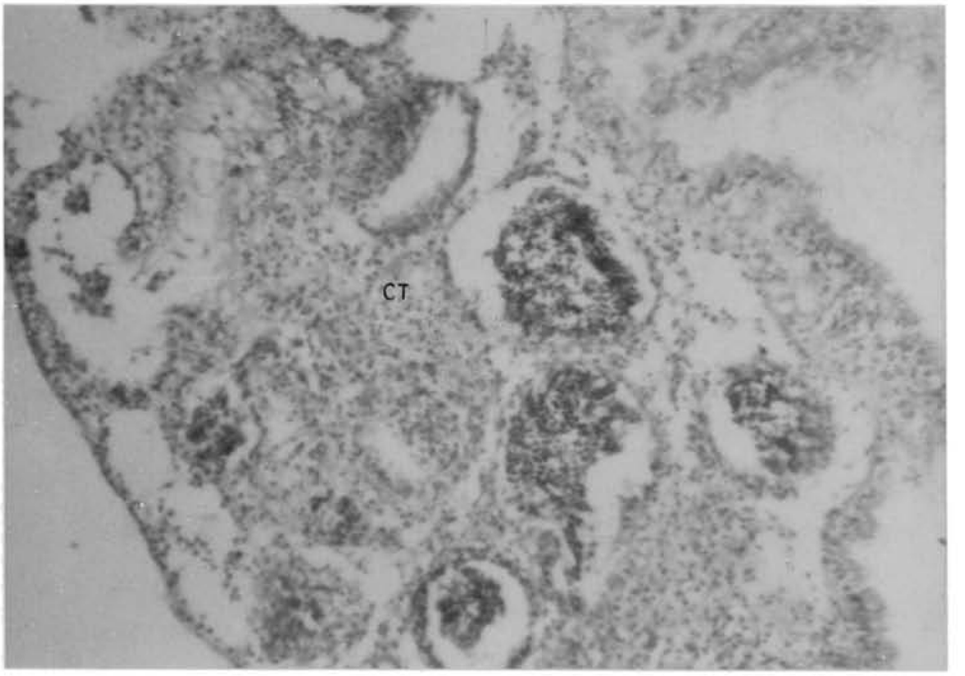


Figure 25. Late spawning stage of male gonad showing the presence of spermatozoa and infiltration of connective tissue (CT).

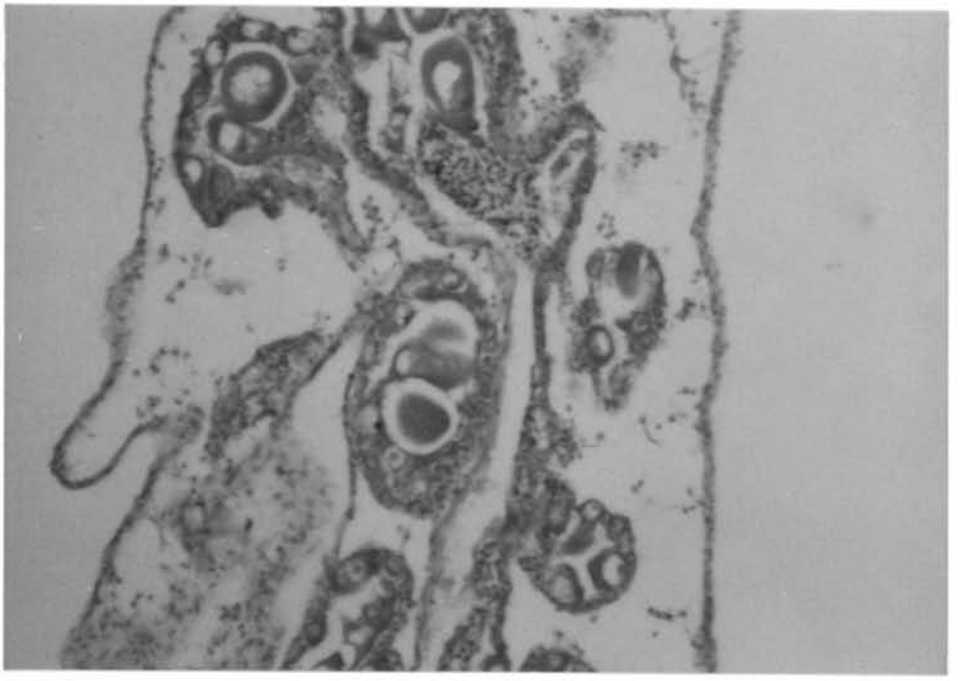


Figure 26. Late spawning stage of female gonad showing regressing oocytes with connective tissue in between.

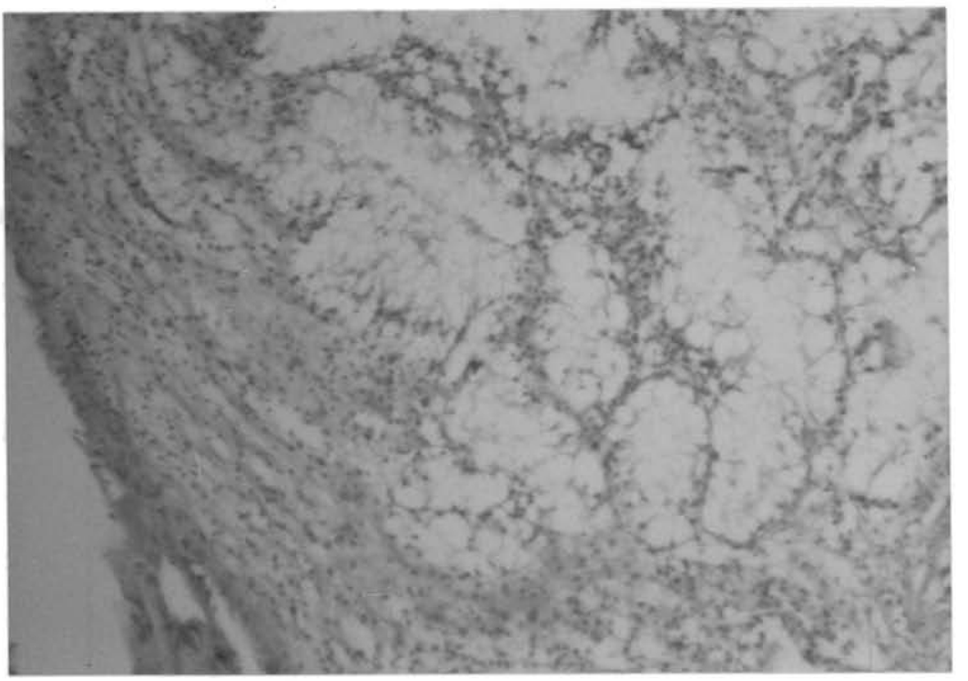


Figure 27. Section of spent gonad gorged with connective tissue.

Male: Spent Stage

In the spent condition (Fig.25) the lumen of the follicles contains residual spermatozoa which are probably cytolysed by phagocytes. Gonads of the spent phase are characterised by contracted follicles, residual spermatozoa in the process of being cytolysed and extremely slight gametogenic activity. Connective tissue of the mantle appears to occupy the space between the follicles. The mantle of the spent individuals become soft, spongy, membranous and semi-translucent.

Female: Developing or Active Stage

This process in most respects is very similar to that of the male especially in early development. Sex differentiation starts with the differentiation of the germ cells in the connective tissue. With the formation of follicles the gametogenic process is initiated. The developing stage is characterised by an increase in the number and size of oocytes

During early development, female follicles contain oogonia and primary oocytes which occur around follicle wall. Oogonia are the initial female germ cells proliferated from the large resting cells, the stem cells, found around the follicular wall. The primary oocytes developed by cell division are larger than the spermatocytes. As the development proceeds the oocytes increase in size, become elongated towards the centre of the lumen and retain an attachment with follicular wall by a slender stalk (Fig. 18) which has a measure of 14 μ. Nucleus of this oocytes migrates to the proximal end. Yolk materials get accumulated in the ooplasm and thus the stalked oocytes enter into a phase of vitellogenesis. Finally these cells detach from the follicular wall and lie free in the lumen. They become more regular in outline.

The oocyte has an average diameter of 34.5 μ with a large nucleus occupying more than half the cell volume. As yolk accumulates the cytoplasmic volume increases so that the cytoplasm stains more deeply than the nucleus. The nucleolus differentiates into an amphinucleus and it is very prominent (Fig.20).

Female: Ripe or Mature Stage

The mature gonad is characterised by the presence of large number of nearly round oocytes in the lumen of the follicles, but oogonia and oocytes are not infrequent (Fig.22). The oocyte has an average diameter of 42 μ. Ooplasm is rich in yolk materials. Mantle of the mature female is thick, flabby and yellow in colour.

Female: Spawning Stage

This stage is characterised by the reduction in density of ova and rounding off, as the pressure within the follicles is reduced. Active discharge of the ripe ova take place, and as it proceeds the central portion of the follicles remain vacant (Fig.24). The colour of the mantle gets reduced to a considerable extent owing to the release of the major part of the ova and finally becomes membranous and translucent.

Female: Spent Stage

Gonad of recently spawned individuals are characterised by shrinkage of the gonadal follicles and the spent females can be readily distinguished by the semi-translucent mantle. Spawning in females is not complete and a few ripe oocytes are always found in the spent gonad which are in different stages of phagocytosis (Fig.26). Presence of large number of phagocytes is observed in this stage for the resorption of the residual eggs by cytolysis.

Table 22. Relationship between size and mantle thickness in males and females of M. senhausia

Size group (mm)	Mantle thickness (in μ) Male	Female
6	99	128
8	114	142
10	128	129
12	132	129
14	192	176
16	259	185
18	192	186
20	300	228
22	392	382
24	399	653
26	534	317
28	290	334
30	-	233

From the results obtained by direct observation and histological studies of the gonad it is revealed that relative thickness of mantle can also be regarded as useful index for assessing the reproductive cycle. The average mantle thickness (expressed in μ) of male and female specimens of different size groups are given in Table 22. The male and female specimens showed difference in thickness. The normal range of mantle thickness of the male was from 99 μ to 534 μ and the females the range was found to be between 128 μ and 653 μ. The maximum average thickness was attained at a length of 24-26 mm and above this, thinning of the mantle was resulted. This might be because of the spawning activity of the organisms. Thickest mantle observed during the study was in female (844 μ) having a length of 24 mm. But in males the thickest mantle (685 μ) was present in mussels of size 26 mm. These two were in mature stage. It indicates the fact that an increase in the mantle thickness generally denoted the development, and a decrease, the spawning. Besides with the gonad development, thickness of the mantle was observed to vary with the size of the animal.

6.4.2 **Histochemistry**

Histochemical analysis revealed that the developing (immature oocytes) stage of the female gonad gave a positive result with Mercury Bromophenol Blue (MBB). The cytoplasm of the oocyte was comparatively strongly stained than the other components. The staining was slightly deeper in mature ova. In the spawning stage also tissue showed positive but a mild reaction. The mantle epithelium showed a reduction in the intensity of staining in mature condition. In male the spermatogonia, spermatocytes and spermatids showed

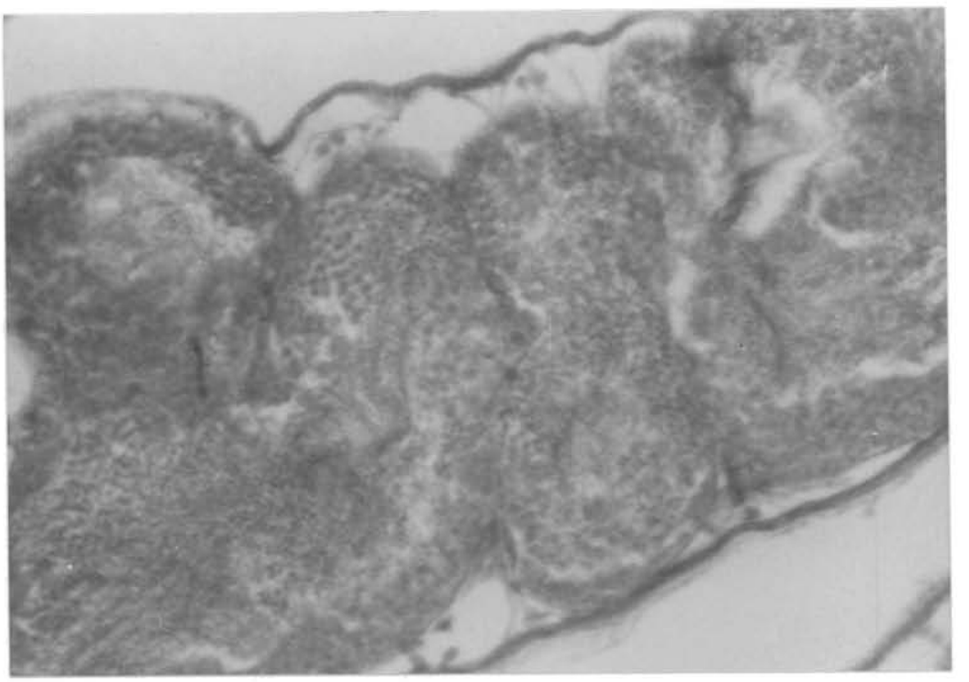


Figure 28. Pattern of staining of male gonad by Mercury bromophenol blue (MBB) for protein.

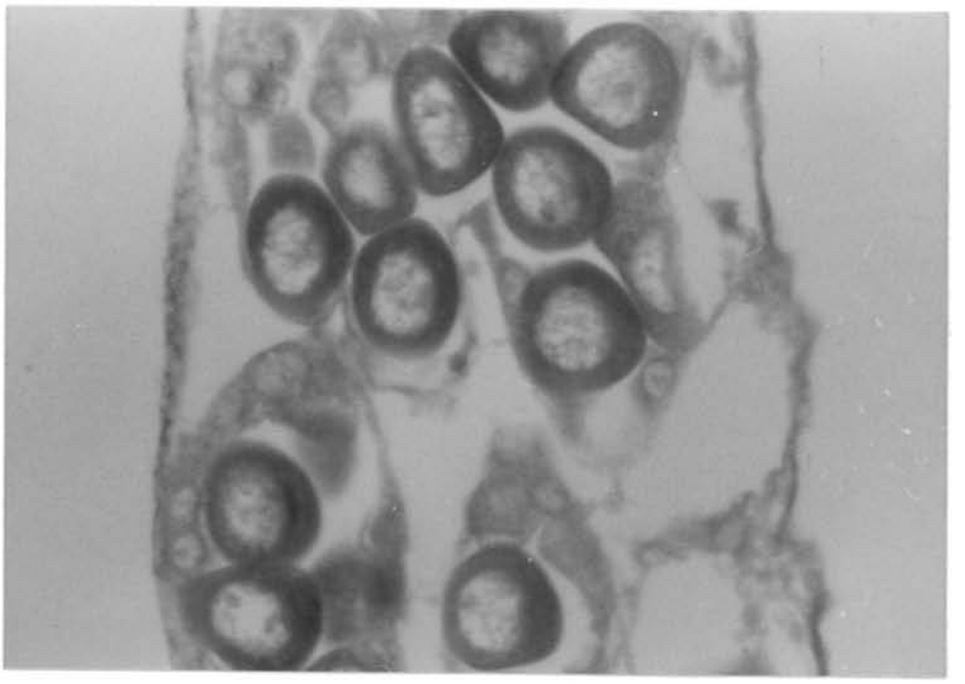


Figure 29. Pattern of staining of female gonad by MBB for protein.

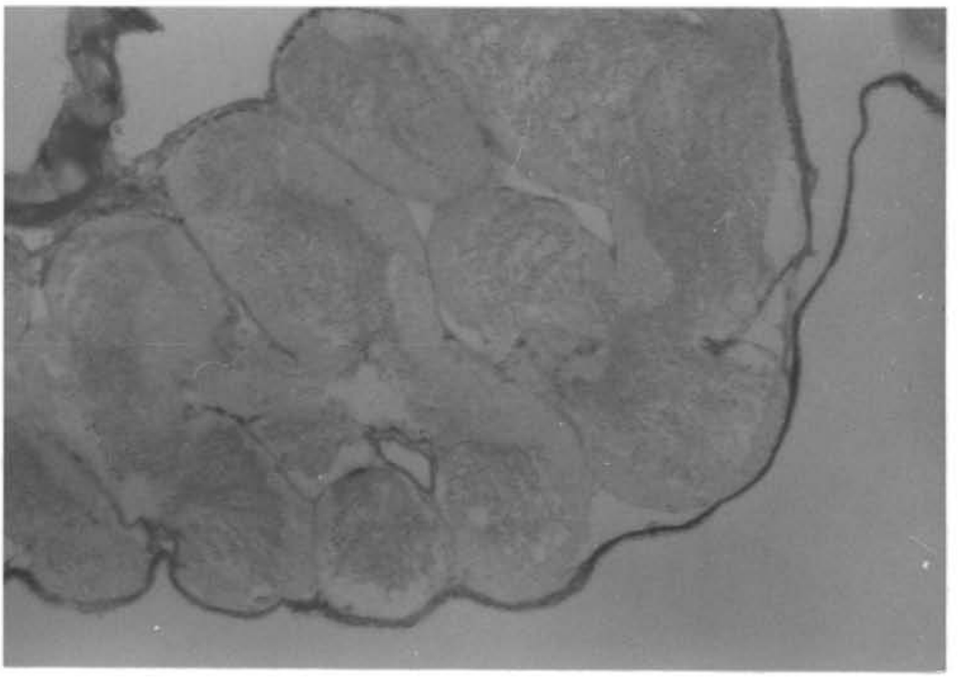


Figure 30. Pattern of staining of male gonad by Periodic acid - schiff's reagent (PAS) for glycogen.

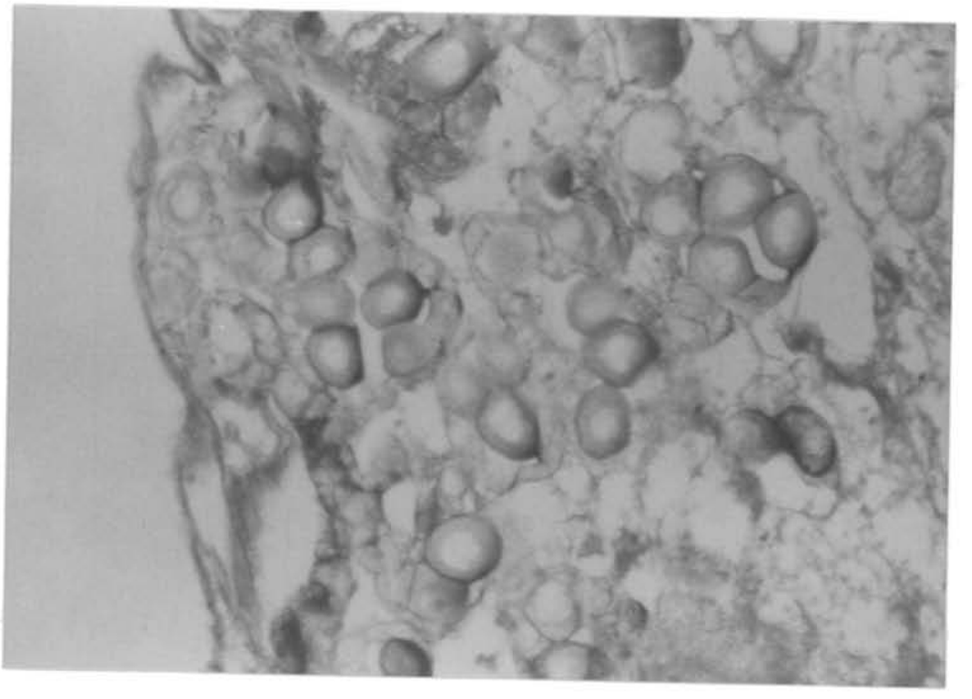


Figure 31. Pattern of staining of female gonad by PAS for glycogen.

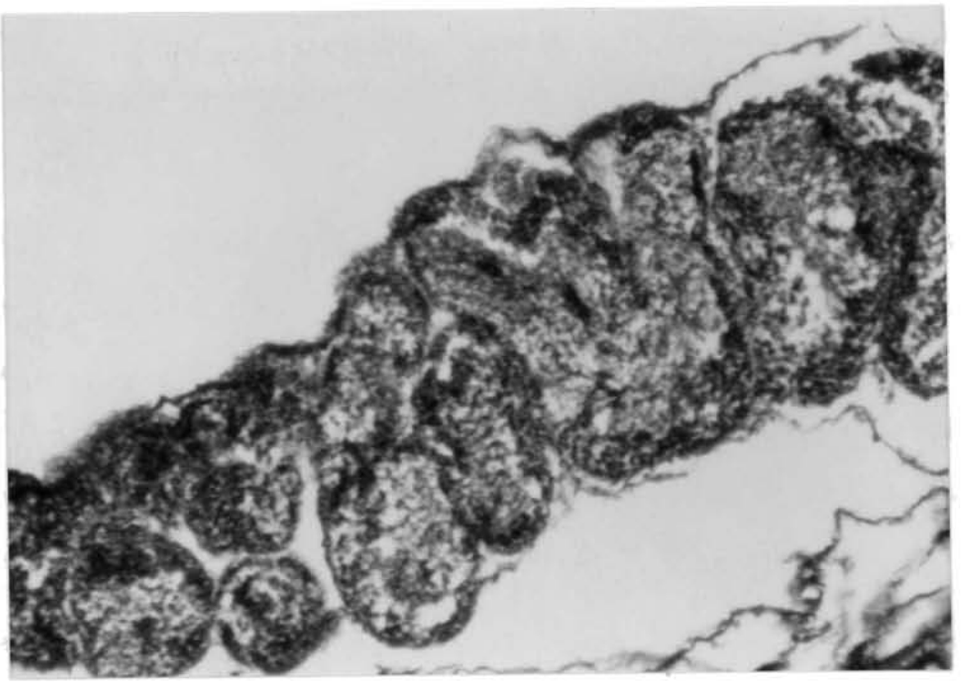


Figure 32. Pattern of staining of male gonad by Sudan black B (SBB) for lipid.

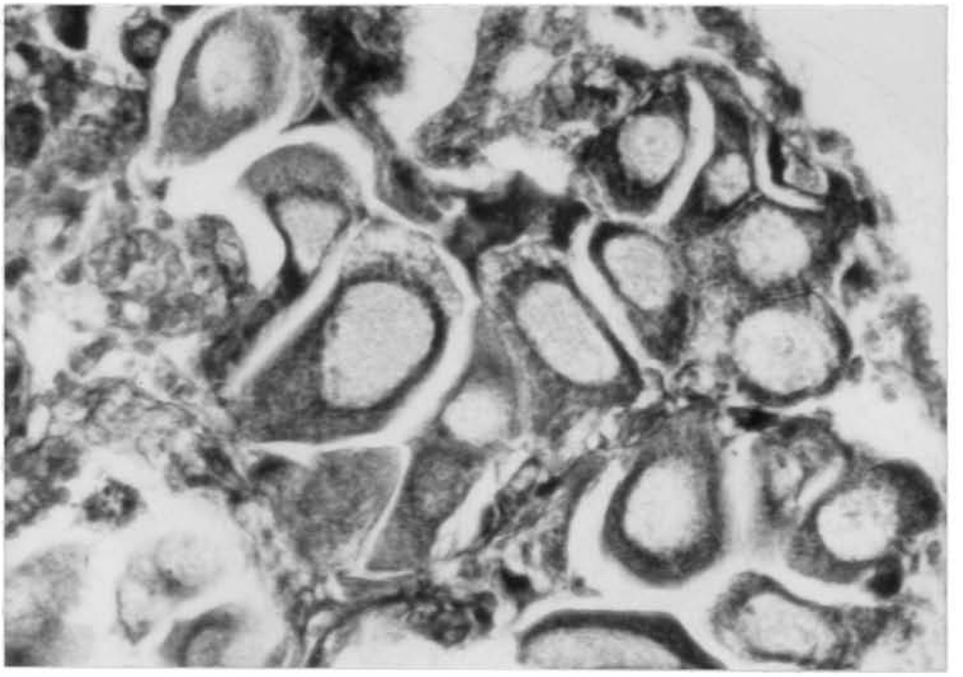


Figure 33. Pattern of staining of female gonad by SBB for lipid.

higher stainability. But in the spawning stage the tissue gave a positive but milder reaction. The mantle epithelium also showed a positive result with MBB.

In the developing stage oogonia and oocytes gave a mild reaction with Periodic Acid Schiff's reagent (PAS). In mature stage, colour intensity was found to be slightly increased. The stainability was slightly reduced in the spawning stage. The male gonad was mildly positive to PAS. The mantle epithelium was positive to PAS. The follicle wall showed a little less stainability.

Generally female gonad was positive to Sudan Black B, (SBB) indicating the presence of lipid in the oocytes. The developing and mature oocytes showed positivity to SBB. The cytoplasm of these inclusions were found to be filled with sudanophilic substances. In stalked oocytes, especially in the region of the stalk, comparatively high staining intensity was noticeable. In the spawning stage the staining intensity, however was found to be faded. In males the staining of the gonadal tissue with SBB showed differential reaction at different stages of maturity. In the developing stage more sudanophilic substances could be observed in the mantle epithelium, spermatogonia and spermatocytes. The mature stage showed a little less stainability than the other stages. The mantle epithelium less stained in this stage. In the spawning stage more sudanophilic substances were found in the follicular wall and mantle epithelium.

6.4.3 **Annual Reproductive Cycles**

In the annual reproductive cycle of M. senhausia studied for a period from February 1988 to February 1989. Some sort of similarity in the develop-

ment of the gonads in the two sexes were observed. The frequency of different stages of the gonad in different months is given in Table 23.

Male

In February 1988 the mussels were in active growth phase. Most of the mussels were in the developing (59.09%) and mature (36.36%) condition. In March and April most of the mussel were in mature and spawning phase. Animal with mature gonads were more (62.51%) in March. In April 21.43% of the male were in developing stage. Others were in mature (32.14%) and spawning (42.86%) phase of the gonad. In this month spawning was at its peak. It was indicated by the presence of few spent mussels (3.57%) with residual gametes. During this period shells of large size group were present in the collection, indicating the mortality of large specimens. In May developing mussels (50%) increased in number. Mature (25%), spawning (18.75%) and a few spent (6.25%) mussels were also present in the sample. In June, that is in low saline period due to southwest monsoon, the development and spawning took place in smaller sizes compared to that in other months. During this period 66.67% of the males were in the developing stage of the gonad, 23.81% in mature stage and 9.52% in spawning condition. In July also the same trend was maintained. But animals with mature gonad (51.85%) increased in number. Developing (37.04%) and spawning (11.11%) were also observed. After a break of three months, in November most of the mussels collected were in mature stage (41.18%). A few mussels with developing (35.29%) and spawning (23.53%) gonads could be seen in the population. During December 44.44% of the population were in developing, 27.78% in mature,

Table 23. Percentage distribution of the different stages of gonad development (1. Developing, 2. Mature, 3. Spawning and 4. Spent) in male and female of M. senhausia in different months.

Month	Gonadal stages of male				Gonadal stages of female			
	1	2	3	4	1	2	3	4
Feb.1988	59.09	36.36	4.55		50.00	40.00	10.00	
March	4.17	62.51	33.33		20.83	54.17	25.00	
April	21.43	32.14	42.86	3.57	11.54	38.46	42.31	7.69
May	50.00	25.00	18.75	6.25	41.67	20.83	20.83	16.67
June	66.67	23.81	9.52		76.47	23.53		
July	37.04	51.85	11.11		50.00	30.00	16.67	3.33
November	35.29	41.18	23.53		25.00	37.50	31.25	6.25
December	44.44	27.78	16.67	11.11	37.50	43.75	18.75	
Jan.1989	15.38	61.54	23.08		25.00	35.00	30.00	10.00
February	45.00	30.00	20.00	5.00	25.00	25.00	20.83	12.50

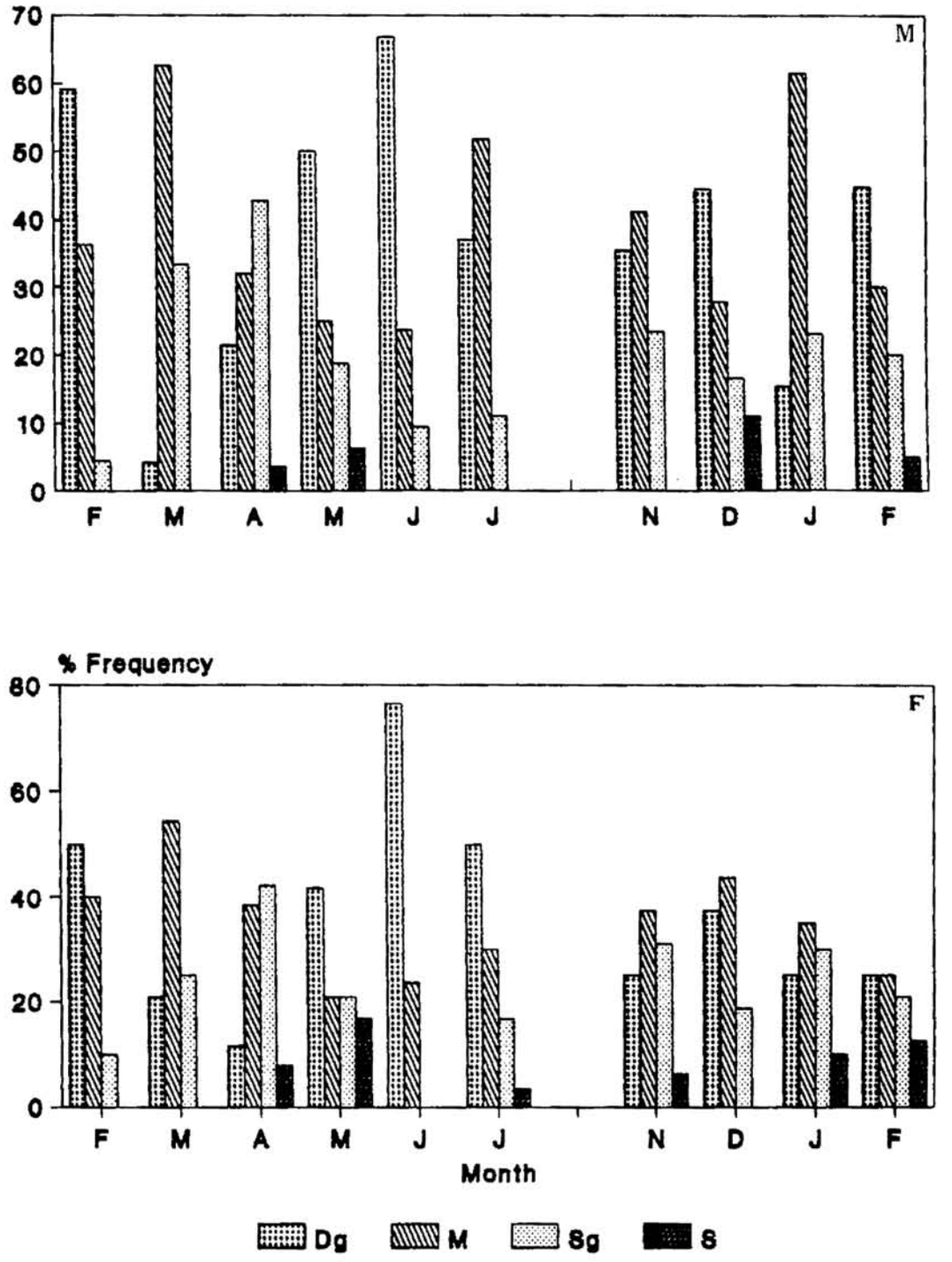


Figure 34. Percentage distribution of different stages of gonad development (Dg - developing, M - mature, Sg - spawning and S - spent) in M. senhausia (Male - M and Female - F)

16.67% in spawning and 11.11% in spent stage. In January 1989 most of the mussels were in mature (61.54%) stage, but a few were observed to be in developing (15.38%) and spawning (23.08%) condition. In February all the stages of development could be observed in the population. That is, 45% developing, 30% mature, 20% spawning and 5% spent mussels could be observed.

Female

In the case of females almost the same trend as in males could be observed. In February 1988 most of the females (50%) were in developing stage of the gonad. Individuals with mature (40%) and spawning (10%) gonad were also encountered in this month. During March about 54.17% of the mussels were in mature stage and a few with developing (20.83%) and early spawning (25%) condition. In April there was an increase in the number of spawning females (42.31%) in the population, while mature females registered only 38.46%. A few mussels in developing (11.54%) and spent (7.69%) stages were observed during this period. In May mussels of developing stage constituted 41.67%. Mussels with all the other stages of gonad development also could be seen in this month. The mortality of older specimens observed during these months resulted in the occurrence of the fewer number of fully spent mussels in the population. In June most of the mussels were in developing (76.47%) and only 23.53% of the mussels were in maturing condition. In July all the four stages were present in the population. After monsoon season, in November most of the mussels were with mature gonad (37.5%). Besides, 25% of the mussels showed developing, 31.25% spawning and 6.25% spent state of the gonad. In December mature (43.75%) mussels increased

Table 24. Distribution of male and female during the period of study

Month	Male	Female	Chi-square
February 1988	22	30	1.2308
March	24	24	0.0000
April	28	26	0.0741
May	32	24	1.1429
June	21	17	0.4211
July	27	30	0.1579
November	17	16	0.0303
December	18	16	0.1176
January 1989	13	20	1.4848
February	20	24	0.3636
Total	222	227	0.0557

in number and developing (37.5%) and spawning (18.75%) mussels also could be seen. During January 1989 along with the developing (25%), mature (35%) and spawning (30%) mussels, few mussels were found to be in spent (10%) stage. In February also mussels with all the four stages of gonad development were present in the population. Developing (25%) and mature (25%) mussels were more in number.

In the mussels of the size group 4-5 mm onwards, developing stages of the gonad were observed. The smallest animal with mature gonad was in the size of 13-14 mm. Spawning was observed to commence in the mussels of length 22-23 mm. There was no distinct difference between male and female at the commencement of different stages, however similar observation could not be found throughout the year. Especially during monsoon season the animal of 2-3 mm size group was found to start the gonad development and the maturity attained in a size group of 8-9 mm. This may be due to the fact that sexual maturity may be attained irrespective of size denoting that the size at which maturity occurred varied most probably according to changes in the environmental condition.

The sex ratio of M. senhausia observed during different months is presented in Table 24. Of the 449 mussels studied during one year period 222 (49.44%) were males and 227 (50.56%) were females. In general sex ratio in different months was more or less the same. The chi-square test of the monthly sex ratio were showed that the male: female ratio was not significantly different from the expected 1:1 ratio.

6.5 DISCUSSION

The mussel M. senhausia is dioecious and showed no trace of sex reversal and hermaphroditism. Bivalves are regarded as a group characterised by gonochorism and 96% of the species included in the class are of separate sexes (Coe, 1943).

M. senhausia showed different prominent gonadal stages like indifferent, developing, mature, spawning and spent. Here the classification is almost in agreement to that of the Seed (1976). He recognised four main stages of gonad development in Mytilus edulis : developing, ripe, spawning and spent. He divided developing and spawning stages into four substages based on minor changes in the development which is not followed in the present study.

In the indifferent and spent condition the mantle is found to be composed of connective tissues like adipogranular tissue and vesicular connective tissue (Leydig cells). According to Lubet et al. (1976) the vesicular cells store large amounts of glycogen and adipogranular cells contain lipid droplets and protein granules. These cells have the common ability to stretch or contract as occasion demands (Tranter, 1958). Several authors have pointed out about the possibility of various cell types that form the mantle tissue. Lubet (1959) described that the mantle of Mytilus galloprovincialis is composed of various blood cells, gametes, adipogranular cells (AG) and vesicular connective tissue (VCT). Lunetta (1969) reported the existence of both AG and VCT cells in Mytilus Spp., but in M. (=Perna) perna he observed only VCT cells which he referred to as interfollicular connective tissue. The occurrence of AG and VCT in the mantle connective tissue has been recorded in Mytilus

edulis (Bayne et al., 1982; Lowe et al., 1982), M. californianus (Kelley et al., 1982), Perna indica and P. viridis (Ajithakumar, 1984).

M. senhausia showed an extended breeding period with a major peak in April-May with intermittent spawning in other months. According to Giese (1959) breeding season of organisms in tropical seas is the most prolonged one. Studies on the marine and estuarine bivalves have revealed the presence of continuous and discontinuous breeding in bivalves of the Indian coasts. George and Nair (1973) observed protracted and asynchronous breeding period with three synchronous peaks in Musculista arcuatula. A prolonged breeding period with two major peaks have been observed in Katelysia opima (Nagabhushanam and Mane, 1975a), Paphia laterisulca (Nagabhushanam and Dhamne, 1977) and Mytilus edulis (King et al., 1989). An extended breeding period with a distinct period of intense activity was noticed in Perna indica (Kuriakose, 1973) and Donax cuneatus (Nagabhushanam and Talikhedkar, 1977a). Extended breeding period was reported in some bivalves like Meretrix meretrix, M. casta, Katelysia opima (Jayabal and Kalyani, 1986a) and Modiolus modiolus (Jasim and Brand, 1989). In Mytilus viridis (Nagabhushanam and Mane, 1975b) and Crassostrea madrasensis (Stephen, 1980a) only two spawning periods were observed in a year.

The bivalve gonad usually enters into a resting stage after spawning (Loosanoff, 1962). But in the present study no resting stage was observed. Throughout the year germ cells in different developmental stages could be seen in follicles of the gonad. Likewise no resting stage was observed in Pecten maximus (Mason, 1958), Musculista arcuatula (George and Nair, 1973),

Perna indica (Kuriakose, 1973), and Paphia laterisulca (Nagabhushanam and Dhamne, 1977). Stephen (1980a) observed a gametogenic cycle of nine months followed by an inactive phase for the rest of the period in Crassostrea madrasensis. In Donax cuneatus an inactive period of three months after spawning was reported by Victor and Subramoniam (1988).

The pattern of reproductive periodicity of organisms shows a relationship with the climatic conditions to which they are exposed. In the present investigation major spawning was observed to be in April-May indicating the fact that increase in salinity facilitated the rapid development of the gonad and spawning. Later sudden decrease in salinity of the ambient medium due to southwest monsoon and resulting freshwater inflow resulted in the mortality of major part of the population in this region. Later at the close of the monsoon, the presence of the mussels in this region helped to reach the conclusion that the population might get replenished by the larvae of the population that exist at the outer end of the barmouth near sea. During this period, with the gradual increase in salinity the mussel started its development. Spawning reached its peak with further increase in salinity.

Studies on reproductive cycle of bivalves reveal that the temperature influences the spawning in temperate waters (Wilson and Hodkin, 1967; Barber and Blake, 1983; MacDonald and Bourne, 1987). But in tropical waters, salinity, rather than temperature appears to play an important role on the breeding cycle. The factors influencing spawning may be quite different from those inducing annual reproductive cycle (Giese, 1959). Increase in salinity has been found to trigger spawning in many bivalves such as Paphia

laterisulca (Nagabhushanam and Dhamne, 1977), Donax cuneatus (Nagabhushanam and Talikhedkar, 1977a), Crassostrea madrasensis (Joseph and Madhyastha, 1984), and Donax cuneatus (Victor and Subramoniam, 1988). In Mytilus viridis (Nagabhushanam and Mane, 1975b), Crassostrea madrasensis (Stephen, 1980a), and Saccostrea cucullata (Sukumar and Joseph, 1988) peak spawning was observed with decline in salinity. But in some other bivalves like Meretrix meretrix, M. casta, and Katelysis opima spawning was found to occur in moderate salinity (Jayabal and Kalyani, 1986a).

The histochemical studies on gonad helps to locate changes of organic components like glycogen, protein and lipid occurring at different periods of the reproductive cycle. The present study revealed that the mantle of M. senhausia is glycolipo-protein in nature. The gonadal tissue showed slight increase in protein content in mature condition. This may be due to the build up of protein in the developing oocytes and spermatocytes. In the spent gonad, the staining property was diminished because of the depletion of protein due to the release of gametes.

Glycogen was found to accumulate in the cytoplasm of the oocytes. During later stages glycogen content showed a reduction. In Mytilus edulis Lowe et al.(1982) and Bayne et al.(1982) observed the transfer of yolk precursor substances from the connective tissue to the developing oocyte in the follicles. In male, only a lesser quantity of glycogen could be noticed inside the spermatozoa, but it occurred in more quantity in the mantle epithelium.

Histochemical observations on the lipid revealed that in males the accumulation of the lipid was little more than in females in different stages

of development. The mature follicles showed only moderate stainability. In the case of females, as the gametogenesis proceeded, the oocytes showed an increase in lipid content. The stalk of the stalked oocytes was found to be more stainable. This may lead to the conclusion that other than the lipid stored in the connective tissue, lipid is also contributed from some other tissue. The mature oocytes contained more sudanophilic granules. Lubet (1959) also observed the appearance of granules in mature Mytilus edulis. Ajithakumar (1984) got almost similar results in the distribution of glycogen, protein and lipid components during the period of gametogenesis of Perna indica and P. viridis.

From the different studies to elucidate the reproductive cycle and its changes in different condition revealed that the M. senhausia is gonochoristic and shows different prominent gonadal stages according to the development. It shows a protracted spawning period with a peak during April-May. The development and spawning is influenced by salinity of the ambient medium. Besides these different organic components like protein, glycogen and lipid also shows variation according to the development of the gonad.

C H A P T E R - VII

BIOCHEMICAL COMPOSITION

7.1 INTRODUCTION

In India coastal and estuarine areas sustain vast resources of molluscs. Bivalve molluscs, a potential source of valuable proteins, carbohydrates and minerals, are abundantly available in the country. Among them edible mussels are of great potential because of their high productivity. These have been exploited by the people of the coastal areas from time immemorial for food and also for their shells. An understanding of its nutritional aspects can lead to better utilisation of the resources. Seasonal cycles of the variation in the biochemical constituents like protein, carbohydrate and lipid are generally attributed to the complex interaction between environmental parameters, food availability, growth and reproductive activity (Bayne, 1976; Sastry, 1979; Gabbot, 1976, 1983). In general, the energy is stored prior to gametogenesis when food is abundant and is utilised in the production of gametes when metabolic demand is high (Bayne, 1976; Gabbot, 1975). The study of variation in energy storage in the form of protein, glycogen and lipid would help in understanding the ecology and overall economy of the species. A sound knowledge of variations in biochemical composition in different stages of growth is essential so that it enables the exploitation of bivalves when their nutritional value is greatest.

7.2 REVIEW

Studies on the biochemical composition of different species of bivalves have, because of their importance as food and their role in the economy, received the attention of scientific workers in several parts of the world.

Most of the works on biochemical composition of marine bivalves have been concerned with the gross changes in protein, lipid and carbohydrate content. Several workers have estimated the organic constituents in the whole tissue of many bivalves like Donax vittatus (Ansell, 1972), Donax incarnatus and D. spiculum (Ansell et al., 1973), Meretrix casta, Sanguinolaria diphos (Wafar, 1974), Abra alba (Ansell, 1974a), Chlamys septemradiata (Ansell, 1974b), Nucula sulcata (Ansell, 1974c), Ostrea edulis (Holland and Hannant, 1974), Astarte montagui (Ansell, 1975), Musculista arcuatula (George and Nair, 1975), Villorita cyprinoides var. cochinensis (Nair and Shynamma, 1975a), Mytilus viridis (Wafar et al., 1976 and Nagabhushanam and Mane, 1978), Donax cuneatus (Nagabhushanam and Talikhedkar, 1977b), Perna viridis (Shafee, 1978), Crassostrea gigas, Ostrea edulis (Mann, 1979), Villorita cyprinoides var. cochinensis, Meretrix casta (Lakshmanan and Nambisan, 1980), Donax trunculus (Ansell, et al., 1980), Villorita cyprinoides (Ansari et al., 1981), Tapes decussatus, and T. philippinarum (Beninger and Lucas, 1984), Mytilus galloprovincialis (Bressan and Marin, 1985), Meretrix meretrix (Jayabal and Kalyani, 1986b) etc.

Giese et al. (1967) suggested that the analysis of various body components for biochemical constituents would be more informative to elucidate the mobilisation of the tissue reserves to the gonad during gametogenesis. Nagabhushanam and Deshmukh (1974) estimated protein, fat, glycogen and water content of different body components such as adductor muscles, digestive glands, foot, gills, gonads, mantle, siphon and rest of the body and whole tissue of Meretrix meretrix. Ansell (1974b) analysed carbohydrate, nitrogen, lipid, carbon and water content of gonad, adductor muscle and mantle tissue of Chlamys septemradiata from the Clyde Sea area. Thompson (1977) has

separated gonad and somatic tissue of Placopecten magellanicus and estimated carbohydrate, lipid, protein and ash content. Salih (1977) observed variation in biochemical composition of different tissue components like gonad, adductor muscle and body entire of Meretrix casta. Pieters et al. (1978) investigated the changes in glycogen, protein and total lipid level of mantle and total tissue of Mytilus edulis and Zurberg et al. (1978) analysed gills, mantle, posterior adductor muscle, hemolymph and combined remaining tissues of this mussel for glycogen, lipid, protein and free amino acids. Taylor and Venn (1979) have given an account of the seasonal variation in weight and biochemical composition of the tissues like adductor muscle, gonad and other tissues of Chlamys opercularis from the Clyde Sea area. Seasonal changes of protein and nitrogen levels in the adductor muscle, gill and midgut gland of the clam Tapes philippinarum were reported by Adachi (1979). Stephen (1980b) analysed variation in water, protein, carbohydrate, lipid and ash content in the adductor muscle, mantle and gonad of Crassostrea madrasensis. Barber and Blake (1981) gave an account of the biochemical changes in the adductor muscle, mantle, digestive gland and gonad tissues of Argopecten irradians concentricus. Thangavelu and Sanjeevaraj (1988) estimated protein, lipid, carbohydrate and water content of the different body components like mantle, gill, adductor muscle, gonad and hepatopancreas of the oyster, Crassostrea madrasensis. Katticaran (1988) reported seasonal biochemical changes in foot, mantle, adductor muscle, gill, digestive gland and gonad of Sunetta scripta.

Among the above studies only in a few cases wet tissue were used for the estimation of different biochemical constituents. A few works carried

out with wet tissue are on Martesia fragilis (Srinivasan and Krishnaswamy, 1963), Crassostrea virginica (Galtsoff, 1964), Lamellidens marginalis (Ahmed et al., 1978), Tapes decussatus and T. philippinarum (Beninger and Lucas, 1984), Villorita cyprinoides var. cochinensis (Sathyanathan et al., 1988) etc.

The mussel M. senhausia occur in good quantity in some areas of Cochin waters and it is utilised as poultry feed and fertilizer. From the studies on maturity stages of this mussel it is revealed that most of the stages of the development could be obtained throughout the year. Three maturity stages recognised are developing, mature and spawning. Animals with gonads in these stages were analysed for different biochemical components like protein, lipid and glycogen. Thus the present study has been undertaken to elucidate the biochemical make up of different body components like mantle+ gonad, foot and whole tissue of the mussel M. senhausia with regard to sex and gonad condition.

7.3 MATERIALS AND METHODS

Collection were made at an interval of fifteen days for a period from April 1989 to April 1990. Mussels within the length range of 10-24 mm selected for the study were cleaned, and acclimated in filtered water of habitat salinity for two days. Individuals of two sexes were separated by the examination of gonad smears microscopically.

Wet weight was measured after washing the tissues with distilled water and removing the excess water with filter paper. In order to determine the dry weight, the weighed samples were dried at 60-80°C until a stable

weight was reached. From these the water content was calculated by the following formula:

$$\frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100 = \% \text{ wet weight}$$

For biochemical estimations several individuals of the same sex with similar gonad condition were pooled, as the weight of the component organs dissected from individual specimen was too small to meet the requirement of different estimations. Wet tissues were used for all the estimations. Total tissue and different tissue components such as mantle+gonad and foot were then used for the analysis of glycogen, protein and lipid.

For the estimation of glycogen the method followed was that of Montgomery (1957). A quantity of 0.2 ml of the tissue extract was added to 1.0 ml of 10% trichloroacetic acid. This mixture was centrifuged for 10 minutes at 2500 rpm. To 1 ml of the supernatant added 2 ml of 95% ethyl alcohol. This mixture was kept undisturbed for about 12 hours in a refrigerator, and then centrifuged at 2500 rpm for 15 minutes. The supernatant was decanted and 0.1 ml of 80% phenol and 2 ml of distilled water were added to the pellet. To this 5 ml sulphuric acid was added and mixed well. After 30 minutes optical density of the colour developed was measured at 490 nm. Calibration curve was prepared using glucose as standard.

Lowry's method (Lowry et al., 1951) was used for the determination of total protein. To 1 ml of 10% trichloroacetic acid added 0.2 ml of the tissue extract and centrifuged at 2500 rpm for 15 minutes. The precipitate was dissolved in 1 ml of 0.1 N sodium hydroxide; 5 ml of alkaline copper

reagent was added to it and mixed well. After 10 minutes, 0.5 ml of Folin's phenol reagent was added and shaken well. After 30 minutes the intensity of the blue colour developed was measured spectrophotometrically at 500 nm. Here bovine serum albumin was used to prepare the standard.

Lipid levels in the tissue was determined by the method of Barnes and Blackstock (1973). Tissue samples were extracted with chloroform methanol mixture. To 1 ml of this extract, 1 ml of methanol, 2 ml of chloroform, 2 ml of chloroform-methanol mixture and 0.2 volume of 0.9% sodium chloride were added one by one, mixed well and allowed to stand for a few hours. The lower phase was separated and transferred into a clean test tube and made up the volume to the original quantity of chloroform added before; 0.5 ml of the extract was taken and dried in a vacuum desiccator over silica gel; 0.5 ml of concentrated sulphuric acid was added to this, mixed well. The test tube was plugged with non-absorbent cotton and placed in boiling water bath for 10 minutes. After cooling, 0.2 ml of the acid digest was taken, added 5 ml of phosphovanillin reagent, mixed well and allowed to stand for half an hour. After this, optical density of the red colour developed was measured at 520 nm. Cholesterol was used for the preparation of the standard curve.

In all cases the values are expressed as μg/mg wet weight. The experiments repeated with several samples.

7.4 RESULTS

The biochemical composition of the total tissue, mantle and foot were analysed on the basis of gametogenesis. The data thus obtained for the three

stages, that is, developing, mature and spawning are presented in Tables 25-27 and in Figures 32-35.

Total tissue

In the total tissue the water content (expressed in percentage wet weight) in the spawning stage was highest (89.25% WW) (Table 25) when compared to female tissue. In maturing condition it was little less (86.03%) than that of the developing stage (87.40%). The water level again increased in the spawning stage. In general there is no appreciable variation in water content in different stages. The average protein level (expressed in µg/mg wet weight) obtained showed high value (56.61 µg/mg) in developing stages. From this level it decreased to 40.18 µg/mg in mature condition, and 34.22 µg/mg in spawning stage. The lowest protein level was observed in spawning stage. Glycogen and lipid also showed the same trend. These two components showed a high value in developing stage (glycogen : 25.71 µg/mg and lipid : 26.60 µg/mg) and low value (glycogen : 12.93 µg/mg and lipid : 18.22 µg/mg) in spawning stage. In both the stages the lipid component was little more than the glycogen content.

Like the male tissue, in female also the water level decreased as the development proceeded. Here the highest level of water content (88.90%) could be noticed in developing stage (Table 25) and the lowest level was found in mature condition (86.23%). But in the spawning stage it was again found to be increasing. Other components like protein, glycogen and lipid showed a decreasing trend as the development proceeded. Glycogen level showed a slight increase (27.14 µg/mg) in females.

Table 25. Details: water content (% wet weight), protein, glycogen and lipid (µg/mg wet weight) components of total tissue in M. senhausia during three phases of gametogenesis DG = Developing, M = Mature, SG = Spawning

Sex	Gonadal condition	Water content	Protein M±SD	Glycogen M±SD	Lipid M±SD
Male	DG	87.40	56.61±5.09	25.71±8.22	26.60±5.71
Female	DG	88.90	56.85±3.80	27.14±9.47	23.22±5.65
Male	M	86.03	40.18±10.57	15.08±5.90	18.99±4.27
Female	M	86.23	37.46±12.78	18.65±11.89	18.95±5.61
Male	SG	89.25	34.22±10.75	12.93±4.62	18.22±4.78
Female	SG	87.38	34.18±17.09	15.94±5.83	14.32±4.32

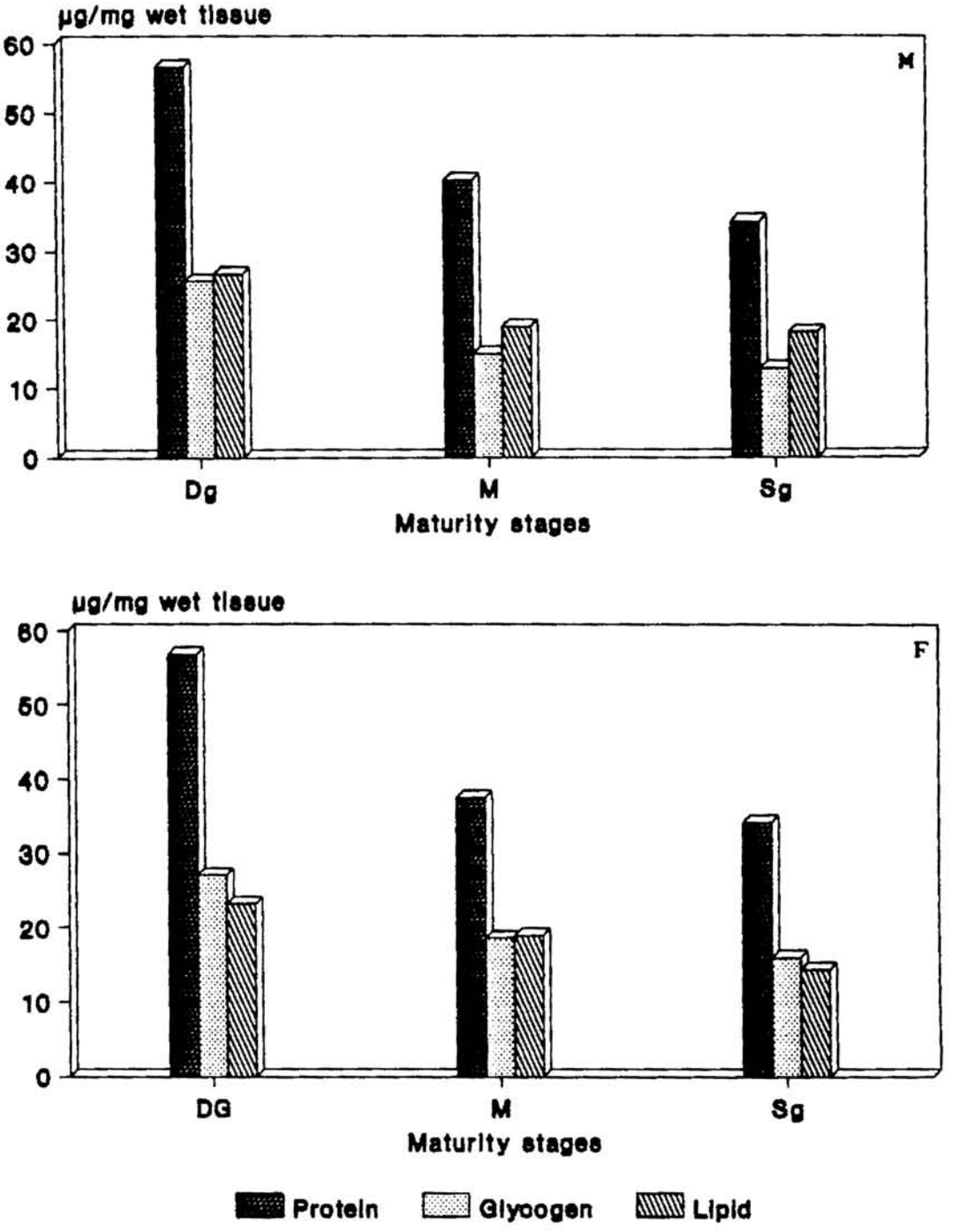


Figure 35. Variation in protein, glycogen and lipid components in the total tissue (M - male and F - female) of M. senhausia during different developmental stages (dg - developing, M - mature and Sg - spawning).

Table 26. Details: water content (% wet weight), protein, glycogen and lipid (µg/mg wet weight) components of mantle+gonad tissue in M. senhausia during three phases of gametogenesis DG = Developing, M = Mature, SG = Spawning

Sex	Gonadal condition	Water content	Protein	Glycogen	Lipid
Male	DG	94.10	56.21±9.83	22.95±10.18	24.67±9.13
Female	DG	89.70	58.42±12.45	23.39±13.17	26.13±6.11
Male	M	82.94	47.52±13.94	17.63±6.66	23.03±6.77
Female	M	83.13	45.67±17.88	15.29±9.74	25.71±5.69
Male	SG	81.30	41.42±12.02	17.31±6.90	17.99±4.18
Female	SG	79.85	41.84±13.95	13.56±4.09	19.33±4.47

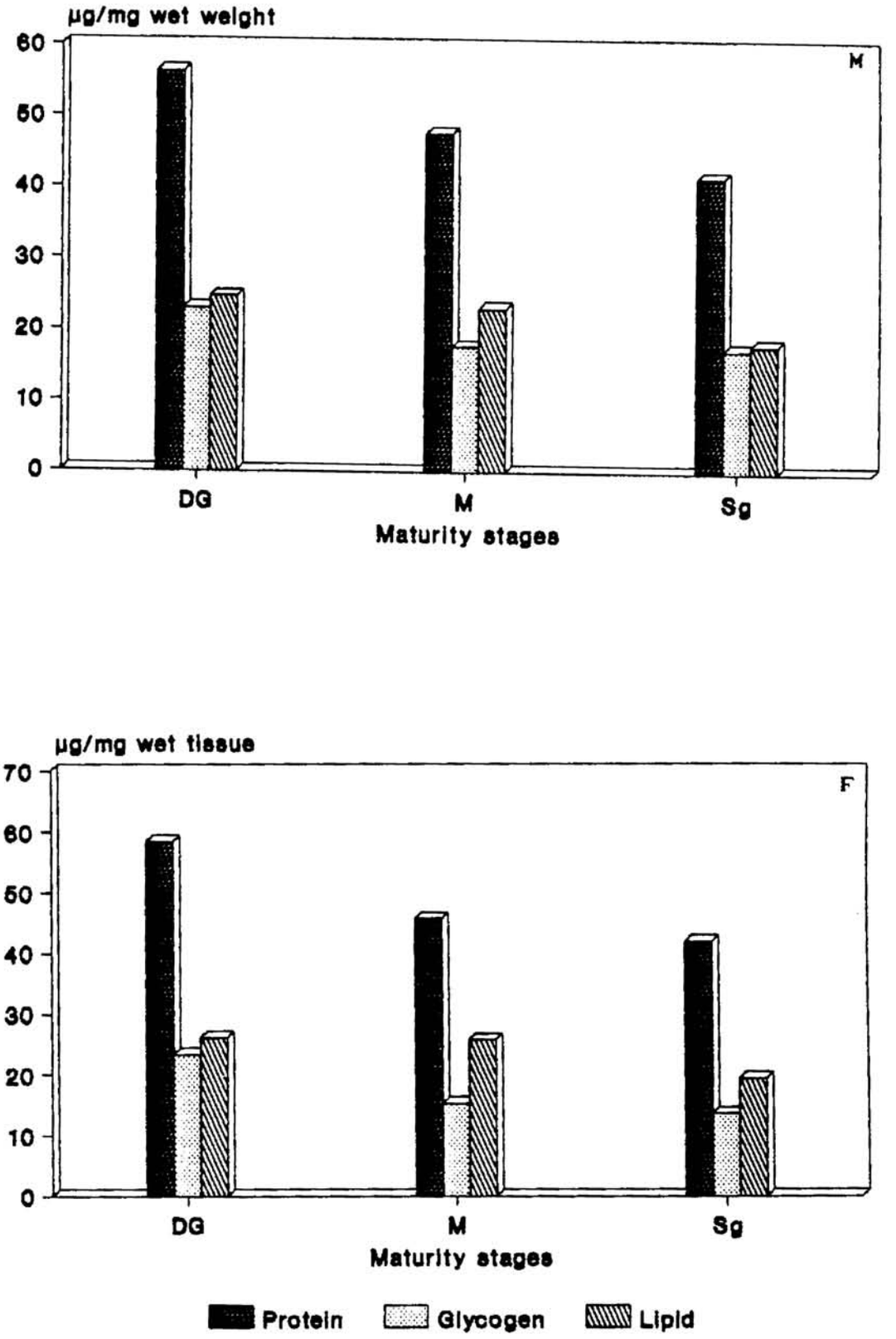


Figure 36. Variation in protein, glycogen and lipid components in mantle + gonad of M. senhausia (M - male and F - female) during different developmental stages (Dg - developing, M - mature and Sg - spawning).

Mantle+Gonad

In the developing stage in male, mantle+gonad maintained a slightly higher water level (94.1%) than in the other body components (Table 26). As gonad maturation proceeded water level showed a gradual decrease. Comparatively higher water decline was observed in mature condition (82.94%). Protein, glycogen and lipid showed a decrease towards the spawning stage. Much higher decrease in protein and glycogen content could be observed in mature condition (Protein from 56.21 to 47.52 µg/mg and glycogen from 22.95 to 17.63 µg/mg). Only slight decrease could be noticed in the case of lipid in mature condition (from 24.67 to 23.03 µg/mg). But in later stage the decrease of lipid was much higher (17.99 µg/mg) when compared to the previous stage. In the case of protein and glycogen also the lowest level could be noticed in spawning condition (protein: 41.42 and glycogen: 17.31 µg/mg).

Protein, glycogen and lipid showed slightly higher value in the tissue of females in developing stage (58.42, 23.39 and 26.13 µg/mg), but the water content was lower (89.7%). All these components showed a decreasing trend as gonad maturation proceeded. Water, protein and glycogen contents decreased to 83.13%, 45.67 µg/mg and 15.29 µg/mg respectively in mature stage, the decrease in lipid level was only slight (25.71 µg/mg). In the spawning stage all these components showed further decrease, which in the case of lipid was little more (19.33 µg/mg) than in others. Water, protein and glycogen levels obtained in this stage were 79.85% WW, 41.84 µg/mg and 13.56 µg/mg respectively.

Table 27. Details: water content (% wet weight), protein, glycogen and lipid (µg/mg wet weight) components in foot tissue of M. senhausia during three phases of gametogenesis

DG = Developing, M = Mature, SG = Spawning

Gonadal condition	Water content	Protein	Glycogen	Lipid
DG	80.80	88.08±24.00	27.74±14.78	27.99±8.35
M	73.08	77.99±25.12	24.14±10.45	25.54±4.29
SG	74.00	70.84±20.13	14.43±3.74	19.75±4.77

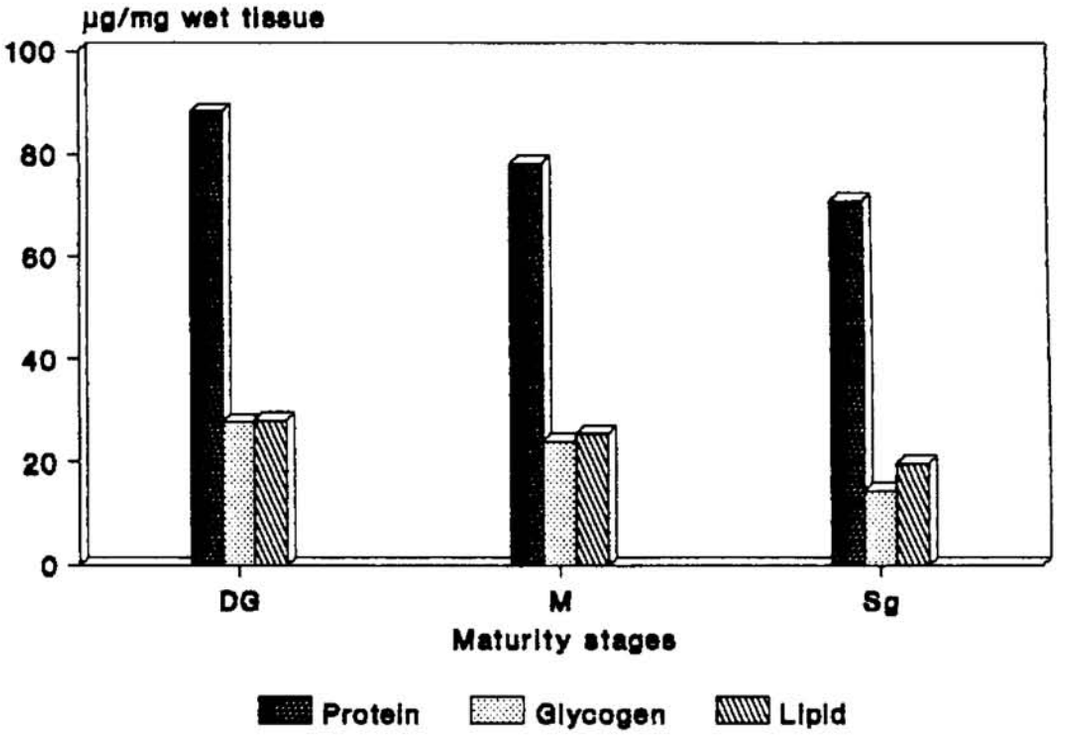


Figure 37. Variation in protein, glycogen and lipid components in foot tissue of M. senhausia during different developmental stages (Dg - developing, M - mature and Sg - spawning).

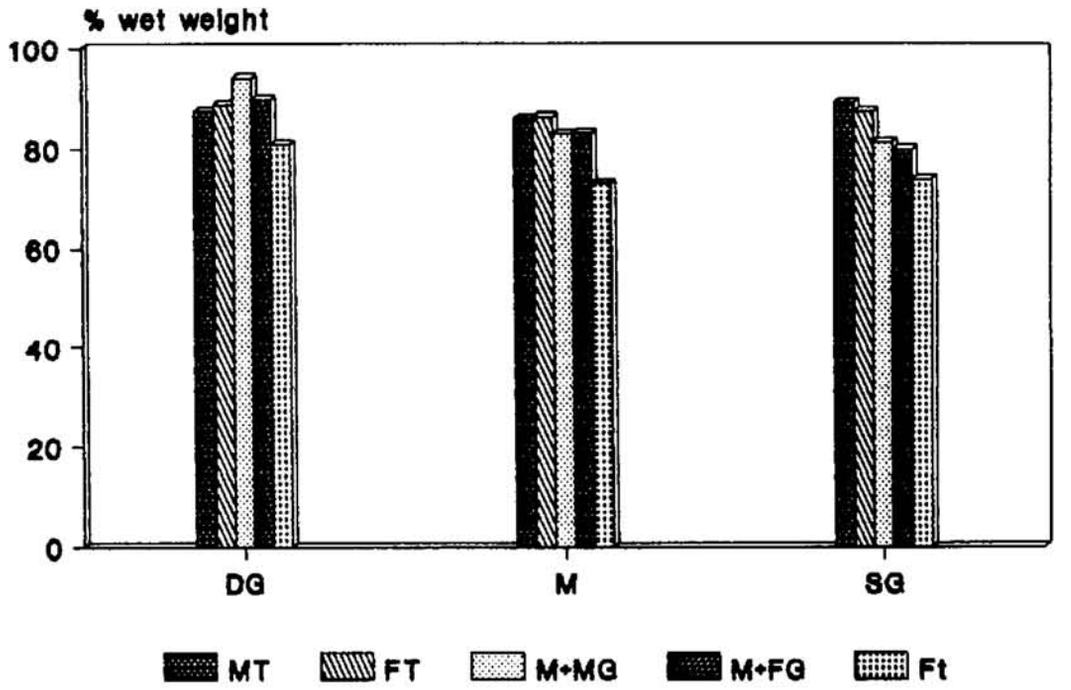


Figure 38. Variation in water content in different tissues like total tissue (male (MT) and female (FT)), Mantle + gonad (male (MG) and female (FG)) and foot (Ft) during different development stages (Dg - developing, M - mature and Sg - spawning).

Foot

Owing to the paucity of enough quantity of foot tissue from each sex, the foot tissue from several mussels were pooled irrespective of sex for this study. In the foot tissue the water content was low when compared to the other tissues (Table 27). It showed variation according to the development of the gonad. High value of water content (80.80%) was observed in the developing stage and low value (73.08%) in mature stage, followed by a slight increase (74%) in the spawning stage. Among the different tissue components, comparatively high protein value (88.08 µg/mg) could be seen in the foot tissue. Protein level showed a decrease with the development of the gonad. Glycogen and lipid also showed similar trend. In the case of glycogen and lipid only slight decrease could be observed in the first two stages (glycogen from 27.74 to 24.14 µg/mg and lipid from 27.99 to 25.54 µg/mg), but in later stages the decrease (14.43 µg/mg and 19.75 µg/mg respectively) in these components were little more.

7.5 DISCUSSION

Variations in the biochemical composition are influenced by different factors like hydrographic conditions, availability of food, growth and reproduction. Knowledge of the reproductive cycle is essential for interpretation of variations in biochemical composition of the tissues (Taylor and Venn, 1979). Observations on M. senhausia revealed that the main period of gonad proliferation and maturation occurred between December and March. Intense spawning activity was observed during March-April with intermittent spawning throughout the year. So the population apparently consists of individuals

with ripe gametes throughout the year. Energy reserves like protein, lipid and glycogen showed marked quantitative changes during different developmental stages. The variation in water content and biochemical composition are associated with the reproductive activity in relation to storage and utilisation of food reserves during growth and reproduction.

The water content in the gonad tissue was higher when compared to the other tissues, and it showed gradual decrease with development. Ansell (1974a) stated that the water content of the bivalve tissue may give an indication of the time of spawning. In the case of total tissue and foot the water level which is comparatively low in the latter showed a decrease in mature stage, and then an increase in spawning condition. High water content was observed during spawning in Villorita cyprinoides (Ansari et al., 1981) and Villorita cyprinoides var. cochinensis (Nair and Shynamma, 1975a). Ansell (1974b) observed lowest water level in the ripe gonad of Chlamys septemradiata, and a considerable increase during spawning period. Salih (1977) observed a low water level in the developing stage in Meretrix casta.

In the present study it is revealed that protein was the major fraction that shows maximum difference in various stages of development. Here it presents a decreasing trend as maturation proceeds. Pieters et al. (1978) observed a decrease of protein in the ripening stage of Mytilus edulis. But Giese et al. (1967) pointed out that in Tivela stultorum tissue protein level remained constant throughout the year. Nagabhushanam and Deshmukh (1974) observed an increase in protein content of Meretrix meretrix in mature condition. Ansari et al. (1981) suggested that the level of protein during

gametogenesis in Villorita cyprinoides is decreased during breeding season and it again rises to a second peak before the second spawning. In Sunetta scripta Katticaran (1988) observed a peak protein value at the commencement of spawning and a gradual depletion during spawning. In M. senhausia depletion of protein value in foot tissue during maturation can be explained as a consequence of mobilisation of protein reserves for utilisation during gametogenesis. Mobilisation of protein from different tissues has been reported in Tapes philippinarum (Adachi, 1979) and Mytilus edulis (Gabbot and Bayne, 1973).

The carbohydrates in bivalves comprised mainly of glycogen (Gabbot and Bayne, 1973) and large amounts are stored in adductor muscle (Taylor and Venn, 1979), mantle (de Zwaan and Zandee, 1972), gonad (Sastry, 1979; Gabbot, 1983) and digestive gland (Thompson, 1977; Barber and Blake, 1981). In the present study the glycogen content was slightly higher in the females. It may be attributed to higher biochemical budget required for oogenesis. The glycogen level showed decreasing trend with the advancement of gametogenesis. This may be due to a massive conversion of glycogen for the development of gametes. This is in agreement with Galtsoff (1964) who observed a high carbohydrate level in immature oyster, Crassostrea virginica and it declined steeply as gametes were formed in the gravid animals. Giese et al. (1967) showed that gonads of Tivela stultorum had the least carbohydrate storage when mature gametes were present. Nagabhushanam and Deshmukh (1974) also noticed a high level of glycogen during the period of gonad development and a fall when the gonads were in mature condition. Similar results were again observed in Mytilus edulis (Pieters et al., 1978) and Crassostrea madrasensis (Stephen, 1980b). Katticaran (1988) observed that total carbohydrate

level in the maturing gonad in Sunetta scripta showed a decreasing trend with the advancement of gametogenesis with the lowest level at the early spawning. But salih (1977) observed an increase in glycogen during spawning period in Meretrix casta. He suggested that the rise may be only an indication of normal metabolic activity consequent on the draining of excess water from the tissues and on the rise in salinity of the surrounding water. In Donax incarnatus Nagabhushanam and Talikhedkar (1977b) noticed highest glycogen content during mid season of gonad ripening. The difference in the storage and utilisation of glycogen reserves reflects the complex interaction between growth and reproductive cycle (Taylor and Venn, 1979).

In the present study lipid component was slightly greater than glycogen content. But its utilization was less during the period of maturation, especially in the mantle+gonad tissue, but later at the commencement of spawning the decline in the lipid content was more when compared to the earlier stage. Salih (1977) reported a fall in lipid content with spawning in Meretrix casta. Pieters et al. (1978) noticed a rapid decrease of lipid during ripening in Mytilus edulis. In Meretrix meretrix Nagabhushanam and Deshmukh (1974) observed low values of lipid due to discharge of gametes during spawning, but a higher value was reported when the gonads were fully ripe. In fully mature stage a peak lipid value was observed in Donax cuneatus (Nagabhushanam and Talikhedkar, 1977b) and Villorita cyprinoides var. cochinensis (Lakshmanan and Nambisan, 1980). But Ansari et al. (1981) found no variation in the lipid content in Villorita cyprinoides.

Thus the amount of protein, lipid and glycogen showed variation in the present study. These three components showed a decrease with the

advancement of gametogenesis in all the tissue components studied. In the case of gonad, utilisation of glycogen was more in mature stage than that of lipid. This may be due to the fact that besides using as an energy source, glycogen reserves are converted into lipid during gametogenesis which are stored in the ripening gametes as reserves to be used subsequently in early embryonic development. During later stages, that is in spawning condition, the depletion of lipid is more with the release of mature gametes. According to Giese et al. (1967) the gonads of the clam accumulates their nutritional reserves for gametogenesis independently of other body components. In the present study the utilization of the energy reserves for the gametogenesis is evident from the observed decrease in protein, lipid and glycogen contents. The water content also showed a decreasing trend in gonad. But an inverse relationship between organic constituents and water level in total tissue and foot is observed during spawning period. This increase may be due to the release of gametes and depletion of other stored organic substances from different tissues of the body. Thus it can be concluded that the water, protein, glycogen and lipid levels vary according to different developmental stages.

C H A P T E R - VIII

SUMMARY

Musculista senhausia (Benson) is found in abundance in backwaters especially in shallow areas. It lives on muddy bottom and builds nests made of mud and byssus threads. The thesis is divided into eight chapters comprising of introduction, taxonomy, study area and its environs, age and growth studies, salinity tolerance and filtration rate, reproductive biology, biochemical composition and summary.

The first chapter, the general introduction, covers the significance of the present study. Because of the close resemblance of this species to Modiolus its systematic position are explained in the second chapter. The genus Musculista belongs to the bivalve family Mytilidae of the phylum Mollusca

The study area is subjected to wide variation in salinity due to southwest and northwest monsoons. The salinity fluctuations affected the distribution of M. senhausia. Substratum is composed of sand, silt and clay sediment. During monsoon, sediment was of silty sand type. The distribution of the mussel is also affected by sediment texture. The most suitable substratum for the mussel bed is found to be the sand silt clay sediment. These form the subject matter of the third chapter.

In the fourth chapter the growth rate of M. senhausia was explained on the basis of the examination of random samples collected at regular intervals for a period from February 1987 to June 1988. From the population structure it is observed that during the period from February to July the mussel showed a growth of 9 mm within three months. But during second

part consisting of seven months from December to June it showed only 5 mm growth. But this observation is actually due to rapid growth, spawning and post spawning mortality of older specimens which was evident in each month's collection. Here the growth of the specimen is masked by the production of more younger ones. But from the modal progression analysis it is revealed that growth rate showed variability, being faster during first five months. After this, growth was poor. During the two periods of study almost the same kind of growth rate could be noticed. Growth rate was high during the first few months after the settlement.

Growth parameters of von Bertallanffy growth equation determined have also shown similar result. From the K values calculated it is revealed that first and second stock showed almost same rate of growth.

Biometric relationships (between height and length, depth and length, total weight and length, flesh weight and length and shell weight and length) have been studied in different months using the method of least squares. In the length-height relationship the mussel showed no difference in growth in different months. But the relationship between length and other parameters showed difference in different months.

The results are correlated with salinity observations. Pre-monsoon season with more or less constant environment is the optimum condition for the growth of the species. After spawning in high saline period of March-April there was high mortality of larger mussels. Along with this settlement of young mussels also occur in the population. During monsoon season a retardation in the growth of the mussel is observed.

Salinity tolerance and filtration rate of M. senhausia are explained in fifth chapter. Experiments on salinity tolerance were carried out with three size groups of the mussel, viz., 9±1 mm (small), 17±1 mm (medium) and 25±1 mm (large) in length. The animals were subjected to different salinities 0, 10, 15, 20, 25, 30 and 35 ppt. The nature of activity and salinity tolerance range are explained. Mussels showed difference in valve closure. byssal attachment and faecal production in different salinities. Studies revealed that small sized mussels could tolerate a salinity range of 10-35 ppt, medium sized mussel, 10-30 ppt and large sized mussels, 10-25 ppt. This revealed that small mussels appeared to tolerate great variation in salinity than the larger ones. The lethal salinities were found to be 7 ppt for small and medium sized mussels and 8 ppt for larger mussels.

The rate of filtering activity was studied by adopting neutral red dye clearance technique. Studies on filtration rate were conducted in different salinities (10, 15, 20, 25 and 30 ppt) and in different size groups (9-24 mm in length). In all cases filtration rate showed an increasing trend with body weight, but weight specific filtration rate showed a decreasing trend. Statistical analysis of the results revealed that filtration rate of the mussels of different size groups were not changing significantly in different salinities.

A detailed account of the reproductive cycle of M. senhausia is given in the sixth chapter which is based on the study of histological preparation of the gonad. Four main stages of the development such as developing, mature, spawning and spent could be recognised in the annual reproductive cycle of both sexes. The breeding period of the mussel is rather protracted

with one major spawning peak occurring during April-May. No resting condition is observed in the reproductive cycle, that is, throughout the year germ cells are present in the gonad.

Histochemical localisation of the storage substances in gonad revealed that the mantle is glycolipo-protein in nature. The protein, glycogen and lipid showed variation in different tissues like male and female germ cells.

Salinity is found to have an influence on reproductive cycle of M. senhausia. During the period of increasing salinity the mussels were gametogenically active. Spawning was observed to be associated with high and relatively stable salinity. Post-spawning mortality of the older specimens was also observed during this period. During monsoon the drastic fall in salinity adversely affects the population in the study area.

Observation on the variation in water content, protein, glycogen and lipid levels in the body entire and in selected component parts (mantle+gonad and foot) are explained in relation to reproductive cycle. In the case of water content, total tissue (male and female) and foot (sexes combined) showed a decrease in mature condition and later in spawning stage it showed an increase. But in mantle+gonad water level showed a decrease with the gonad development. The protein, glycogen and lipid level of all the tissues studied showed a decreasing trend. But the variation in protein and glycogen were more in mature stage than in spawning condition whereas the lipid of the different tissues like mantle+gonad and foot exhibited only a slight decrease in mature condition. Later in spawning stage the difference was more. it

is concluded that different organic constituents are utilised for gametogenesis which is an energy-demanding process. These form the subject matter of the seventh chapter.

The summary forms the eighth chapter of the thesis followed by the list of references cited in the text.

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