STUDIES ON THE BIOLOGY OF THE WEDGE CLAM *DONAX INCARNATUS* (GMELIN) FROM THE MALIPPURAM BEACH OF KERALA

THESIS

SUBMITTED TO
THE COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

MARINE BIOLOGY

UNDER THE FACULTY OF MARINE SCIENCES

By

SUNILA GEORGE

DEPT. OF MARINE BIOLOGY, MICROBIOLOGY AND BIOCHEMISTRY
SCHOOL OF MARINE SCIENCES
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 016

MARCH 2000

Dedicated to My Loving Parents

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried out by Smt. SUNILA GEORGE under my supervision and guidance in the Department 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 thereof has been presented for the award of any other degree, diploma or associateship in any University.

30.3.2000

Dr. K. Y. MOHAMMED SALIH
Professor,
Dept. of Marine Biology, Microbiology and Biochemistry,
School of Marine Sciences,
Cochin University of Science and Technology
Kochi -16.

Kochi - 682 016
March, 2000

DECLARATION

I, Sunila George, do hereby declare that this thesis entitled "STUDIES ON THE BIOLOGY OF THE WEDGE CLAM *DONAX INCARNATUS* (GMELIN) FROM THE MALIPPURAM BEACH OF KERALA" is a genuine record of the research work done by me under the supervision of Dr. K. Y. Mohammed Salih, Professor, Department 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.

Sunila George

KOCHI - 682 016
March, 2000

SUNILA GEORGE

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

I am greatly indebted to my supervising teacher Dr. K. Y. Mohammed Salih, Professor, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin for suggesting the topic, constant guidance and encouragement throughout the period of my research work.

My special thanks are due to Prof. (Dr). N. R. Menon, Director, School of Marine Sciences, Cochin University of Science and Technology, for providing me all the necessary facilities, constant help, encouragement and critical improvement and suggestion in the thesis.

I remember with gratitude the help and encouragement given by Dr. A. Mohandas, Director, School of Environmental Studies and Dr. K. Suresh for the help in preparing photomicrographs.

I am thankful to Shri. H. Krishna Iyer, Senior Scientist, C. I. F. T, Cochin and Dr. M. Sreenath, Senior Scientist, C. M. F. R. I, Cochin for the help rendered in statistical analysis of the data.

My unfeigned thanks to Dr. K. Rengarajan, Senior Scientist, C. M. F. R. I. and Shri. M. R. Boopendranath Senior Scientist, C. I. F. T, Cochin for critically evaluating the manuscript and for their valuable suggestions during the preparation of the thesis.

It is ineffable to record my gratitude to late Dr. Radha, P. S. whose unflinching help and co-operation have helped me to make this thesis come out in its present form. It was her affection towards me that has helped to overcome the innumerable difficulties I faced during the tenure of my research.

My friends, Dr. Nandini Menon, Dr. Shiny Sreedhar, Dr. T. K. Maqbool, Dr. Abdulla Bava, Dr. P. G. Suresh, Dr. Ajit Joseph, Dr. P. J. George, Mr. Suresh Kumar, Mr. Padmasenan and Mrs. Zeena, K. V. whose co-operation, help and support have played no small part in the completion of this thesis are gratefully remembered.

I thank the authorities of Cochin University of Science and Technology for awarding me a fellowship during the initial period of my study.

No acknowledgement will be complete without expressing my gratitude towards my loving parents and brother who have seen me through the worst of my troubles. I must also thank my husband Mr. Kurian Mathew and my little son for having persevered with me.

SUNILA GEORGE

CONTENTS

PREFACE

Molluscs comprise of a heterogeneous group of animals of great diversity. The group includes less familiar coat - of - mail shell, Amphineura; familiar forms like spirally twisted, single shelled, gastropods; two valved, bivalves; curious cephalopods (squids, cuttle fishes, octopuses) and the elephant tusk shells, scaphopods. Molluscs inhabit different ecosystems such as land, fresh water bodies, backwater and estuarine areas, mangroves, intertidal regions, littoral down to deep waters in the marine region. According to Subba Rao (1991), the number of molluscan species recorded from different regions of the world is between 80,000 and 100,000. From India, a total of 3271 species, formed of 1900 gastropods, 1110 bivalves, 210 cephalopods, 41 polyplacophores and 20 scaphopods have so far been recorded (Appukkuttan, 1996).

Imprinted in the Indian mythology and legend, in folklore, in social customs and traditions, in trade and handicrafts, as currency and medicine, the molluscan resources of India have been traditionally exploited along the coastal belt for food, export, shell-industry, shell-crafts and ornamental purposes from ancient times. However, organized fishery has been limited to only a few resources of chanks, pearl oysters and cephalopods, the exploitation of other gastropods and bivalves being remaining, by and large,

at sustenance level. At present, exploited molluscan resources contribute to only 4-5% of the total fish landings of India as against about 8-9 million tonnes (13%) of world molluscan production.

The bivalves belonging to Class Lamellibranchiata or Pelecypoda or Bivalvia of the Phylum Mollusca, are the widely distributed and better known among the various classes of shelled molluscs and comprise of a large group of specialised and laterally compressed forms. The Class includes oysters, mussels, clams, cockles and certain less familiar species groups. India has extensive bivalve resources in the coastal and estuarine waters and are utilised as food or a source of lime or for cement or for decorative shell-craft articles. In recent years, they form a delicacy and luxury food item in Japan, USA and Western Europe.

Among the exploited bivalve resource of India, clams occupy top position with an annual production of 50,000 tonnes. Kerala ranks foremost accounting for about 72% of clam landings (Narasimham, 1993), the Vembanad and Ashtamudi lake regions contributing to the bulk of the landings.

Several species of clams belonging to the families Arcidae, Venuridae, Corbiculidae, Tridacnidae, Solenidae, Mesodesmatidae, Tellinidae and Donacidae are exploited along the Indian coast. While the species such as

Meretrix casta, *M. meretrix*, *Katelysia opima* and *Villorita cyprinoides* are the major ones exploited from India, *Anadara granosa*, *Paphia malabarica*, *P. laterisulca*, *Gafrarium tumidum*, *Mesodesma glabratum*, *Tellina* sp., *Donax faba*, *D. cuneatus*, *D.incarnatus*, *Mactra voilacea*, *Tridacna maxima*, *T.crocea* and *T.squamosa* are the other important clams supporting the fishery. Among these, the wedge clam, *D.incarnatus* is fished in significant quantities in the Malippuram region in Vypeen Island, near Kochi, central Kerala.

With the increasing demand for animal protein food to meet the food requirement of burgeoning human population, and in the context of decline in the land area for agriculture, the urgent need of appropriate strategies to enhance the exploitation, production and utilization of fish and shellfish resources through capture and culture means from different water bodies is emphasized. In this scenario, organized exploitation of molluscan resources is receiving greater attention. Although, the exploited molluscan resources of India, in general, are able to withstand the fishing pressure because of their high fecundity, reproductive capacities and greater larval production, the natural fluctuation in abundance, pollution and environmental hazards and indiscriminate fishing would adversely affect the stocks. A comprehensive knowledge on the biology, population characteristics, and the biotic and

abiotic factors influencing the resource has, therefore, become essential not only for sustained exploitation but also for formulating rational managerial measures to maintain a healthy stock position. Although several aspects of the biology and ecology of commercially important species of *Donax* supporting the fishery along certain regions of the Indian coast have been studied, a perusal of the literature reveals that no detailed information on the biology of *D. incarnatus* exploited from the Central Kerala coast is available. As this species contributes at present to a significant seasonal fishery along the Malippuram coast, and has great potential to improve the fishery through culture and sea ranching, it was selected for investigation on certain aspects of its biology and ecology. The results of these investigations are embodied in this thesis complex.

The thesis is presented in five Chapters following a Preface and General Introduction.

The first chapter describes the characteristics of the species and its environment. The distribution of the species in the study area is presented in relation to the environmental factors.

The second chapter deals with the age and growth of *D. incarnatus*. The growth rate of the species is compared with that recorded for the species in other areas and the variation discussed.

The third chapter presents the results of the studies on the reproductive cycle of the species on the basis of detailed microscopic and histological observations of gonads. The different maturity stages are described. The spawning season and the peak breeding period of the species are determined.

The biochemical composition of *D. incarnatus* is given in the fourth chapter. Variations in different organic constituents such as protein, glycogen and lipid are correlated with the reproductive cycle of the species.

The results of observations on salinity tolerance of the species and filtration rate in different test salinities are presented in the fifth chapter.

The salient findings of the present study are discussed in the light of the earlier works and gaps in the knowledge pointed out in the general discussion.

It may be mentioned that the results of these investigations have considerably enhanced the existing knowledge on the biology and distribution/availability pattern of *D. incarnatus* in the Malippuram region. The species occurs in good concentration during October - March/April, and disappears from the area during late premonsoon and monsoon months. Recolonising the area in September, it grows fast in the subsequent months. The life span of the species is estimated to be about an year. Studies on the reproductive biology

of the species have revealed that there are two spawning peaks, the major peak in February - March and minor peak, in December. The salinity regime of the area influences the reproductive activity. These observations form the original contribution in the thesis. The information on variation in water content, protein, glycogen and lipid levels in relation to reproductive cycle has helped to a better understanding of the gametogenic activity and spawning of the species. Similarly, the findings on salinity tolerance and filtration rate have shown that small sized clams exhibit greater tolerance range than larger clams, and grow at a faster rate with active metabolism. It is hoped that these information would considerably add to the present knowledge of the basic facts which are relevant to the improvement and management of the clam fishery of this region.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

In the total world production of molluscs by harvest of wild stocks, clams/cockles rank next to squids and octopus, the world landings of clams being 925,622 tonnes in 1996 forming about 16% of total molluscan production (FAO, 1998). In terms of production through aquaculture source, clams occupy third place after mussels and oysters. The major clam producing countries are China (main land), India, Japan, Indonesia, Thailand, Bangladesh, Vietnam, USA, Korean Republic, Norway, Philippines, France, Chile and China (Thaiwan) (FAO, 1999). In India, clams support a sustenance fishery in the estuaries and backwaters of Kerala, Karnataka, Goa, Tamilnadu and Andhra Pradesh.

A general survey of the literature on clams and cockles of India reveals that the early works pertain mainly, as in the case of fishes and shellfishes, to faunistic investigations. Among these, the contributions of Nevill (1877), Melvill and Ambercrombie (1893), Melvill (1893), Smith (1904a,1904b, 1906), Preston (1909, 1910, 1911, 1914, 1915, 1916), Annandale and Kemp (1916), Hornell (1917, 1922, 1949a,b,c, 1951), Gravely (1941), Satyamurti (1952), CMFRI (1969,1974) are the most significant.

Information on the clam resources and on the biology of economically important clams is available from a number of contributions (Hornell, 1916; Rai, 1932, 1933; Rao, 1941; Rao, 1952, 1958, 1963; Rao, et al., 1962; Rao, 1967; Jones, 1968; Abraham, 1953; Nayar, 1955; Ranade, 1964; Deshmukh, 1972; Ansell *et al.*, 1972; Alagarswami and Narasimham, 1973; Parulekar *et al.*, 1973; Salih, 1973; Mane, 1974; Harkantra, 1975; Nair, 1975; Nagabhushanam and Talikhedhar, 1977; Nair *et al.*, 1978; Mane and Nagabhushanam, 1979; Joseph and Madhyasta, 1982; Reddy, 1983; Sreenivasan, 1983; Chatterji *et al.*, 1984; Thippeswamy and Joseph 1988; Thangavelu and Poovannan, 1994 and Joe and Narasimham, 1995). Certain symposia and National seminars conducted on molluscan/ shellfish/ marine fisheries R & D (MBAI, CMFRI, 1987) also contain valuable information, on the resources, biology, population and farming of clams among other commercial species of molluscs. Most of the biological investigations carried out so far relate to age and rate of growth, maturation and spawning and the environmental changes influencing the growth and reproduction.

The importance of ecological studies on the distribution and survival of clams is well recognized. The noteworthy contributions on the ecology, density, distribution and abundance of clams of India are by Nayar (1955), Alagarswamy (1966), Durve and Dharmaraj (1969), Desai (1971), Philip

(1972), Ansell *et al.* (1972a), Ansell and Trevallion (1972b), Dwivedi *et al.* (1973), Parulekhar *et al.* (1973), Achuthankutty (1976), Ayyappan Nair *et al.* (1978), Mane and Nagabhushanam (1979), Ramachandra *et al.* (1981), Joseph and Santha (1988), Alongi (1990) and Joe and Narasimham (1995). These studies have indicated that the nature and type of substratum, wave action, temperature and salinity regimes of the area largely determine the distribution, abundance, migration and burrowing behavior of the clams.

Physiological responses of clams in relation to temperature, salinity, oxygen consumption, starvation and behaviour have been the subject matter of several studies. The most valuable among these works from India are those of Rao (1952), Ansell (1973), Ansell and Sivadas (1973), Ranade (1973), Mane (1975), Nair and Shynamma (1975b) and Mane and Talikedkar (1976). While Ansell and Sivadas (1973) and Mane and Talikedkar (1976) studied the effect of temperature on the metabolic rate of *Donax vittatus*, and resipiratory rate of *D.cuneatus* respectively, the influence of salinity on the growth of *Ketelysia opima* at Madras was noted by Rao (1952); on *Mertrix casta* at Adayar backwater (Madras) by Abraham (1953); on *D. cuneatus* at Palk Bay by Nayar (1955) and on *K.opima* at Ratnagiri by Mane (1974b). The capacity of clams to tolerate fluctuations in salinity was reported by Prasad(1922) and Nair and Shynamma (1975b) on *Villorita cyprinoides var.*

Cochinesis, Talikhedkar and Mane (1976) on *D. cuneatus*, Salih (1978) on *M.casta*, Ranade and Kulkarni (1973) on *K. opima*, Thampuran *et al.* (1982) on *Sunetta scripta*, Sundaram and Shafee (1989) on *M. meretrix* and Ram Mohan and Velayudhan (1995) on *Paphia malabarica*. Ranade (1973) and Ansell (1973) studied the effect of temperature and salinity on the oxygen consumption of *Meretrix meretrix*, *Katelysia opima* and *Donax vittaus*. Later Mane (1975) reported the oxygen consumption in *K. opima* in relation to environmental changes and physiological factors such as body size, salinity, temperature, pH, oxygen tension, starvation and diurnal rhythm.

Seasonal changes in the biological constituents and chemical changes during the reproductive and growth phases and in storage and utilisation of reserves, though not studied intensively and extensively, have been reported in certain species of edible clams. Thus Ansell (1972) observed that in *D. vittatus*, spawning which occurs in the early summer, results in a marked fall in the mean body weight. Nagabhushanam and Dhamme (1977) recorded relatively high water content in *Paphia laterisulca* during monsoon, suggesting loss of salts from the body and gain of water in low salinities. Similarly, Nagabhushanam and Talikhedkar (1977b) observed decline in glycogen content in September in the wedge clam, *D. cuneatus* and also during the maturation of gonad. Seasonal variations in energy, organic carbon

and lipid contents in *D. incarnatus* were described by Balasubramanian *et al.* (1979). Index of condition which measures the meat quality/physiological condition was studied in *Donax faba* (Alagarswamy, 1966) and in *Meretrix casta* (Durve and George, 1973). Mane (1974a) and Krishnakumari *et al.* (1977) reported the percentage edibility of *M. casta* and *K. opima* from Goa.

Clams are by far harvested from the wild till recently. Though they are cultured in several countries such as China, Thailand, Malaysia, Indonesia, Singapore, U.K. and Australia, the technology of culture is not as advanced as in the case with oysters and mussels. However, with the increasing demand for animal protein food, and since clams feed low in the food web and are efficient converters of primary production into nutritious food, and form cheap source food, their culture prospects has received greater attention in recent years. As a result of the researches carried out in India during the past three decades by several maritime Institutes, particularly the Central Marine Fisheries Research Institute, Kochi, a package of technology for the culture of blood clam, *A. granosa* and venerid clam, *P. malabarica* is now available. However, it is yet to be commercialised (Narasimham, 1980, 1991; Narasimham and Laxmilatha, 1996).

The above brief review of literature and those given under each of the chapters reveal that not much information is available on the ecological and biological aspects of clams of the Central Kerala coast. In consideration of the increasing importance of clams in rural economy, in the export trade, as an animal food and as an ideal eco-friendly candidate for culture and its prospects, the present study involving the ecological and biological aspects of *Donax incarnatus* (Gmelin) from the Malippuram beach, near Kochi was undertaken. The information/ data collected would be useful for rational exploitation and management of this valuable resource.

MATERIAL AND METHODS

The details of material/data collected, methods followed for the analysis of samples, processing of data and the experiments conducted to determine the salinity tolerance and filtration rate in the present investigation are given in the concerned chapters. Briefly these were as follows.

Random samples of *D. incarnatus* were collected regularly once a fortnight from February 1990 to February 1991 from Malippuram beach using a wooden frame of 20 cm^2 fixed on the shore. The clam samples thus obtained were used for growth and age determination and for studies on reproductive activity.

Soil samples were collected from the intertidal areas during low, mid and high tides for texture analysis following the conventional sieving procedure.

Data on temperature, salinity and dissolved oxygen of the shore waters were collected for a period of 13 months from January 1990 to February 1991. The seawater samples were collected in a bucket and the temperature was noted immediately using a mercury thermometer. Dissolved oxygen and salinity of the seawater samples were estimated by Winkler's and silver nitrate titration methods respectively.

Morphometric measurements of clam were taken using a Vernier caliper, and the weight measurement by an electric balance.

To determine the growth, the length data pooled monthwise and grouped at 2 mm class intervals, were analyzed for modal progress and ELEFAN I programme applied to fit von Bertalanffy growth equation.

Regression equation ($y = a + bx$) was employed for morphometric data.

Gonadial smear was examined under microscope to note the sex and maturity stage. Standard histological techniques were employed to study the gonadial maturation process and activity.

Biochemical analysis of tissues relating to protein, glycogen and lipid were performed following the method of Lowry et al. (1951), Kemp and Vankitz (1954) and Barnes and Blackstock (1973) respectively.

Selected, healthy clams of different size were used for salinity tolerance experiments and for determining the filtration rate. Standard procedures were followed in the conduct of these experiments.

CHAPTER I

Chapter I

SPECIES DESCRIPTION AND STUDY AREA

Introduction

The Genus *Donax* belongs to the Family Donacidae within the Superfamily Tellinacea of the Phylum Mollusca, one of the important phyla within the animal kingdom. It constitutes a group of animals of laterally compressed form which are adapted to live in the sandy substratum of the intertidal region.

Among the six Classes of Phylum Mollusca, Class Bivalvia, also known as Lamellibranchiata or Pelecypoda, with approximately 31,000 living species (Russell - Hunter, 1979) form the second largest class and in some way the most highly modified of all the molluscs.

The Family Donacidae includes the bivalves living in marine habitats. Among molluscs on a world-wide basis, species of *Donax* form by far the most dominant group in the infauna of sandy beaches. *Donax* is predominantly tropical in its distribution. Approximately 75% of the living species are found in tropical waters, 21% are mainly warm temperate in their distribution and only 4% extend their distribution into cold temperate areas (Ansell, 1983). The commonest species found in the east and west coast of

India are *Donax incarnatus, D. cuneatus, D. faba, D. scortum* and *D. spiculum.* Of these species, *D. incarnatus* is selected as candidate species for the present investigation from the Malippuram beach, a small fishing hamlet of Vypeen Island near Kochi in Kerala. They are characterised by having a large, anteriorly displaced foot and two separate moderately elongate mobile siphons posteriorly. They are burrowing bivalves and they are known for tidal migration.

Description of the species

The shell is rather high and sharply triangular in outline, strongly inequilateral with a short, steeply sloping posterior side and a long, gradually inclining dorsal margin in front of the umbo (Fig. 1.1).'Ventro - posteriorly, the outline is rather angulated. Anteriorly, the shell is more or less narrowly rounded. The lunule is narrow, greatly elongated and slightly depressed and external ligamentary area behind the umbo is short and rounded. The area marked by the keel and the portion of the surface behind it are conspicuously sculptured with somewhat undulating, concentric ridges. The rest of the surface is very finely, closely and radiately grooved. The surface presents a smooth and glossy appearance, especially towards the umbones. On the inner surface, the muscle impressions and area outside the pallial line are pale fleshy pink, while the area within it including the area of the pallial sinus is

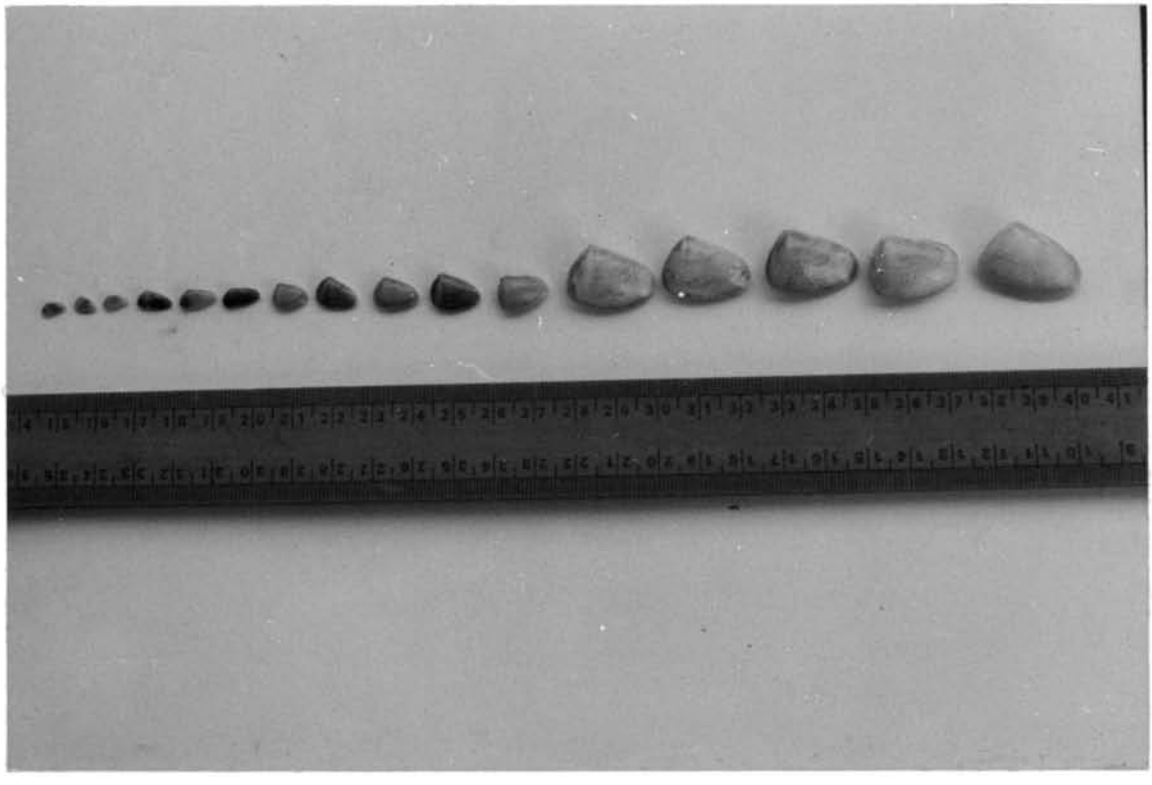


Fig. 1.1. Difference sizes of *Donax incarnatus* - lateral view

opaque and chalky white. The margin of the inner surface is finely grooved. The outer surface is pale fleshy pink or even whitish, tinged slightly with pink and variously banded with darker reddish or pinkish red concentric bands. The umbonal area is usually deeply tinted with pink (Fig. 1.2).

THE ENVIRONMENT

The study area

Malippuram (10^0 02' N, 76^0 13' E), a coastal fishing village within Elamkunnapuzha Panchayat having an area of 11.52 sq. kilometres with a population of 51197 is situated in the Vypeen Island (9^0 58' - 10^0 11' N, 76^0 10' -76^0 15' E) in the central part of Kerala, South India. This 26 km long island is a narrow strip of land lying parallel to the main land and is separated from it on the eastern side by northern extension of the Vembanad Lake (Cochin backwater). The Cochin and Azhikode Barmouths form the southern and northern boundaries of the Island, while on the western side is the Arabian Sea. Malippuram village is situated 7 km north of the southern tip of the Vypeen Island and its beach is extended to a length of about 3 km. (Fig. 1.3).

Beach erosion is experienced strongly and heavily at the Malippuram beach during active Southwest monsoon months (June-August) when the sea becomes rough. Also during November-December months and sometimes

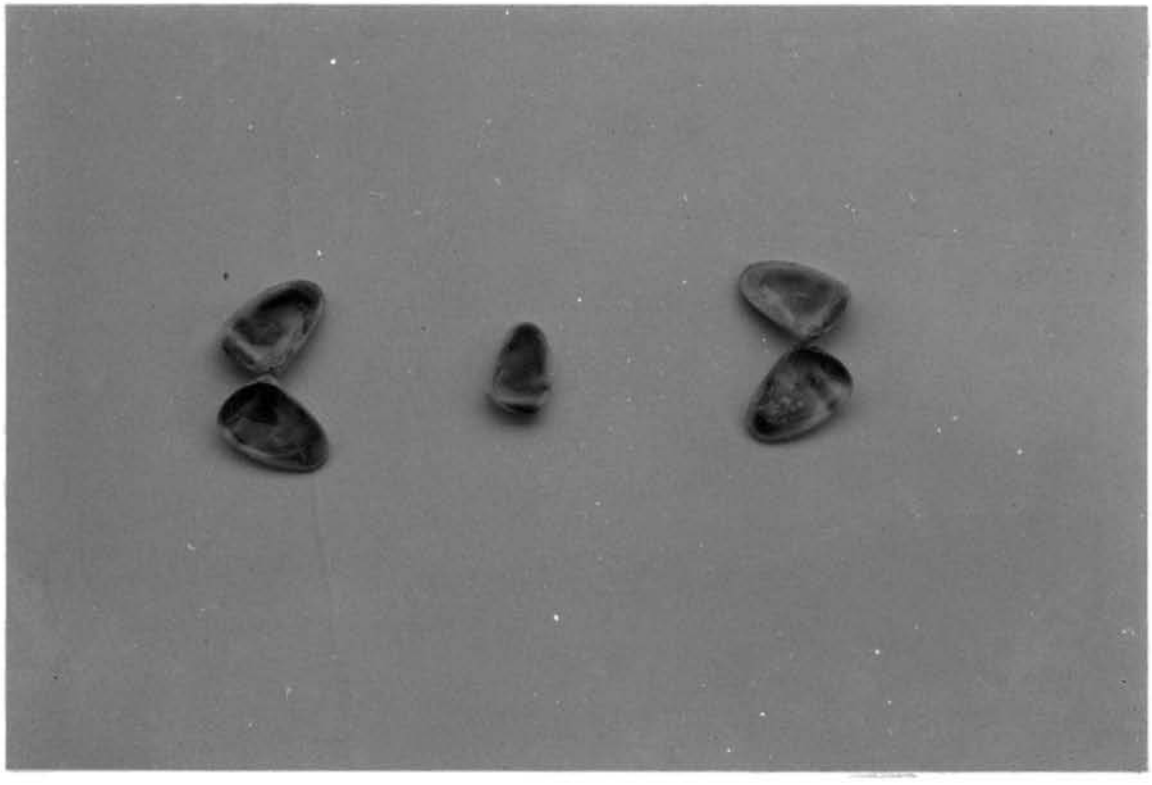


Fig. 1.2. Shells of *Donax incarnatus* - inner view

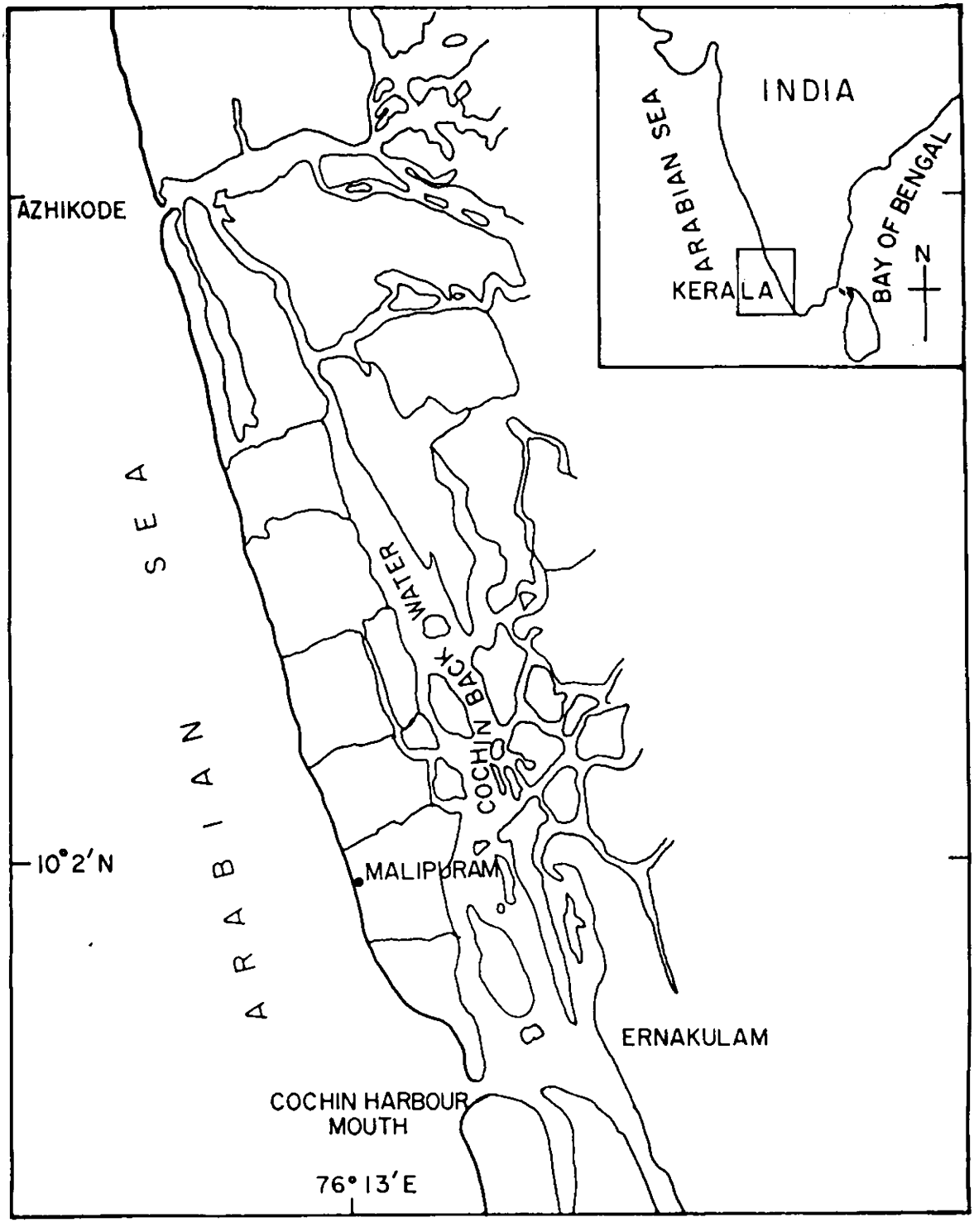


Fig. 1.3 MALIPPURAM IN VYPEEN ISLAND, THE STUDY AREA

extended even upto the middle of January, a high water phenomenon locally known as 'vrichikaveli' is prevailing in this area and all along the Vypeen coast when the tidal amplitude is found to be high to a height of more than one metre and as a result seawater flows over the beach inundating the low lying land area. During the other part of the year, tidal amplitude is only within the range of 0.46-0.76 metre. Inorder to prevent the erosion of land, anti-sea erosion wall by using large granite blocks has been abutted along the Malippuram coast leaving a gap of about 200 m for fishing activities. Because of this construction, the shoreline has been widened to some extent in the entire length of Malippuram coast (Fig. 1.4).

The tides in the region of Cochin are of a mixed type with a strong semi-diurnal influence; diurnal inequality is large. Wave action is continuous; waves result from ocean swell from the southern Arabian sea. The currents along the Kerala coast flow northwards during November to January and southwards from late May to early September. Coastal areas are particularly affected by salinity changes from the great freshwater outflow from the backwaters and land drainage during Southwest monsoon, extending from May to September (Darbyshire, 1967; Trevallion *et al.*, 1970).

Samples of the wedge clam *Donax incarnatus* (Gmelin) were collected from this area. Owing to the rough weather, beach erosion and salinity

Fig. 1.4. Malippuram beach - a natural view

dilution, they were not available during late premonsoon and monsoon months in the intertidal area. Clams might have either perished or migrated to the mid-littoral zone and even extended to sub-littoral zone where the salinity is congenial to the species. The wedge clam is found in abundance in the surface sandy soil to a depth of 5-7 cm. During September, thick mat like bed of young ones of this species was seen along the beach (Fig. 1.5).

HYDROGRAPHY

An understanding of the seasonal variation of important physico-chemical factors such as soil texture, temperature, salinity and dissolved oxygen are essential prerequisites for the interpretation of the distribution, abundance, settlement and various behavioural responses of animal population.

The substratum

The substratum is sandy - mixed with fine black sand particles. Samples for sediment analysis were collected from the intertidal area of Malippuram beach. For this purpose, sand samples were collected during low tide, mid tide and high tide for textural analysis. These samples were coned and quartered and washed with freshwater to make it free of salt and then treated with dilute Hydrochloric acid to remove the shell fragments. It was rinsed with water again and again to free the sand grains from acid traces and

Fig. 1.5. Thick carpet like bed of *Donax incarnatus* from the study area

then oven dried. The samples were then subjected to granulometric analysis by following the conventional sieving procedure (Krumbein and Petti John, 1938). The grading of sand showed fine, medium and coarse grains at 91.15%, 8.57% and 0.28% during the premonsoon and postmonsoon periods, while during monsoon months they were 86.10%, 9.23% and 4.62%, respectively. Very fine sand and silt and mud were found to be negligible (0.05%). This shows that the wedge clam preferred a substratum having fine sand particles. Since Malippuram beach has been subjected to monsoon dominated erosion and postmonsoon deposition, the fauna recolonised after the monsoon. This closely relates with the studies carried out at Calangute beach in Goa (McLusky *et al.*, 1975) and Shertalai beach in Kerala (Ansell *et al.*, 1972a, 1973). Desai (1971), Dwivedi *et al.* (1973), Achuthankutty (1976) and Ramachandra *et al.* (1981) also found that the distribution and abundance of clams is related to grain size distribution of the sediment, the clams being most abundant in the habitat with fine to medium sand.

Hydrology

Hydrographical structure is subjected to great changes depending on the seasons. Climatologically, the annual cycle consists of premonsoon, monsoon and postmonsoon seasons. Hydrological factors such as salinity, temperature and dissolved oxygen were studied for a period of 13 months

from January 1990 to February 1991 (Table 1.1). The salinity was estimated by titration against silver nitrate using Potassium chromate as indicator. Based on the observation, the year can be divided into premonsoon (January to May), monsoon (June to August) and postmonsoon (September to December) periods. The salinity during the period of study was in the range of 11.7 and 34.9 ppt, the minimum being in August and maximum in April. But certain days in June, July and August it showed a sharp decline to the level of 11.7-15.4 ppt. Similarly, the temperature was in its maximum in April (33^0C) and minimum (25^0C) in July. The dissolved oxygen (DO_2) was estimated by following Winkler method. The level of DO_2 was in the range of 2.8 and 5.6 ml/l, the minimum during June and maximum during September. During the monsoon season due to beach erosion and salinity fall, *D. incarnatus* were not available. This might be attributed either to mortality or emigration or both these processes. It is observed that, generally, during the low saline period the macrofauna of the intertidal region migrate into the lower mid-littoral zone and even to sub-littoral zone where some congenial saline regime prevails (Ansell and Trueman, 1973). The absence of *D. incarnatus* during the monsoon and recolonisation thereafter is found to be due to salinity fluctuations. During the low saline period (monsoon) the normal filtration and oxygen consumption and feeding is curtailed to almost

Table 1.1. Hydrological data on salinity, temperature and dissolved oxygen during the period 1990 -'91

Month	Salinity (ppt)	Temperature (°C)	Dissolved oxygen (ml/l)
February 1990	32.5	31.2	3.9
March	34.2	31.5	4.1
April	34.9	33.0	4.5
May	33.2	32.0	3.6
June	15.4	28.6	2.8
July	12.1	25.0	3.8
August	11.7	26.6	4.8
September	25.8	29.8	5.6
October	29.2	30.8	3.8
November	22.3	28.5	4.4
December	29.5	30.5	4.7
January 1991	30.5	31.3	3.6
February	33.0	31.5	3.0

nil level. This may force the animal to emigrate to the saline subtidal level. The temperature might not be a major factor causing mortality or disappearance as it was always within the range of 25-33^{0}C. McLusky *et al.*, (1975) opined that the dominating factor of the annual monsoon, which by its influence on the salinity, the particle size composition and slope of beach, created widely fluctuating conditions causing the death or emigration of the macrofauna. In the present study, after the monsoon season, repopulation of *Donax incarnatus* was noticed. This closely agrees with the observation of Panikkar (1969) who had postulated the concept of partial or complete destruction of tropical fauna during the Southwest monsoon followed by an annual repopulation of fauna in postmonsoon. Such a phenomenon is a characteristic feature of tropical estuarine and coastal biotopes (Parulekar *et al.*, 1980).

Chlorophyll a concentration

Primary production and standing crop of phytoplankton of the west coast of India have been studied by Subrahmanyan, 1959; 1959a; 1960; Nair *et al.*, 1968 and Chennubhotla, 1969. Gopinath *et al.* (1974) made observations on the phytoplankton of Cochin backwater. All these investigations revealed that all along the west coast of India phytoplankton production is at its highest during Southwest monsoon.

However, Dehadrai and Bhargava (1972) made measurements of chlorophyll a for a period of 9 months (September 1969 to May 1970) in the coastal waters from Goa to Bombay and observed that chlorophyll a is found to increase during postmonsoon period. Nair *et al.* (1985) estimated the surface chlorophyll concentration in the inshore waters of Cochin region and found that chlorophyll a concentration is high during premonsoon season. Radhakrishna (1989) reported high phytoplankton production during postmonsoon months extending up to March - April and low in the Southwest monsoon period from the coast of Maharashtra. Gopinath (1981) indicated two phytoplankton production peaks during January and May in the inshore sea off Cochin. Trevallion *et al.* (1970) made observations on the sandy beach of Cochin region during premonsoon period and reported the amount of chlorophyll attached to sand grains was very small and in the water it varied between 0.9 and 13.0 µg/l.

Detailed studies on the production of chlorophylla in the inshore waters of Cochin region were made by Balachandran *et al.* (1989) during the period 1987 and 1988. The data collected from the near shore station (10 m) reveal that the surface chlorophylla is subjected to monthly variations. During 1987, the chlorophylla indicated three seasonal peaks, a high primary peak during premonsoon and secondary and tertiary peaks of low orders

during postmonsoon and monsoon periods respectively. The highest value of 3.6 mg/m^3 was observed in March and lowest value of 0.24 mg/m^3 in May. Seasonal chlorophyll a production in monsoon months was of low magnitude (0.64 mg/m^3 in June, 1.92 mg/m^3 in July and 1.49 mg/m^3 in August). During August 1988, chlorophyll a was observed to be totally absent. They have concluded that the production of chlorophyll a has no influence on hydrographic factors, particularly salinity and dissolved oxygen.

The above studies indicate that the basic productivity of the inshore waters of the Cochin region is relatively low during the monsoon months from June to August/September, and the productivity increases with the normalisation of the environmental factors in the postmonsoon months. The non-availability of *D. incarnatus* during the monsoon months and the recolonisation and increased abundance in the postmonsoon and premonsoon months in the Malippuram beach thus closely follows these physico-chemical and biological changes of the ecosystem.

CHAPTER II

Chapter II

STUDIES ON AGE AND GROWTH

Introduction

The knowledge on different aspects of age and growth of fishes and shellfishes is increasingly applied in the fishery biological research to improve their capture and culture fisheries. Information on the growth at different phases of life, relative growth of different body parts and age structure of the population is essential to understand the population/stock characteristics and for scientific interpretation of the fluctuations in the population in space and time. Similarly, an understanding of the relationship between the environmental factors and the growth provides greater insight into the dynamics of the population structure in nature.

Age and growth of fishes and shellfishes are studied at present following four important methods. These are (1) Peterson's method of length-frequency analysis, (2) studies on the markings, rings in the hard parts of the body, (3) rearing of animals in captivity under controlled conditions and (4) mark-recovery experiments. Among these, as in the case of fishes, length-frequency analysis method is, by and large, employed for molluscs, though it has certain limitations mainly due to distribution and availability of certain year classes in the fishery.

Shell markings and growth rings are also used to study the growth of molluscs including clams by several workers. Although encouraging results are obtained in these studies, seasonality of growth and environmental factors are found to influence the ring formation. Tagging experiments are used only on a limited scale in molluscs in view of the scarce recovery of the tagged specimens. With the development of culture techniques, rearing of molluscs in captivity to study the growth is followed in recent years. However, growth manifestations in this situation depends largely on the suitable environmental and rearing condition, and feed provided, and often, may not reflect the growth of the animal in nature.

In recent years, several growth modals (Devarajan, 1983) are applied to the basic data on length and weight to estimate the age and growth.

As the information on age and growth of *D. incarnatus* is generally scarce and not available from the Cochin region, the present investigation is taken up.

REVIEW OF LITERATURE

Information on the growth of bivalves has been provided by several workers. In *Meretrix casta*, Abraham (1953) used the measurement of marked clams, analysis of natural populations, laboratory-reared clams, and the coaxial rings on the shell, and found that in the laboratory conditions, the

growth rate was very low due to the inadequate food supplies, but in natural conditions it was found to be adversely affected by crowding. Durve and Raja (1965) reported differences in the dimensional relationships of the *Meretrix casta* from two localities and opined that this could be attributed to widely differing environmental conditions prevailing there. Durve (1970) studied the growth pattern of *M. casta*, collected from the marine fish farm at Mandapam Camp, in relation to its length, depth and height. Length-frequency studies on the backwater clam *Meretrix casta* inhabiting the Cochin Barmouth show that the growth is moderate during the premonsoon period, fast during the postmonsoon period and poor during the monsoon period (Salih, 1973). In *Katelysia opima*, Mane (1974a) used length-frequency method to study the growth pattern of the clam. He also studied the allometric relationships, discussed the significance of disturbance on rings and reported a retardation of growth during monsoon season (low salinity). Harkantra (1975) pointed out that high salinity and temperature accelerated growth in *Meretrix casta* in Kali Estuary, but no growth could be recorded in low temperature-saline conditions. His observations were based on the length-frequency distribution and the appearance of regular annual rings on the shell and agreed with the observations of Mane (1974b) and Salih (1977). Salih (1977) studied 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 recorded a minimum monthly average growth in length during monsoon season. Conan and Shafee (1978) reported an absolute annual increase in shell height of *Chlamys varia* using von Bertalanffy growth models. Mane and Nagabhushanam (1979) studied the growth of *Paphia laterisulca* at Kalbadevi Estuary, Ratnagiri and reported that it could attain a size of 50 mm at the end of three and half years. Shafee (1980) studied that 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) gave a brief account of the allometric relationship of *Sunetta scripta* in Cochin waters. Sreenivasan (1983) made some observations on the growth of the clam *Meretrix casta* (Chemnitz) transplanted in Vellar estuary. Chatterji *et al.*, (1984) reported the average monthly growth increment in shell length of *Villorita cyprinoides* and its relationship with other growth indices such as 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. 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* were determined by the length-frequency study and various morphometric and length-weight relationships (Narasimham, 1988b). He also estimated the parameters of the von Bertalanffy growth equation and found a comparatively faster growth rate in the species. Rao (1988) found out the length-weight relationship of *Meretrix casta* and *Paphia malabarica* and other dimensional relationship of *M. casta*. He also 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 (1988 b) opted von Bertalanffy equation for growth in length in *Anadara granosa* from the Kakinada Bay. Growth of *Meretrix casta* was studied by plotting the sizefrequency histograms and from the length-weight relationship in relation to some physico-chemical parameters such as sand excavation and deposition of mining waste in the estuarine belt and salinity (Modassir, 1990). Blay Jr. (1989) worked out the shell morphometrics, length-weight relationships and length distribution 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 populations were probably due to environmental influences resulting in different growth rates. Shiny (1991) determined growth parameters using von Bertallanffy growth equation in *Musculista senhausia*. Biometric

relationships in the mussel *M. senhausia* were determined by Shiny and Radhakrishnan (1995). They also described the relationships using regression analysis of pair of variables. In *Villorita cyprinoides* collected from Vembanad lake, Joe and Narasimham (1995) have estimated the relative length of the animal by using von Bertalanffy growth-equation. Comparison of shell length - meat weight relationship of ocean quahog *Arctica islandica* from three geographical areas in Iceland was studied by Thorainsdottir and Johannessoa (1996). Growth rate of hairy mussel *Trychomya hirsuta* was estimated by applying von Bertalanffy equation using length measurements (Goggin, 1997). Kripa (1998) determined the growth rate of rock oyster *Saccostrea cucullata* (Born) by using von Bertalanffy equation.

Nayar (1955) studied the growth of *Donax* (*Latona*) *cuneatus* from length-frequency distribution, different allometric relationships and growth rings and he suggested that it was not uniform throughout the year. Growth of various meristic characters of *Donax incarnatus* in relation to ecological parameters has been investigated by Nair *et al.* (1978). Thippeswamy and Joseph (1991) estimated the growth rate of *Donax incarnatus* by the analysis of size-frequency data by Pauly's integrated method and calculated theoretical pattern of growth using von Bertalanffy equation.

MATERIAL AND METHODS

In the present study, the methods adopted are the length-frequency analysis, allometric relationship between different dimensions and von Bertalanffy growth equation by ELEFAN I (Electronic Length Frequency Analysis).

The study on the growth rate of *D. incarnatus* was based on fortnightly random samples collected from Malippuram beach during the period from February 1990 to February 1991. The clams were collected using a wooden frame of 20 cm^2, which was fixed on the shore. All the sand with the clams from the enclosed space to a depth of about 10 cm beyond which the clams seldom burrow, was sieved so as to allow the sand to pass through the meshes (1 mm) leaving the clams in the sieve and kept it in water of habitat salinity. Length, breadth and depth of each clam were measured with vernier callipers correct to one-tenth of a millimetre. The greatest anterio-posterior measurement was taken as length; the maximum distance between the hinge and ventral (free) margin of the valves as breadth and the greatest distance between the outer surface of the two valves measured in a direction perpendicular to the anterio-posterior axis as depth. Inorder to determine the flesh weight and dry weight, the clams were kept in aerated water of habitat

salinity for 24 hours to defecate and weights were determined to the nearest 0.1 mg in an electric balance.

Shell length was taken as the standard measurement for determining age and growth of the clam *D. incarnatus*. The frequency distribution of length was assessed by taking the class interval as 2 mm. The monthly size frequencies were then converted into percentage of total number of animals present in the sample, which formed the basis for the interpretation of population structure. By using ELEFAN I, progression of the modal value was noted and determined growth of the clam.

The relation of breadth, depth and weight on length was studied by fitting the regression equation of the type.

$$Y = a + b X$$

The original equation used for the study of length – breadth, length – depth, length – flesh weight and length – dry weight is of the form '$Y=ax^b$'. To determine the value of the parameters 'a' and 'b', the above equation is reduced to a straight line by taking logarithm. The logarithmic lines are fitted to the above data (length – breadth, length – depth, length – flesh weight and length – dry weight).

When required logarithmic transformation was applied, here 'Y' is the dependent 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 independent variable in all the studies. The allometric relationships between length and breadth, length and depth, length and flesh weight and, length and dry weight were studied.

The coefficient of correlation between length and breadth, length and depth, length and flesh weight and length and dry weight were calculated using Pearsons formula. Covariance analysis of the different allometric relationships of different methods were determined (Zar, 1974).

It is assumed that the growth in length follows the von Bertalanffy Growth Factor (Bertalanffy, 1938, 1957) which is given by $L_t = L_\infty (1-e^{-k(t-t_o)})$ where 'L_t' is the length at age 't', 'L_∞' is the theoretical maximum length, 'k' is the growth coefficient. Here 't_o' is assumed to be zero because ELEFAN routine (Pauly and David, 1981) does not estimate it.

RESULTS

Population structure and growth by length - frequency analysis

The length frequency distribution of the wedge clam, *Donax incarnatus* during the period from February '90 to February '91 when the specimens were available, are presented in frequency Table 2.1 and by percentage frequency histogram in Fig 2.1. Estimated growth for three sets of parameters by ELEFAN I were shown in Table 2.2. The distribution shows the distinct modes in most of the months.

As seen in Fig 2.1, during premonsoon period, in February 1990, the samples revealed only adult clams with 16 mm and 18 mm mode. This is in close agreement with the observations in the Chapter 3 where majority of them were in partially spawned phase and mature phase. In March, the peak value of the adult clams is at the size 20 mm. The mode at 16 mm observed in February had shifted to 20 mm in March resulting a growth of 4 mm in one month. A small percentage of smaller specimens could be seen along with the larger ones in the two months. Mode at 20 mm of March was traced to 22 mm in April resulting a growth of 2 mm.

After the break for monsoon, smaller size specimens measuring between 3 mm and 9 mm dominated the sample in September (*i.e.* 45.97%). This indicates new recruits entering the population. The principal modal size

Table 2.1. Percentage frequencies of *D. incarnatus* of different size groups for different months (Samples not available from May to August)

Class (mm)	February '90	March	April	September	October	November	December	January '91	February
3-5	-	-	-	4.51	-	-	-	-	-
5-7	-	-	2.11	22.31	-	4.06	-	-	-
7-9	2.3	4.04	-	19.15	4.12	2.19	-	-	2.18
9-11	4.12	6.05	5.20	6.04	11.26	2.12	4.10	8.19	7.26
11-13	7.08	8.15	2.36	11.20	23.11	0.56	3.16	25.26	4.19
13-15	21.91	2.62	8.04	21.15	20.06	18.12	2.04	28.04	12.31
15-17	27.24	21.26	10.13	8.36	16.19	23.61	17.21	11.06	9.81
17-19	22.15	16.10	14.05	3.31	19.04	21.04	23.06	4.18	25.14
19-21	6.03	26.23	22.11	2.03	2.13	18.19	28.27	9.31	29.14
21-23	5.5	6.19	26.08	2.04	3.09	3.63	11.04	14.31	-
23-25	3.2	4.36	5.4	-	-	6.31	6.18	-	6.08
25-27	0.61	3.08	2.3	-	-	0.04	3.04	-	3.15
27-29	-	2.02	2.16	-	2.14	0.12	3.06	-	-

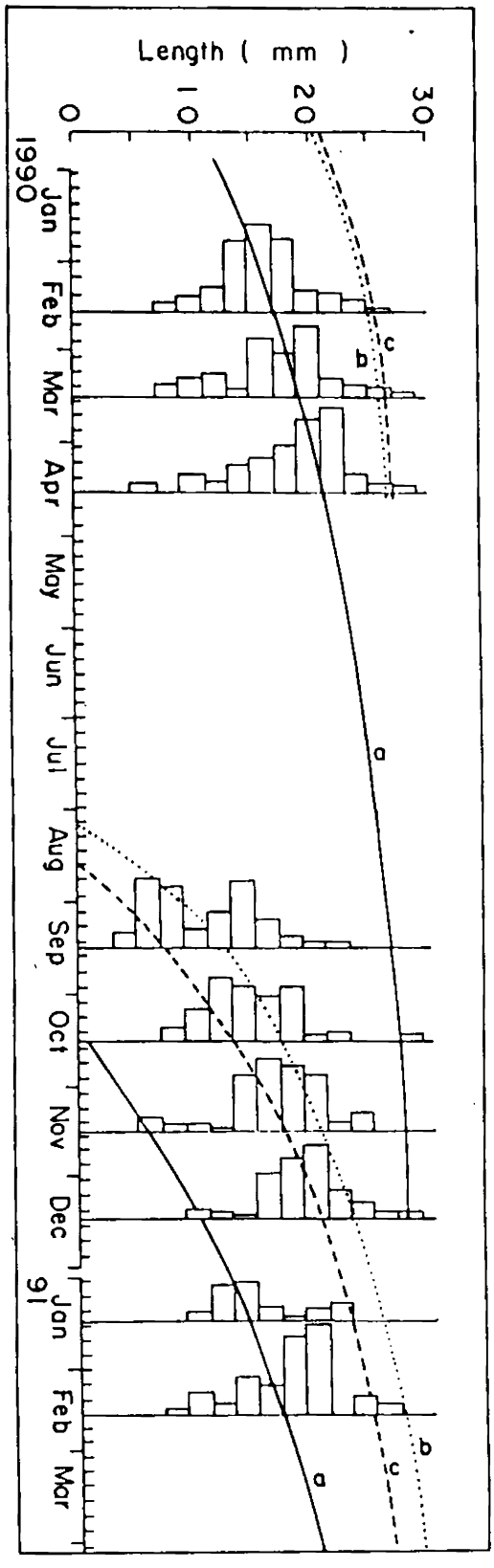


Fig. 2.1 ESTIMATED GROWTH PROGRESSION FROM ELEFAN I

(Samples not available from May to August)

Table 2.2. Details on the estimated growth parameters for three sets of curves by ELEFAN I in *D. incarnatus*

Month	$L\infty = 29.2$; $k = 2.6$ (a)	$L\infty = 29.1$; $k = 2.7$ (b)	$L\infty = 29.5$; $k = 3.2$ (c)
January	13.99	22.98	22.13
February	17.00	24.24	23.88
March	19.21	25.15	25.11
April	21.19	25.98	26.15
May	22.73	26.58	26.93
June	24.02	27.10	27.54
July	25.01	5.22; 27.50	28.00
August	25.84	10.11; 27.83	1.29
September	26.51	14.01	3.01
October	27.03	17.01	12.98
November	5.71; 27.46	19.49	16.91
December	10.23; 27.80	21.40	19.82

of calms in the month was at 6 mm, and a secondary mode at 14 mm. The main mode of 6 mm in September shifted to 12 mm in October indicating an increment of 6 mm during a month. This mode (12mm mode) further progressed to 16 mm size group in November to 20 mm in December 1990 registering a growth rate of 4 mm per month.

In January '91, the main mode was noticed at 14mm and this shifted to 20 mm in February '91. Maximum size observed during the period of study was 28.2 mm.

It is observed from the Fig 2.1 that the growth was relatively faster in these months following the recolonisation of the ground after monsoon in September, being 4mm per month. As the season advanced, the growth rate slowed down to 2 mm per month as observed during March-April. Recruitment of young clams (measuring less than 9 mm) to the population was seen during February, March, April, September and November indicating a continuous breeding habit for the population.

***Fitting of von Bertalanffy Growth Equation Using* ELEFAN I**

Growth parameters 'L_{∞}' and 'k' values were estimated using ELEFAN I method in FISAT programme (FAO and ICLARM). The recruitment pattern was estimated using the appropriate programme in FISAT.

Using the ELEFAN I, three growth progressions could be identified (Fig 2.1) resulting in three sets of L_∞ and k values (Fig 2.1a; 29.2 mm, 2.6; 29.1 mm, 2.7 and 29.5 mm, 3.2). Although there was not much difference in the estimate of L_∞, it was observed that the value of 'k' ranged from 2.6 to 3.2 annually. This could be attributed to the fact that the last set of parameters were estimated relating from the smallest observed modal length (Fig 2.1). The estimated growth progression in length for the three sets are given in Table 2.2. It can be seen that at higher length, the progression was more or less similar. Besides since the last set of parameters considered growth progression for the smallest length, it could be taken as indicative of the growth pattern of the species concerned. Hence, the best estimate of L_∞ and k were taken as $L_\infty = 29.5$ and k= 3.2 (per year).

Dimensional Relationships

The relationship between different parameters were worked out by regression analysis. In all the cases, the shell length was considered as basic index to establish the relationship. The shell length - shell breadth, shell length - shell depth, shell length - flesh weight and shell length - dry weight relationships showed a linear growth pattern. The a, b and r values calculated are given in Tables 2.3, 2.4, 2.5 and 2.6. Interrelationship between different dimensions are given in Figures 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 and 2.10.

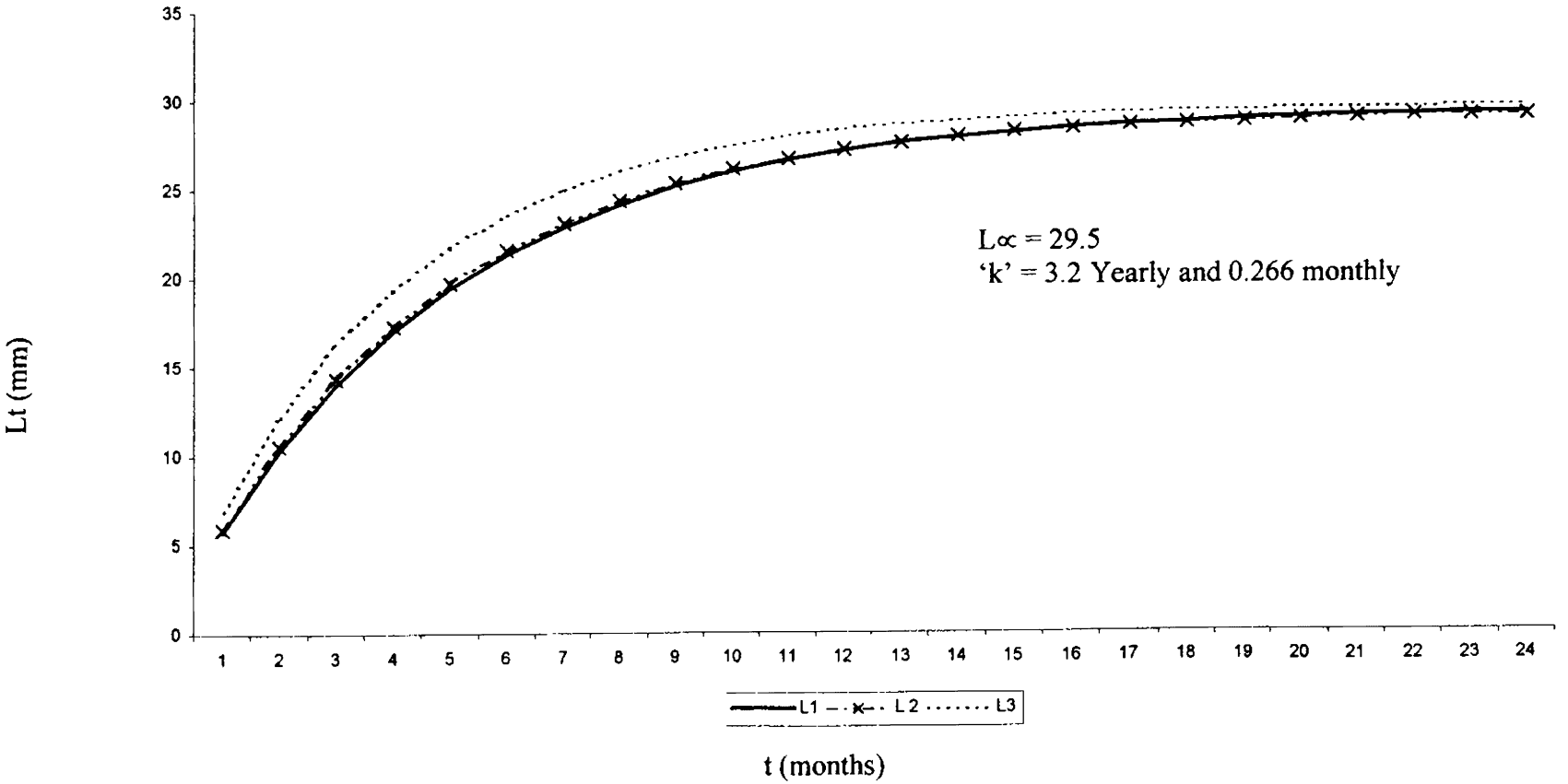


Fig 2.1a. Von Bertlanffy growth curve for *Donax incarnatus* inhabiting the Malippuram beach.

Table 2.3. Allometric relationship between shell length and breadth

(Samples not available from May to August)

Month	N	Log a	b	r
February '90	164	-0.2231	0.7090	0.7013
March	264	-0.1387	0.7893	0.8871
April	180	-0.1385	0.9830	0.9746
September	323	-0.4425	1.2391	0.9881
October	264	-0.5125	1.2988	0.8915
November	264	-0.6192	1.3133	0.8703
December	170	-0.1300	0.9841	0.9349
January '91	264	-0.9131	1.5901	0.9764
February	100	-0.1572	1.0413	0.8897

Table 2.4. Allometric relationship between shell length and depth

(Samples not available from May to August)

Month	N	Log a	b	r
February '90	164	-0.2721	0.4875	0.7874
March	264	-0.4904	1.0750	0.8832
April	180	-0.6381	1.1776	0.9626
September	323	-0.5038	1.0704	0.9576
October	264	-0.6720	1.2270	0.8991
November	264	-0.5089	1.0426	0.8000
December	170	-0.5102	1.0903	0.9107
January '91	264	-0.8555	1.3542	0.9695
February	100	-0.4469	1.0413	0.8166

Table 2.5. Allometric relationship between shell length and wet flesh weight

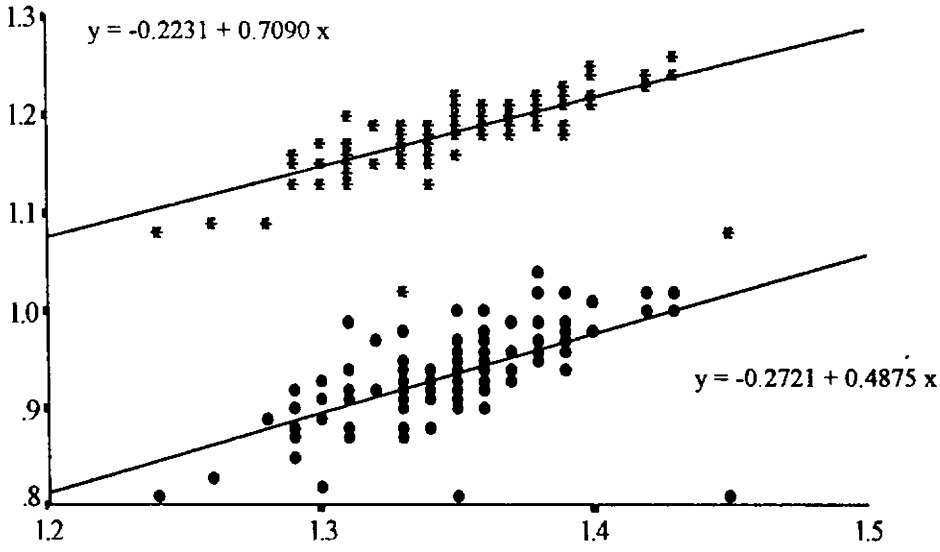
(Samples not available from May to August)

Month	N	Log a	b	r
February '90	164	-3.1616	2.0432	0.6607
March	264	-5.7272	3.9889	0.6123
April	180	-3.4972	2.3088	0.9104
September	323	-4.9511	3.3245	0.9595
October	264	-4.6414	3.0065	0.5698
November	264	-5.4759	3.6139	0.6555
December	170	-5.6354	0.7089	0.7757
January '91	264	-5.7588	3.9769	0.9424
February	100	-3.7707	2.4462	0.7763

Table 2.6. Allometric relationship between shell length and dry flesh weight

(Samples not available from May to August)

Month	N	Log a	b	r
February '90	164	-3.3093	1.7533	0.3069
March	264	-4.5061	2.6140	0.4391
April	180	-2.9512	1.4540	0.5402
September	323	-5.8531	3.7058	0.9595
October	264	-4.0048	2.0706	0.4048
November	264	-5.4759	3.2570	0.6555
December	170	-5.0761	2.8658	0.6507
January '91	264	-5.7588	2.7791	0.9424
February	100	-5.1255	3.1028	0.4429



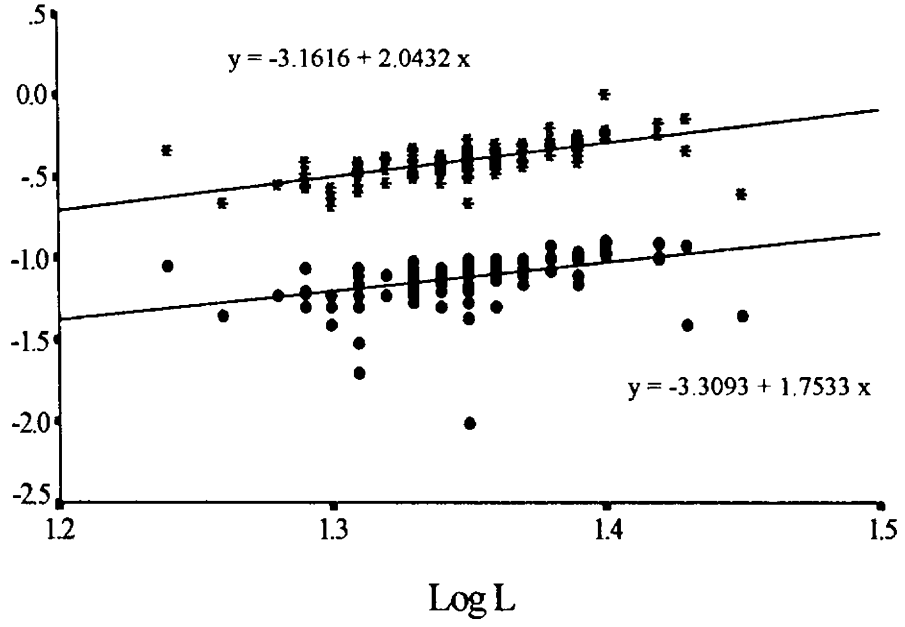
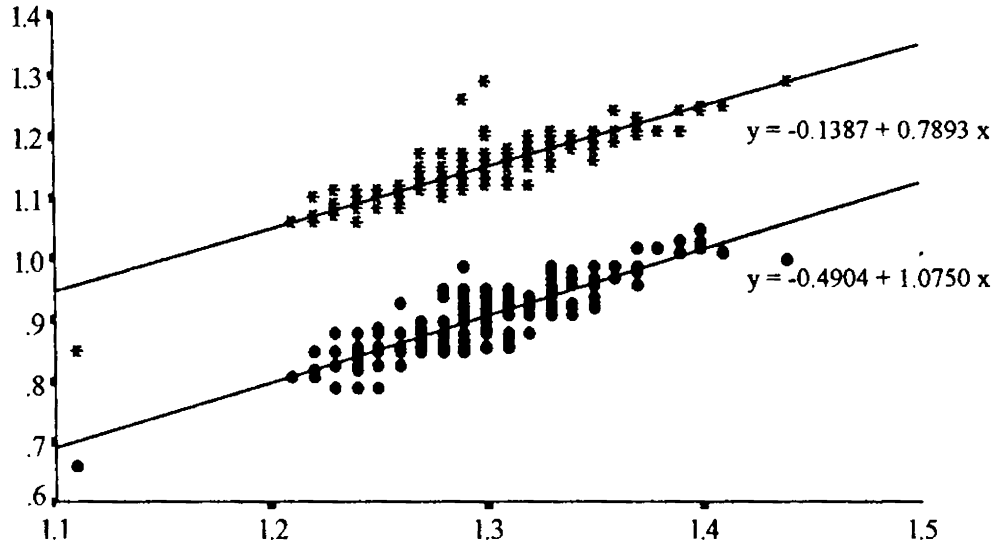


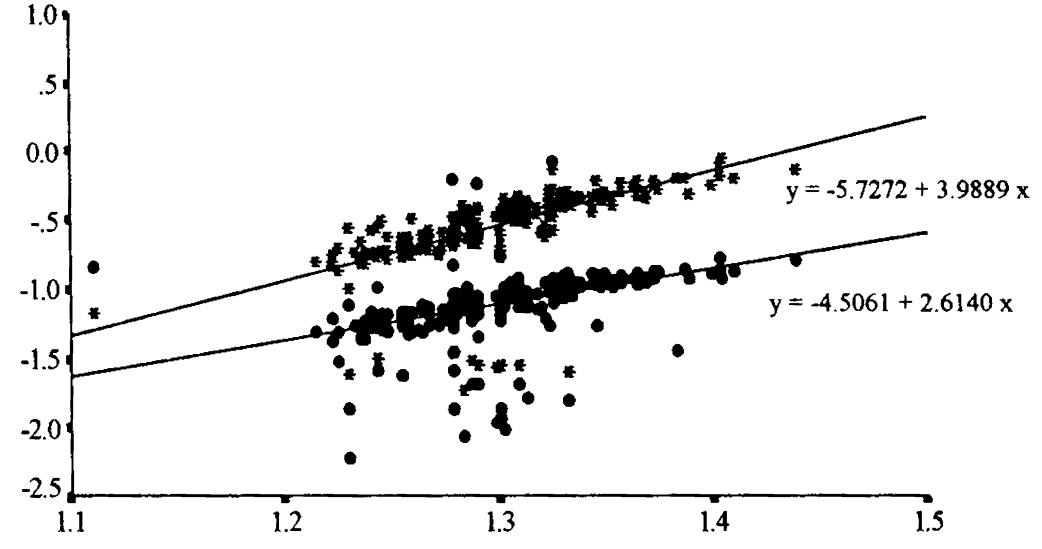
Fig 2.2 Length - breadth, depth, wet flesh weight and dry flesh weight (February 1990)



Log L

* Breadth

• Depth

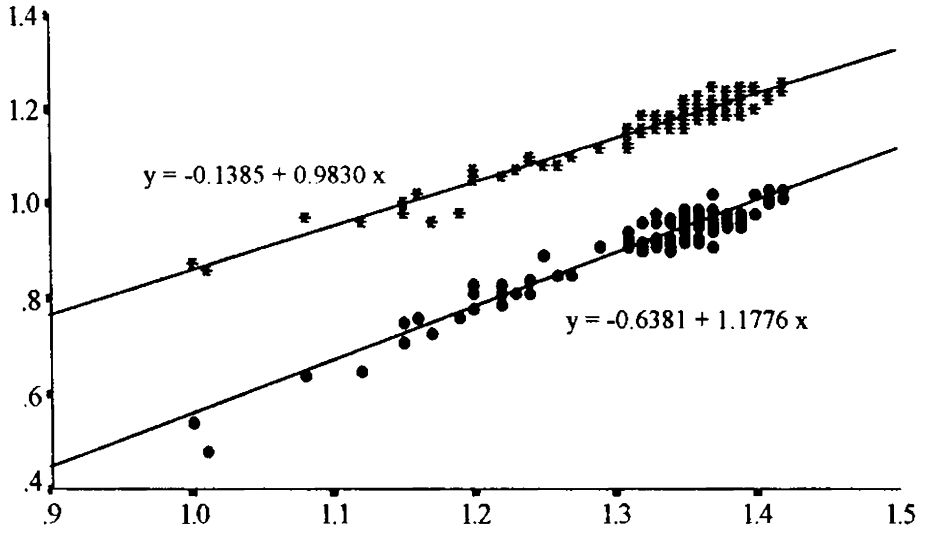


Log L

* Wet flesh weight

• Dry flesh weight

Fig 2.3 Length - breadth, depth, wet flesh weight and dry flesh weight (March 1990)



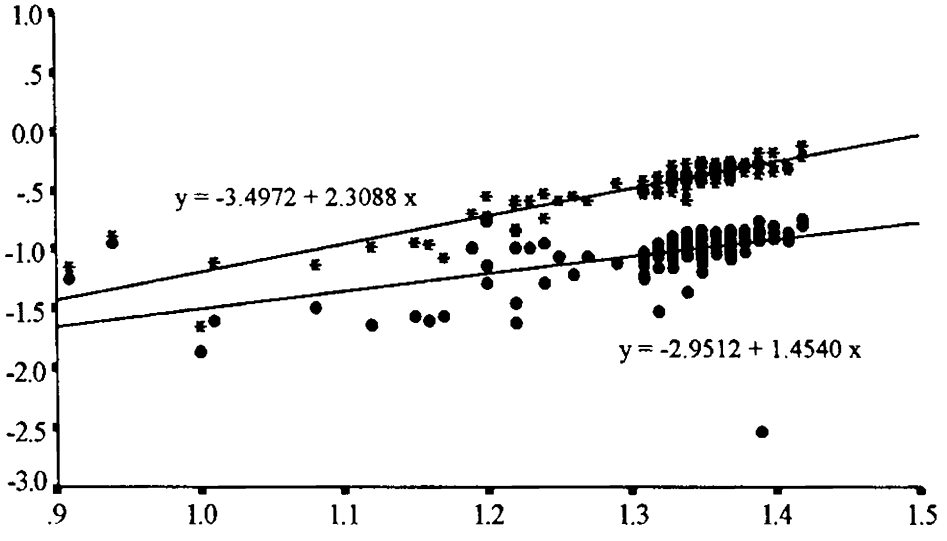
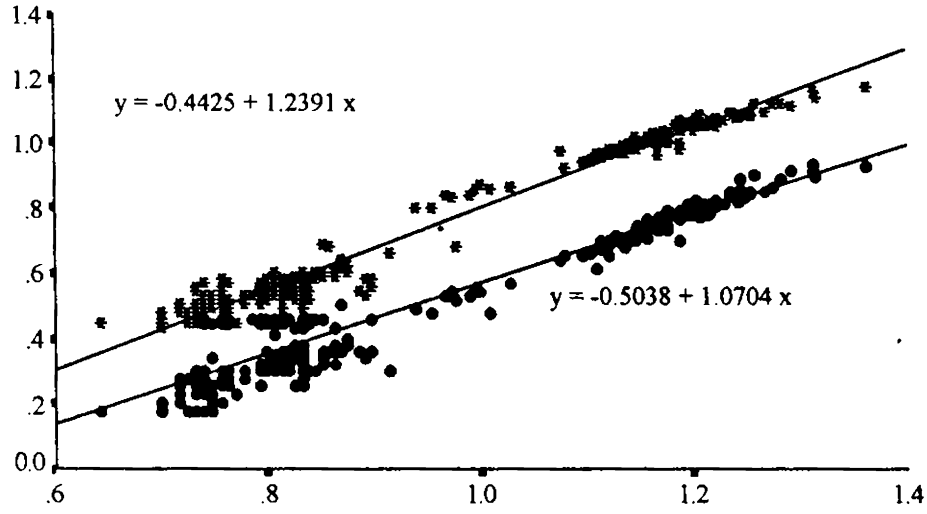
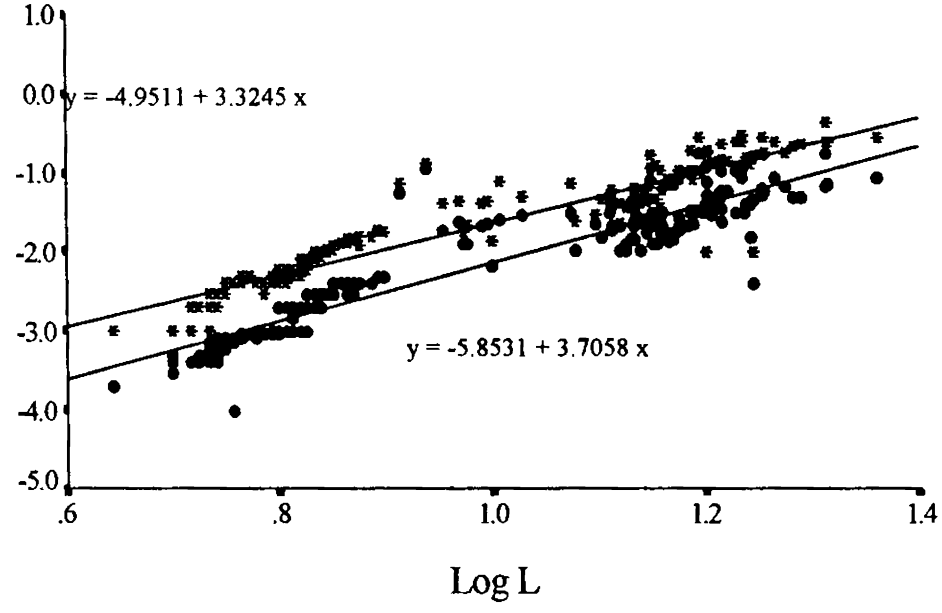


Fig 2.4 Length - breadth, depth, wet flesh weight and dry flesh weight (April 1990)

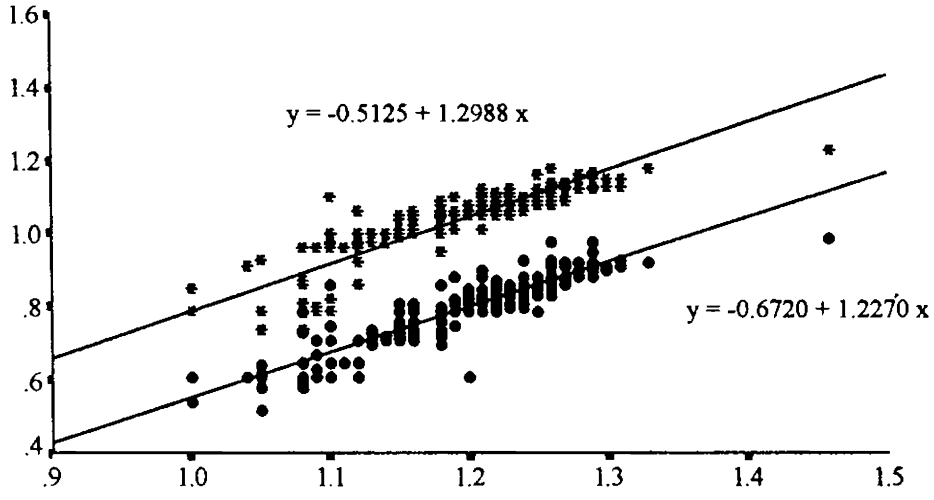


* Breadth
• Depth



* Wet flesh weight
• Dry flesh weight

Fig 2.5 Length - breadth, depth, wet flesh weight and dry flesh weight (September 1990)



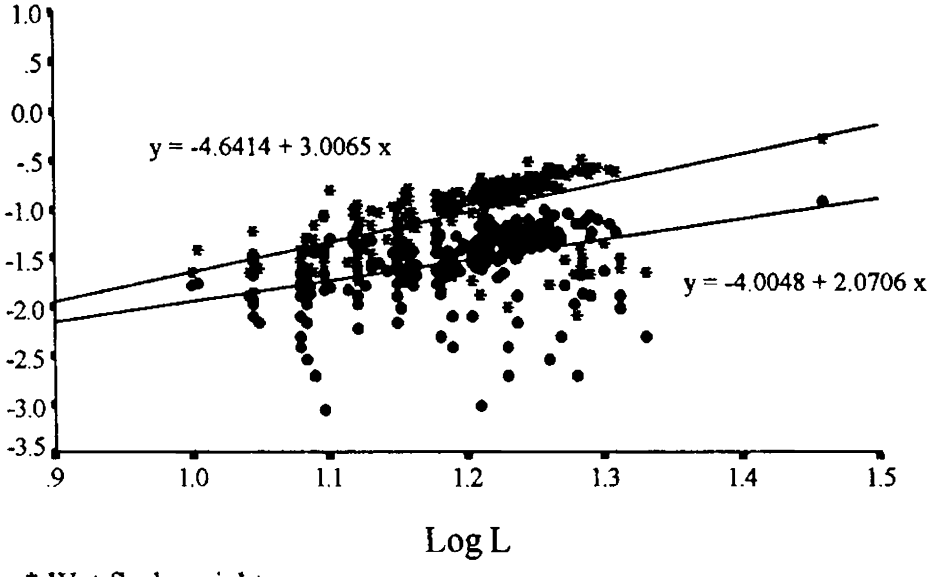
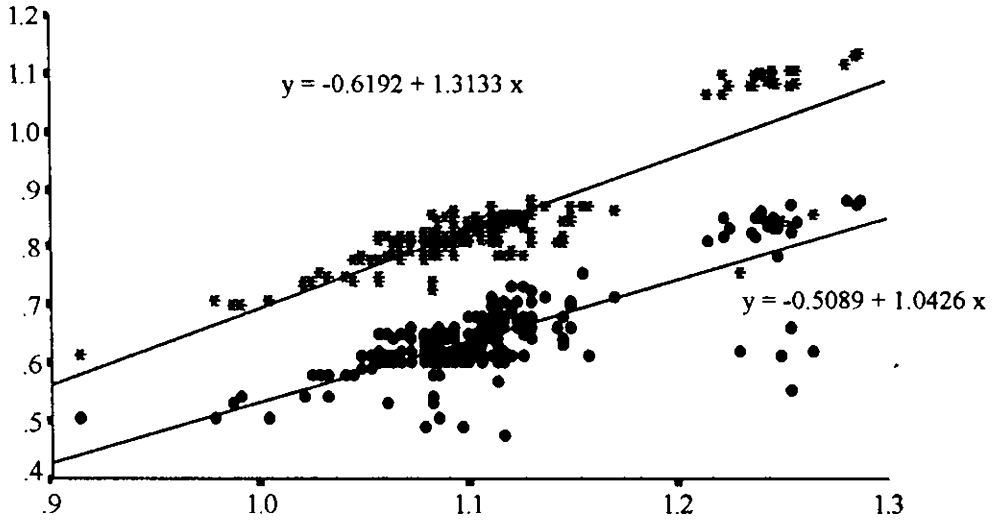
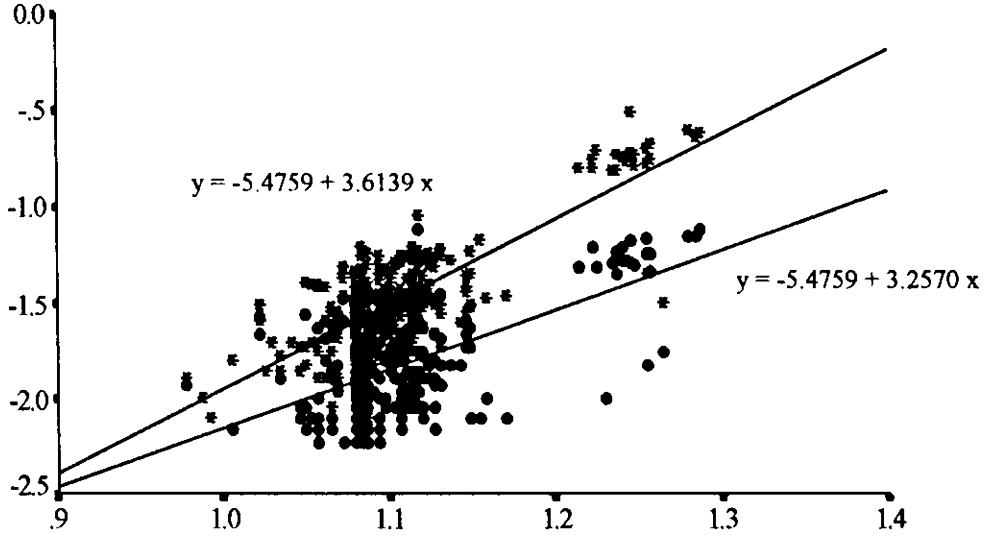


Fig 2.6 Length - breadth, depth, wet flesh weight and dry flesh weight (October 1990)



Log L

* Breadth
• Depth

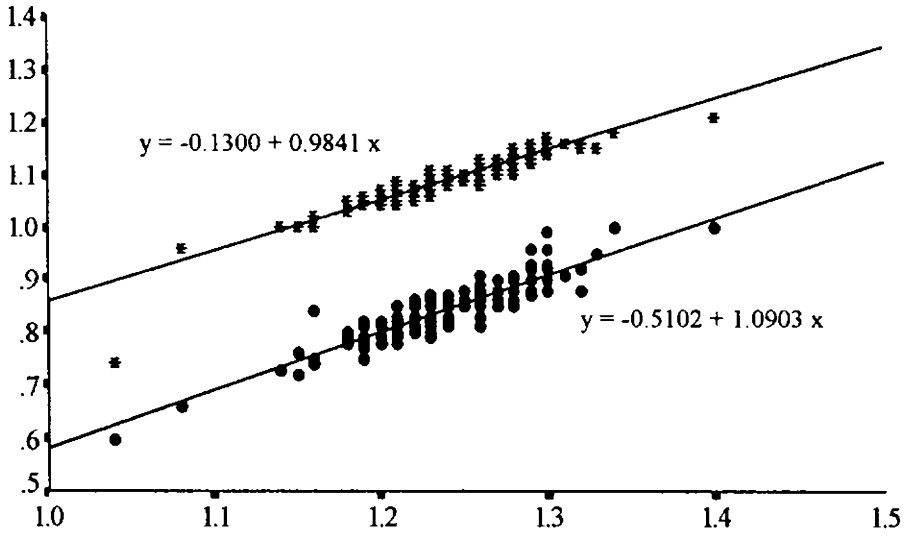


Log L

* Wet flesh weight
• Dry flesh weight

Fig 2.7 Length - breadth, depth, wet flesh weight and dry flesh weight

(November 1990)



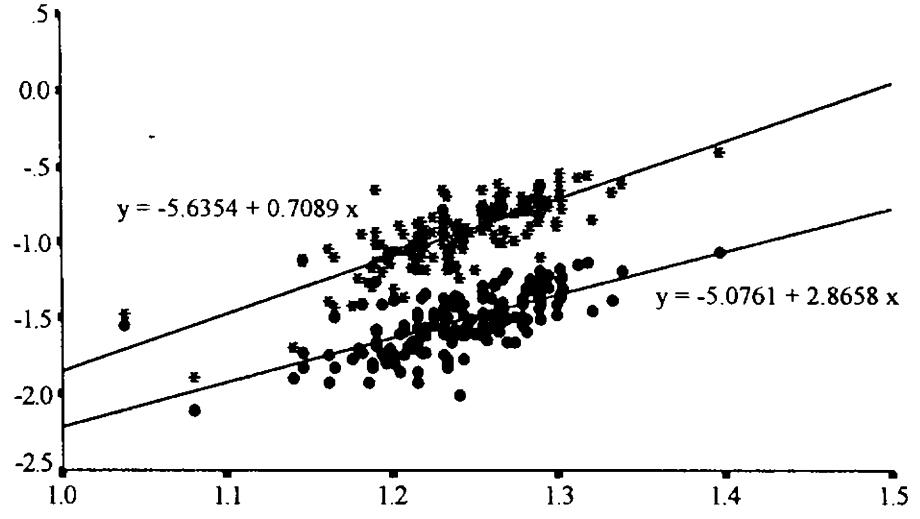
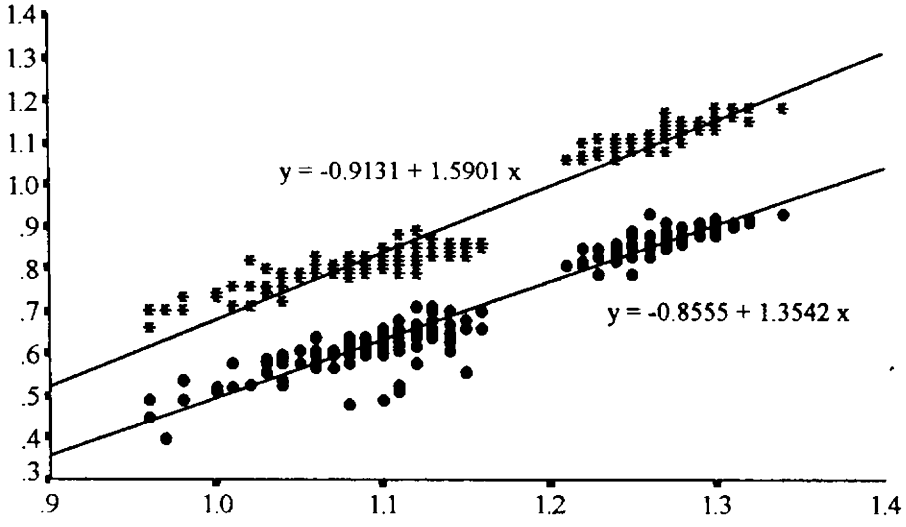
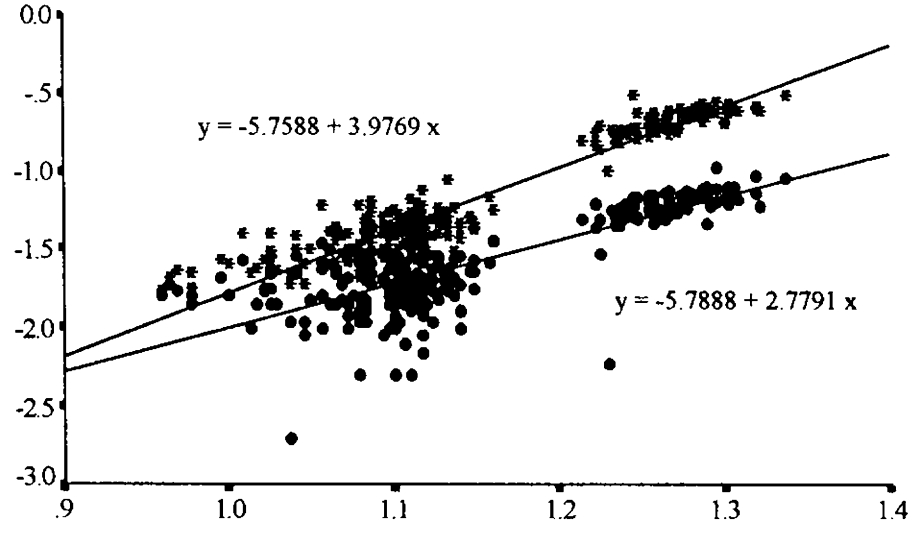


Fig 2.8 Length - breadth, depth, wet flesh weight and dry flesh weight (December 1990)



Log L

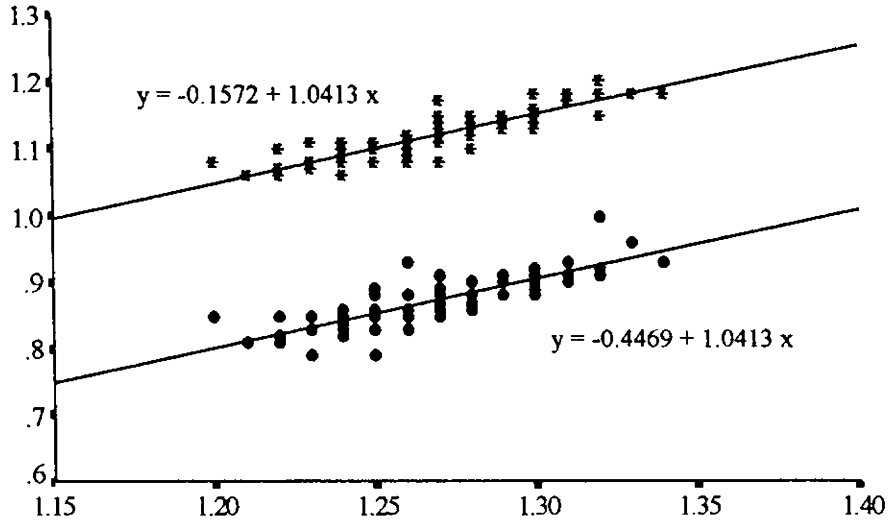
* Breadth
• Depth



Log L

* Wet flesh weight
• Dry flesh weight

Fig 2.9 Length - breadth, depth, wet flesh weight and dry flesh weight (January 1991)



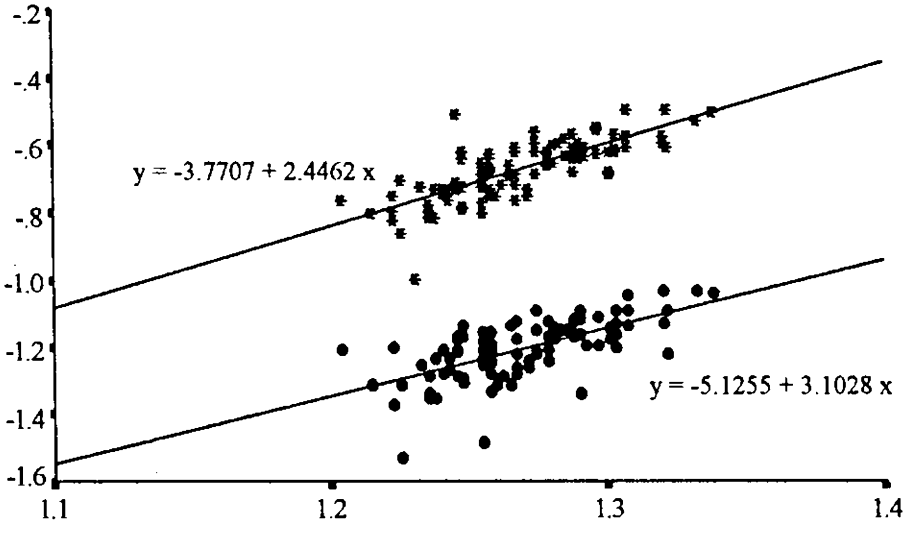


Fig 2.10 Length - breadth, depth, wet flesh weight and dry flesh weight (February 1991)

Covariance analysis of the various relationships between dimensions by month is given in Tables 2.7, 2.8, 2.9 and 2.10. Covariance analysis of the linear regression of logarithm of breadth on logarithm of length during different months were significant. ($P<0.01$). Here the growth in breadth and growth in depth showed variation with length. Growth rate was high during January followed by September, October and November. Lowest growth rate was noticed in February.

The analysis of covariance between length and flesh weight showed a significance at 0.01% level. This indicates that the rate of growth varied between months. The maximum growth rate were observed in January, March, September, October and November and lower growth rate were observed in February, covariance between length and dry weight also showed a significance at 0.01% level, between the regression coefficients. Here the maximum growth were observed in the month of September followed by November and February and slow growth were observed in the month of April.

DISCUSSION

The age and growth of *D. incarnatus* have been estimated by length frequency analysis and by ELEFAN I method. Seasonal allometric relationships were also determined to find out the relative growth rate.

Table 2.7. Analysis of Covariance of linear regression of logaritham of shell breadth on logaritham of shell length

(Samples not available from May to August)

							Deviation from regression		
Month	Σx^2	Σy^2	Σxy	N	df_1	Reg.Co.eff.	df_2	SS	ms
February '90	0.1685	0.1723	0.1195	164	163	0.7090	162	0.0875	0.0005
March	0.4567	0.5680	0.4518	264	263	0.7893	262	0.1211	0.0005
April	1.1139	1.1331	1.0949	180	179	0.9830	178	0.0568	0.0003
September	11.5789	18.2087	14.3440	323	322	1.2391	321	0.4319	0.0013
October	1.2486	2.6501	1.6216	264	263	12988	262	0.5440	0.0021
November	0.8335	1.8981	1.0947	264	263	1.3133	262	0.4604	0.0018
December	0.3558	0.3942	0.3501	170	169	0.9841	168	0.0496	0.0003
January '91	2.2003	5.8356	3.4987	264	263	1.5901	262	0.2724	0.0010
February	0.0770	0.1251	0.0802	100	99	1.0413	98	0.0416	0.0004
Pooled	18.0332	30.9852	22.6550	1993	1984	1.2563	1975	2.0653	0.00105
							1983	2.5230	0.0010
					Difference between slopes		8	0.4577	0.0570

F(8,1975) = 54.4881

Table 2.8. Analysis of Covariance of linear regression of logaritham of shell depth on logaritham of shell length

(Samples not available from May to August)

							Deviation from regression		
Month	Σx^2	Σy^2	Σxy	N	df_1	Reg.Co.eff.	df_2	SS	ms
February '90	0.1685	1.1395	0.0822	164	163	0.4875	162	1.0994	0.0068
March	0.4567	0.6766	0.4909	264	263	1.0750	262	0.1489	0.0006
April	1.1139	1.6671	1.3117	180	179	1.1776	178	0.1223	0.0007
September	11.5789	14.4667	12.3943	323	322	1.0704	321	1.1995	0.0037
October	1.2486	2.3257	1.5320	264	263	1.2270	262	0.4460	0.0017
November	0.8335	1.4155	0.8690	264	263	1.0426	262	0.5117	1.8554
December	0.3558	0.5099	0.3879	170	169	1.0903	168	0.0870	0.0005
January '91	2.2003	4.2925	2.9796	264	263	1.3542	262	0.2575	0.0010
February	0.0770	0.1251	0.0802	100	99	1.0413	98	0.0416	0.0004
Pooled	18.0332	26.6186	20.1278	1993	1984	1.1162	1975	3.9139	0.00199
							1983	4.1529	0.6021
						Difference between Slopes	8	0.2390	0.0299

$F(8,1975) = 19.6945$

Table 2.9. Analysis of Covariance of linear regression of logaritham of flesh weight on logaritham of shell length

(Samples not available from May to August)

							Deviation from regression		
Month	Σx^2	Σy^2	Σxy	N	df_1	Reg.Co.eff.	df_2	SS	ms
February '90	0.1685	1.6135	0.3444	164	163	2.0432	162	0.9099	0.0056
March	0.4567	19.3838	1.8216	264	263	3.9889	262	12.1175	0.0462
April	1.1139	7.1641	2.5717	180	179	2.3088	178	1.2267	0.0069
September	11.5789	139.0005	38.4942	323	322	3.3245	321	11.0347	0.0344
October	1.2486	34.7648	3.7539	264	263	3.0065	262	23.4788	0.0896
November	0.8335	25.3339	3.0123	264	263	3.6139	262	14.4480	0.0551
December	0.3558	8.4717	1.3467	170	169	3.7827	168	3.3745	0.7326
January '91	2.2003	39.1817	8.7505	264	263	3.9769	262	4.3820	0.0167
February	0.0770	0.7634	0.1884	100	99	2.4462	98	0.3026	0.0031
Pooled	18.0332	275.6774	60.2847	1993	1984	3.3429	1975	71.2747	0.0361
							1983	74.1466	0.0374
				Difference between slopes			8	2.8719	0.3589

F (8, 1975) = 9.9441

Table 2.10. Analysis of Covariance of linear regression of logaritham of dry flesh weight on logaritham of shell length

(Samples not available from May to August)

							Deviation from regression		
Month	Σx^2	Σy^2	Σxy	N	df_1	Reg.Co.eff.	df_2	SS	ms
February '90	0.1685	2.8875	0.2955	164	163	1.7533	162	2.3694	0.0146
March	0.4567	16.1862	1.1937	264	263	2.6140	262	13.0658	0.0499
April	1.1139	8.0699	1.6195	180	179	1.4540	178	5.7152	0.0321
September	11.5789	176.2531	42.9094	323	322	3.7058	321	17.2383	0.0537
October	1.2486	32.6718	2.5853	264	263	2.0706	262	27.3188	0.1043
November	0.8335	38.8790	2.7148	264	263	3.2570	262	30.0371	0.1146
December	0.3558	6.9022	1.0196	170	169	2.8658	168	3.9804	0.0237
January '91	2.2003	24.9655	6.1150	264	263	2.7791	262	7.9713	0.0304
February	0.0770	3.7761	0.2390	100	99	3.1028	98	3.0347	0.0310
Pooled	18.0332	310.5913	58.6918	1993	1984	3.2547	1975	110.7310	0.0561
							1983	119.5699	0.0603
						Difference between slopes	8	8.8389	1.1049

$F(8,1975) = 19.6945$

In the present study, samples collected in February, March and April revealed dominance of medium and large clams and again during September-February. Young clams were also seen in most of the months, indicating its continuous breeding pattern. Decrease percentage occurrence in number of small-sized clams may be related to the mortality of young clams due to overcrowding.

Specimens were not available during May-August due to high mortality and extensive environmental disruption caused by annual monsoon. This closely agrees with Mc Luscky *et al.*, (1975) in *D. incarnatus* in the sandy beaches of Goa. Another reason for the disappearance of this clam is due to its migratory behaviour from low to high saline condition. However, confirmation of this fact requires further detailed observations during monsoon season.

During September, fresh recruits were added to the adult population. The growth were faster during the following months indicating that in the early phase of life, growth rate is faster and retardation of growth occurs as age increases. During premonsoon season, the growth rate was moderate while during postmonsoon season, the growth rate was fast. This may be due to the adequate food availability and nutrient rich water after the monsoon season. Along the Indian coast, the maximum primary productivity is

observed during postmonsoon season (October-January) and medium to low during premonsoon (February-May) and monsoon respectively (Quasim, 1977).

There was not much difference in L_{∞} values (29.1 - 29.5) for the estimated three sets of growth curves, which indicates a similar growth pattern. The observed maximum size of *D. incarnatus* in the present area of study was 28.2 mm which closely agrees with the estimated theoretical maximum length of 29.5 mm. The coefficient 'k' value in the present study (2.6-3.2/year) does not show much variation, which indicates similar growth pattern. The slight changes obtained in the value may be due to the small modal length observed during September.

Based on the data collected, it can be concluded that the life span of this species is hardly one year. This observation closely agrees with the findings of Tippeswamy and Joseph (1991) in *D. incarnatus* from Panambur beach.

Knowledge of allometry in shell and soft body characters is essential to fully understand the growth of the species (Gould, 1966). Allometry in the shell length-breadth and shell length-depth variables are linearly related and shows that short individuals are narrow (less breadth) and low (less thickness), and inversely, long individuals are wide (more breadth) and broad

(more thickness). This clearly reflects the fact that length, breadth and width are influenced by one general attribute, that is variation in size. However, some individual of the same length show different breadth and width and these differences constitute shape variation. Thus, proportionate change in the shell dimensions resulted in retaining the wedge shape.

For this species, shape is probably of major adaptive significance, because of the importance of rapid burrowing in the surf beaten intertidal sandy shore where the environmental factors fluctuate. According to Wilbur and Owen (1964), a variety of environmental factors are known to influence shell form in bivalves. Thus, shape rather than size, generally provides more precise information on the dimensional relationships. Similar result was observed by Tippeswamy and Joseph (1992) in *D. incarnatus* from Panambur beach.

From the present observations, it can be seen that linear relationship exists between length and flesh weight. This is because as age increases the weight of clams also increases. Although morphometric relationship between length and flesh weight showed linear growth pattern, variation in this relationship can be explained on the basis of the differences in different phase of life. The maximum value seen in September coincided with the period when there were large numbers of young ones. This indirectly was

suggestive of the breeding season of the clams. The increase in growth in the rest of the months (January, March, October and November) may be largely due to somatic tissue growth and accumulation of food reserve before sexual maturity. This closely agrees with the observations of Shiny and Radhakrishnan (1995) in *Musculista senhausia* from Cochin backwater.

Although length-dry tissue weight relationship showed linear relationship, significant variations were observed between months. The high growth value observed in the month of September, November and February may be due to the presence of new recruits and maturing ones in the population. Low value observed in the month of April was due to the presence of spent animals encountered in the population.

It is well known that in tropical waters, changes in temperature were negligible and therefore salinity has been found to influence the growth of marine clams (Nayar, 1955). In the present study, due to extensive disruption caused by annual monsoon, data were not available. Mortality of animals were confirmed by observing dead shells during monsoon season. Here low salinity may be a factor for the mortality of the animals.

From the present study, it can be concluded that more or less similar growth pattern is observed in different months though a faster growth rate

was observed during postmonsoon due to the recruitment of small sized clams in September, which grew at a faster rate initially.

Growth studied by length frequency distribution and by using ELEFAN I method supports this observation. However, the allometric relationship between different dimensions studied presents difference in different months. It is apparent that while shell length - shell breadth and shell length - shell depth relationships tended to be stable in *D. incarnatus* population, some differences occurred in other allometric relationships, which could be attributed to physiological and ecological variations.

CHAPTER III

Chapter III

REPRODUCTIVE BIOLOGY

Introduction

A thorough knowledge on different aspects of reproduction is important and essential for understanding the annual recruitment to the population and to formulate suitable managerial measures to sustain the population in nature. Information on maturation process, breeding habits and the biotic and abiotic factors influencing the reproduction is imperative to develop suitable technologies for hatchery production of seed and for the culture of the species.

The process of reproduction in bivalves involves germ cell differentiation, gonad development, maturation, spawning, fertilisation, larval development and seed production. The pattern of reproduction differs from species to species according to various intrinsic and extrinsic factors. They may occur in a regular pattern resulting in annual or semi-annual cycle and continuous spawning can also occur with prominent peaks in particular seasons. In addition, environmental variables mainly salinity and temperature, are relied upon as synchronisers of the basic seasonal rhythm of gametogenesis.

Production of gametes in most of the marine bivalves requires a great deal of energy, suggesting a close relationship between the reproductive cycle and energy availability for growth (Bayne, 1985). Gonochorism is seen in majority of bivalves. The gametes are discharged through gonadal duct into the mantle cavity and from there into the surrounding water along with the exhalent water.

After the external fertilisation, larval development takes place in the ambient medium. Some bivalves exhibit hermaphroditism, sex reversal, incubation of developing young ones, etc. Failure in the reproductive activity may result in serious damage to the population structure thereby productivity. Thus, an understanding of the reproductive biology of the bivalves is essential for the proper management, culture and judicious exploitation of the resources. Several methods of assessing the course of reproductive cycle in marine bivalves have been employed. This includes direct observations on spawning in natural or laboratory populations or the occurrence of mature gonad in the population. Observations on gonadal smears and histological studies are resorted to understand the maturation process and to delineate the maturity stages. Relative abundance of developing, mature and spent population and larval abundance yield valuable information on spawning season, spawning intensity and spawning success.

REVIEW OF LITERATURE

Information on the breeding cycles of bivalves is extensively available from Indian waters. Abraham (1953) made detailed observations on the biology of the clam *Meretrix casta* regarding growth, breeding habits, longevity and mortality in the Adayar Estuary at Chennai (formerly Madras) backwaters. A comprehensive study on the reproduction of Australian pearl oyster *Pinctada albina* was conducted by Tranter (1958 a,b,c) which included primary gonad development, gametogenesis, breeding and sexuality and provided valuable information on the cytological aspects of reproduction. Mason (1958) reported the gonadal development, spawning, fertilisation, development of larvae and spat of *Pecten maximus* .

Durve (1964, 1965) made investigations on the seasonal gonadal changes and spawning in *Meretrix casta* and in the edible oyster, *Crassostrea gryphoides* from Bombay waters. A detailed investigation on the growth and reproduction of the clam, *Donax faba* in the Gulf of Mannar was carried out by Alagarswami (1966). Annual reproductive cycle of *Donax cuneatus* of the Madras Coast was studied by Rao (1967) based on the seasonal gonadal changes. Reproductive cycle of estuarine bivalve, *Musculista arcullata* was described by George and Nair (1973). Nagabhushanam and Mane (1975) reported on the reproductive biology of

mussel *Perna viridis* from Bhatia creek, Ratnagiri. Rao *et al.* (1975) studied the spawning, fertilisation and larval settlement of *Mytilus (=Perna) viridis.* Studies were conducted on the seasonal gonadal changes in the clam *Paphia laterisulca* (Nagabhushanam and Dhamne, 1977). Salih (1977) gave a detailed account on the breeding activity of the clam *Meretrix casta* off Cochin barmouth. Studies conducted on the reproductive biology of the wedge clam *Donax cuneatus* by Nagabhushanam and Talikhedkar (1977a) revealed an extended spawning cycle with no resting period .

Detailed observations on the gametogenic stages, reproductive cycles, spawning periodicity, size at first maturity and sex ratio of the oyster *Crassostrea madrasensis* were made by Joseph and Madhyastha (1982, 1984) from Mangalore Coast. Observations on gametogenesis and breeding of oyster *Ostrea edulis* were reported by Wilson and Simons (1985) on the west coast of Ireland. They formulated an equation for predicting the onset of maximum ripeness of oyster populations with the help of histological studies. Jayabal and Kalyani (1986a) reported the reproductive cycles of three commercially important bivalves *Meretrix meretrix, M. casta* and *Katelysia opima* of Vellar Estuary. A comparative study of the reproductive cycle of the soft- shelled clam *Mya arenaria* in Long Island Sound were done by Brousseau (1987). Discussing on the reproductive cycle of the hard clam

Mercenaria mercenaria in Wassan Sound, Georgia, Hefferman *et al.* (1988) found a synchronised polymodal breeding pattern. Narasimham (1988a) reported that the blood clam *Anadara granosa* in Kakinada Bay spawns throughout the year with two to four reproductive peaks.

Aspects of gametogenesis and spawning of the carpet-shell clam *Ruditapes decussatus* were reported by Shaffi and Daoudi (1991). Baron (1992) investigated the reproductive cycles of *Stactidea striata, Grafrarium timidum and Anadara scapha* . A comparative study was carried out by Xie *et al.* (1994) on the gametogenic cycles of the manila clam *Tapes philippinarum* and carpet shell clam *Tapes decussatus.* The gonadial developmental phases of the red clam *Megapitaria aurantiaca* were categorised into five stages using histological techniques (Garcia-Dominguez *et al.*, 1994). Etim (1996) elucidated that *Egeria radiata* spawns once in a year during the peak of the rainy season in the Nigerian waters. Sebastian (1997) observed that the black clam *Villorita cyprinoides* at Cochin backwater breeds twice a year with peak spawning activity in June-July and January-February.

Environmental differences resulted in different physiological responses with respect to timings of development and developmental pattern. Nagabhushanam and Mane (1975) correlated seasonal variation of

reproductive cycle of *Mytilus viridis* with fluctuations in temperature and salinity of the area. Increase in temperature and salinity soon after the monsoon appeared to promote gametogenesis and initiate spawning in *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977a). Nagabhushanam and Dhamne (1977) observed that the spawning stimulation in *Paphia laterisulca* appeared to be due to the sudden increase in salinity. Stephen (1980a, b) revealed that the influence of salinity during different seasons synchronised the gametogenic pattern in *Crassostrea madrasensis.* In *Saccostrea cucullata*, Sukumar and Joseph (1988) opined that an increasing salinity triggered the maturation while low saline condition initiated spawning. On the other hand, Victor and Subramoniam (1988) noticed an influence of low salinity and temperature on active gametogenesis in *Donax cuneatus* in Madras waters and high salinity and temperature on spawning. According to Robinson (1989), in *Crassostrea gigas*, optimum temperature and optimum salinity for larval rearing were 26^0C and 25 ppt respectively after studying the reproductive cycle and conditioning trials. Newell *et al.* (1989) studied the factors regulating reproduction and recruitment in populations of *Crassostrea virginica* and found that the low salinity has an adverse effect on survival rate. Sphigel (1989) observed that gametogenesis in *Ostrea edulis* occurred in salinity as high as 41 ppt.

Though *Donax incarnatus* is a species with considerable importance as a nutritious and proteinous food, very little work on the reproductive biology has been carried out so far. Hence, the present investigation has been taken up to elucidate the gametogenic pattern and the influence of environmental factors on the reproductive biology. More emphasis has been given to the studies of gonadal smears and histology.

MATERIAL AND METHODS

Samples were collected every month during the year 1991, except during monsoon period when no samples were available from the collection ground at Malippuram coast. Specimens of different size groups were used for the study. The clams were maintained in the laboratory conditions for 24 hours in filtered (environmental) seawater collected from the sampling site. The detailed microscopic observations of the individual gonad were made for both sexes for the description and classification of the developmental stages of the gonad.

Standard histological technique was used to assess the reproductive cycle. Twenty animals arbitrarily selected with respect to age and visible stage of gonad development were excised and fixed in Bouins fixative for 24 hr. The tissue was then washed for 5 minutes in running water, dehydrated in graded ethanol, embedded in paraffin wax, serial sections of 7μ were made

and spread on slides smeared with Mayer's albumen, stained in Mayer's hematoxylin, counterstained with eosin and mounted in the D.P.X mounting medium. Slides were examined under light microscope and classified into different developmental stages. Examination of the sections made from monthly samples and on different maturity stages furnished detailed information on the reproductive cycle including the actual period of spawning in the study area. The male - female ratio was recorded in each month and Chi-square test was conducted.

RESULTS

Histology

Development of Gonad

In *Donax incarnatus*, the gonad envelops the ramified digestive gland and the loops of intestine. It develops seasonally to greater proportions, swelling the visceral mass. The ripe gonad is cream or yellowish in appearance.

Donax incarnatus is found to be gonochoristic with no sign of sexual dimorphism. There is no indication of the existence of sex reversal or hermaphroditism in samples observed during the course of the study. The maturation process of gonad is classified into five main stages in addition to

an indeterminate stage and termed as (i) early gametogenesis, (ii) late gametogenesis, (iii) mature, (iv) partially spent and (v) spent stage based on the cytological examinations of the gonad. Both male and female clams follow a similar pattern in the gonadal change.

Indeterminate Stage (I_o)

No gametogenesis is discernible and the sex is indistinguishable. Most of the gonad consists of interfollicular connective tissue between the follicles. The follicle is usually expanded and the follicular wall is dominant.

Male : Early gametogenesis (MD_1)

The proliferation and differentiation of the small earliest cells form stem cells which are distributed around the follicular wall is seen. Proliferation of follicles become more conspicuous and interfollicular tissue is present, but reduced (Fig. 3.1). Follicles contain definitive spermatogonia, and spermatocytes separate from follicle walls.

Late Gametogenesis (MD_2)

Follicles larger, become packed together. The follicles deeply penetrate the visceral mass and the follicular walls contain predominantly spermatogonia (Fig. 3.2) with spermatocytes and a few spermatids radiating into the lumen of follicles.

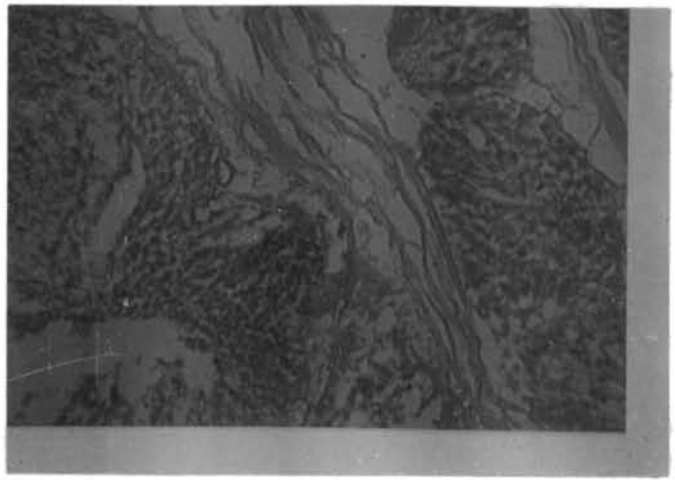


Fig. 3.1. Micrograph of early gametogenic stage of male gonad with proliferation of follicles and interfollicular tissue - MD_1

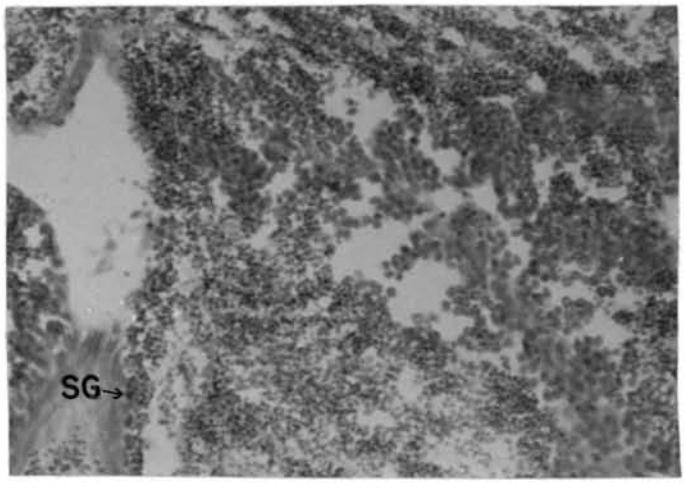


Fig. 3.2. Micrograph of late gametogenic stage of male gonad with spermatogonia (SG) - MD_2

Mature (MD_3)

The follicles contain closely packed sperm masses making appearance as streaks at various places in the gonad tissue (Fig. 3.3). They contain mainly spermatozoa. Spermatozoa aggregate in bands projecting into the lumen with their basophilic heads directed towards the periphery and sperm tails directed away from the wall towards the centre.

Partially spent (MR_1)

Follicles at various degrees of fullness are seen. 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. Connective tissue starts developing between the follicles (Fig. 3.4).

Spent stage (MR_2)

In the spent condition the gonad is characterised by contracted follicles and the lumen of the follicles contain residual spermatozoa, which are partially cytolysed by phagocytes (Fig. 3.5). Interfollicular tissue appears to occupy the space between the follicles.

Female: Early Gametogenesis (FD_1)

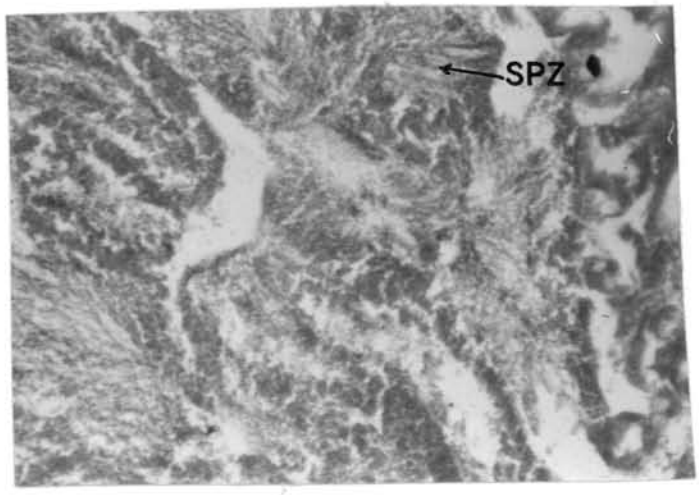


Fig. 3.3. Micrograph of mature male gonad with fully packed sperm masses (SPZ) - MD_3

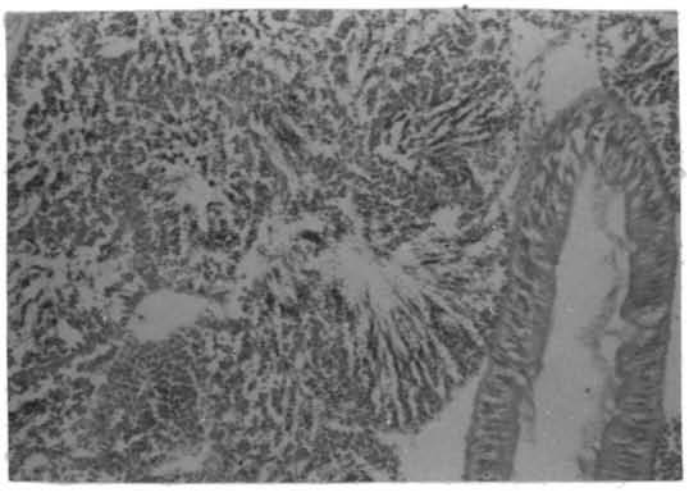


Fig. 3.4. Micrograph of partially spent stage of male gonad with moderate quantity of spermatozoa - MR_1

This process in many respects is very similar to that of the male. Sex differentiation starts with the differentiation of the germ cells in the connective tissue. Follicles appear as scattered patches in the gonad. Follicular wall is lined with oogonia, primary and secondary oocytes (Fig. 3.6). Oogonia are the initial female germ cells proliferated from the large resting cells ' the stem cells ' found around the follicular wall.

Late Gametogenesis (FD_2)

Follicles contain half-grown oocytes in the lumen and are attached to the wall by stalks; thin vitelline membrane is seen around some oocytes (Fig. 3.7). Follicular wall is lined with very a few oogonia and young oocytes; interfollicular tissue is seen.

Mature (FD_3)

Gonad is thick and attains larger size. Follicles contain full-grown and nearly round oocytes in the lumen of the follicles (Fig. 3.8). Free ova with nuclei are also found in the lumen. Pedunculated secondary oocytes are few and attached to the follicular wall.

Partially Spent Stage (FR_1)

This stage is characterised by the reduction in density of ova and rounding off as the pressure within the follicles is reduced (Fig. 3.9). Active

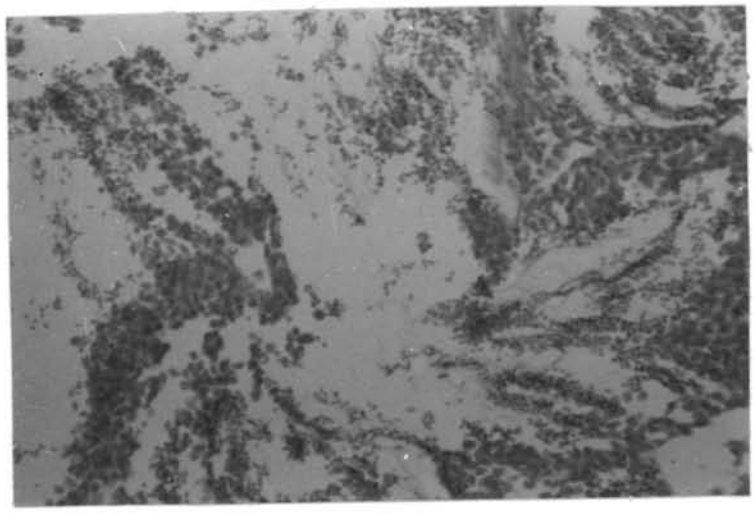


Fig. 3.5. Micrograph of spent stage of male gonad with residual spermatozoa and interfollicular tissue - MR_2

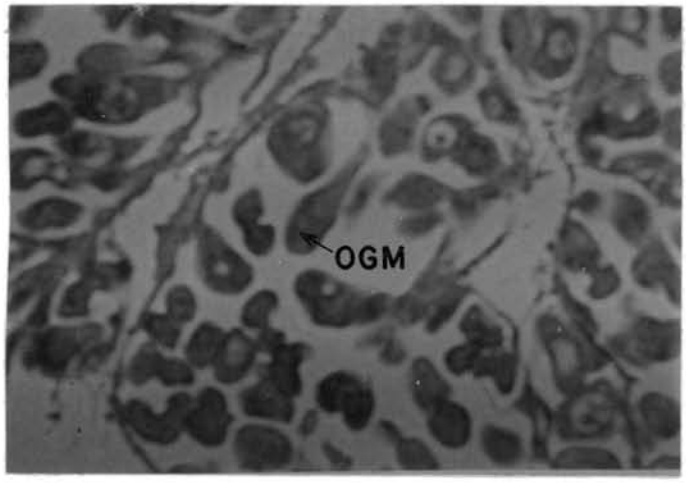


Fig. 3.6. Micrograph of early gametogenic stage of female gonad with developing oocytes (OGM) - FD_1

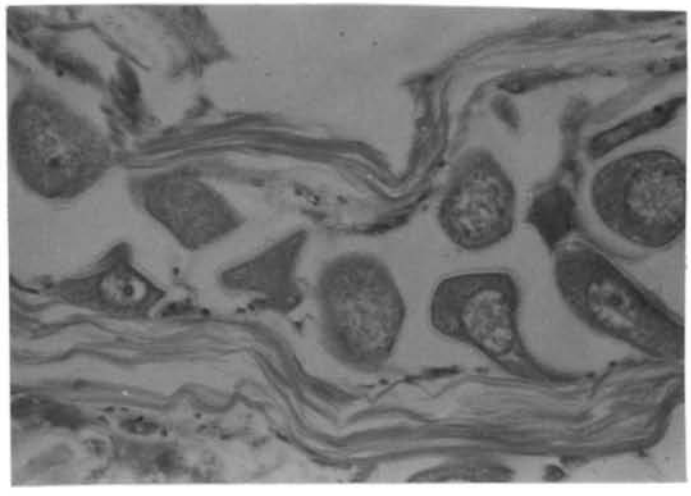


Fig. 3.7. Micrograph of late gametogenic stage of female gonad with pedunculated oocytes - FD_2

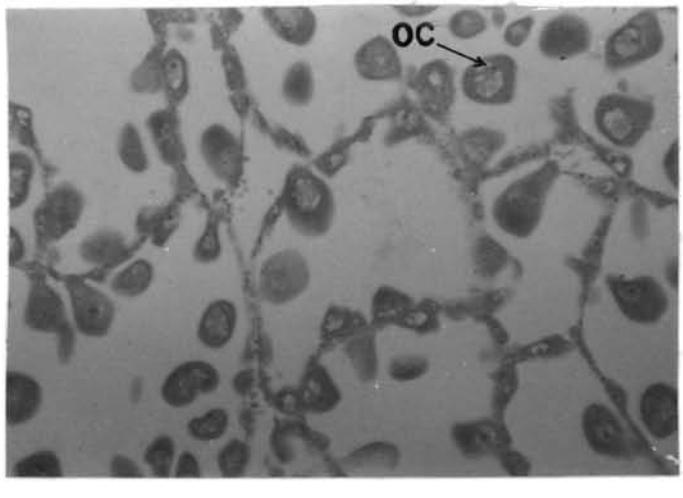


Fig. 3.8. Micrograph of female gonad with fully grown and nearly round oocytes (OC) - FD_3

discharge of ripe ova takes place and as it proceeds, the central portion of the follicles remain vacant

Spent Stage (FR_2)

Gonad remarkably shrunkens in this stage and is in different stages of phagocytosis. Spawning in females is not complete. Interfollicular tissue is seen (Fig. 3.10). Phagocytes present in this stage are for the resorption of the relict eggs by cytolysis.

In the present study the males were found to be in abundance only in April. In all other months, females were predominated (Table 3.1). As the clams reach 13-14 mm., they become sexually mature.

Annual Reproductive Cycle

In the annual reproductive cycle of *Donax incarnatus* studied over a year period has shown some sort of similarity in the development of the gonads in the two sexes. The percentage distribution of different stages of the gonads in different months is given in Table 3.2 and Fig. 3.11and 3.12.

In January, the male clams were in late gametogenesis phase. Most of the clams were in the developing (45%) and mature condition (30%) while a few of them were still found in the spawning and spent phase. In February and March, most of the males were in mature and partially spawned phase.

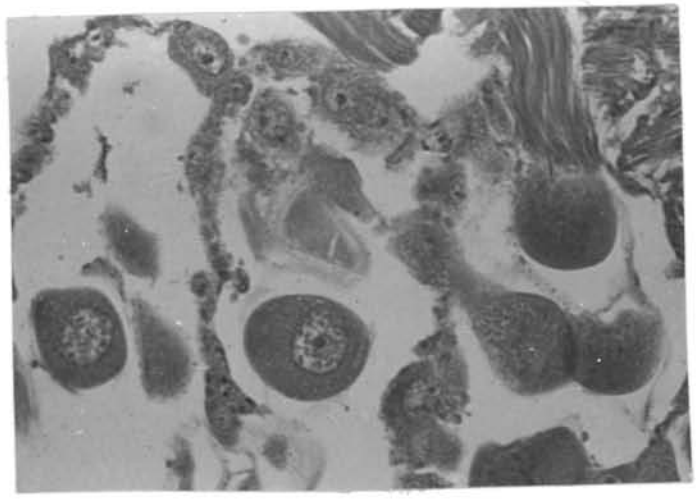


Fig. 3.9. Micrograph of partially spent female gonad with reduction in density of ova - FR_1

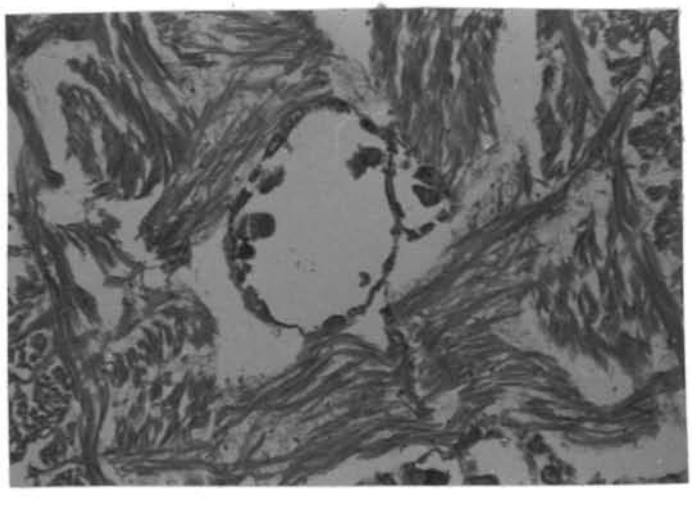


Fig. 3.10. Micrograph of spent female gonad showing regression of oozytes with connective tissue - FR_2

Table 3.1. Distribution of male and female *D. icarnatus* during 1991
(Samples not available from May to August)

Month	Male	Female	Chi-square
January	35	40	0.33
February	30	34	0.25
March	35	27	0.56
April	38	42	0.20
May - August	Nil	Nil	Nil
September	35	37	0.05
October	42	46	0.18
November	31	41	1.39
December	41	44	0.11
	287	311	

Table 3.2. Percentage distribution of the different stages of gonad development in male and female of *D. incarnatus* in different months
(Samples not available from May to August)

	Gonadal stages of male						Gonadal Stages of female					
Month	IND.	MD_1	MD_2	MD_3	MR_1	MR_2	IND.	FD_1	FD_2	FD_3	FR_1	FR_2
January '91		10.00	45.00	30.00	10.00	5.00		9.09	50.00	22.7	13.64	4.55
February			5.00	30.00	55.00	10.00			4.55	31.8	50.00	13.64
March		5.5	11.11	27.77	55.55	5.55		5.00	15.00	20.00	50.00	10.00
April	9.09	4.56	4.55	18.18	18.18	45.45	4.16	4.16	4.16	12.5	20.83	54.16
September	47.83	4.35	4.35	21.74	17.39	4.35	50.00	4.16	4.16	25.00	12.5	4.16
October		4.17	16.66	45.83	25.00	8.33		3.57	17.86	42.86	25.00	10.71
November	4.76	9.52	57.14	19.05	9.52		3.70	7.41	48.14	18.51	14.81	7.41
December		4.55	4.55	31.8	50.00	9.09		4.16	4.16	33.33	50.00	8.3

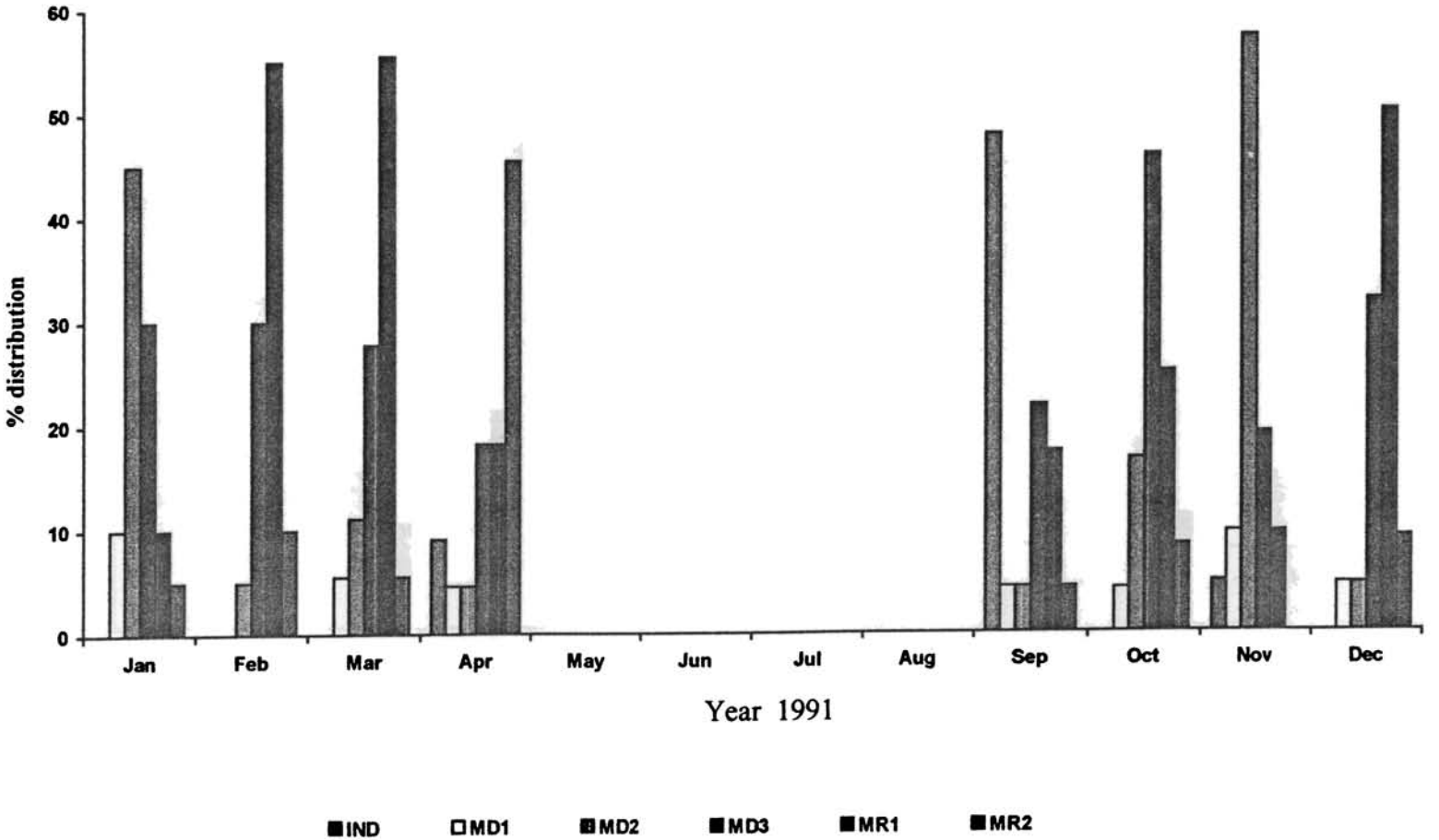


Figure 3.11 Percentage frequency of gonadal phases in *D. incarnatus* (Male)
(Samples not available from May to August)

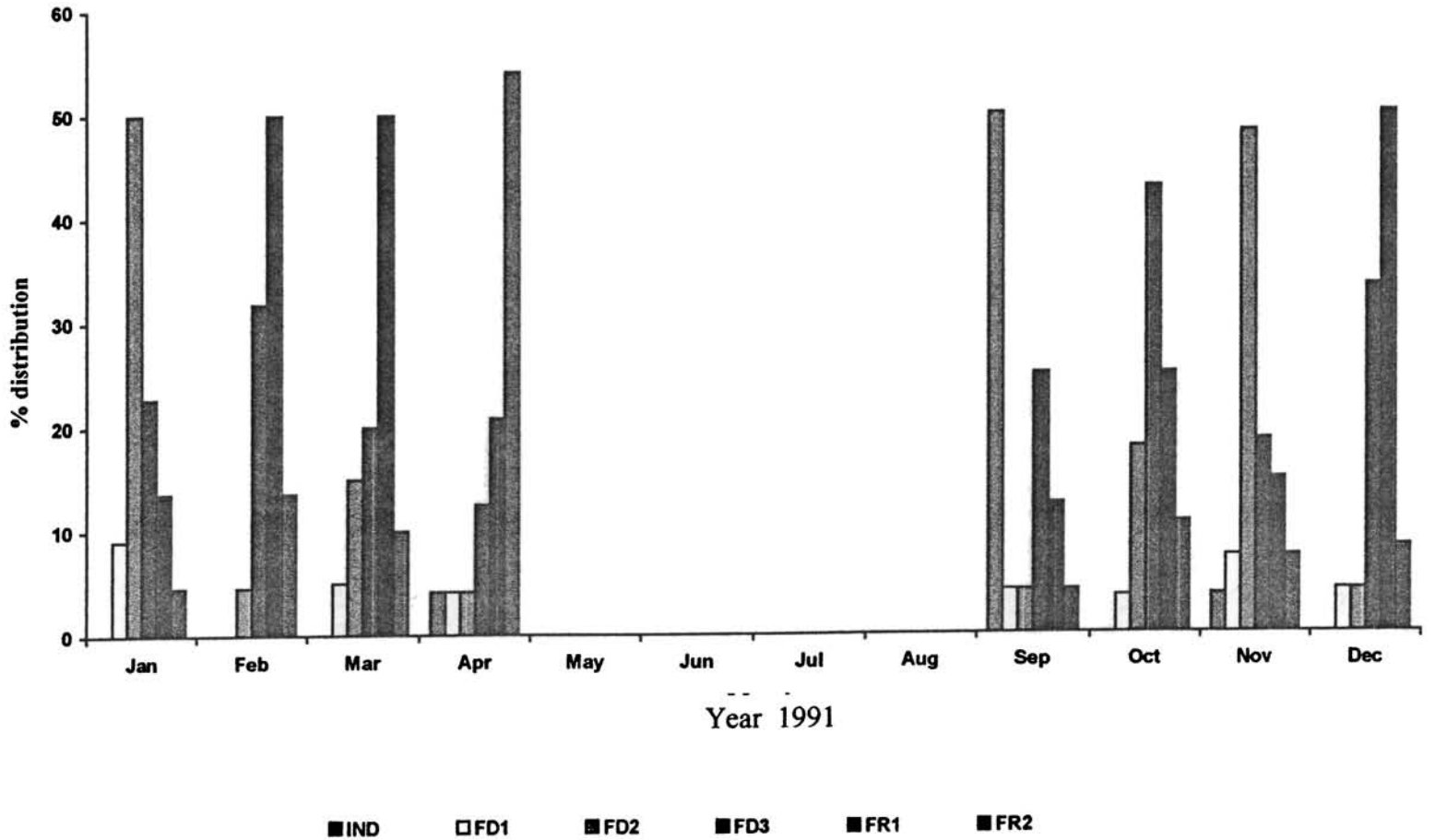


Figure 3.12. Percentage frequency of gonadal phases in *D. incarnatus* (Female)
(Samples not available from May to August)

Individuals with partially spawned condition were more (55%) in February and March, followed by mature phase (30%). Almost same percentage of mature and spawning phases was observed during February and March; the spawning phase was at its peak during these months. It was indicated by the presence of few spent clams (10% and 5.55%) with residual gametes. During April, 45.45% of the males were in spent phase with relict spermatozoa and spermatids in the lumen of the follicles. Others were partially spawned (18.18%) and mature (18.18%) condition.

After a break of few months, in September (Postmonsoon) most of the male clams collected were in indeterminate stage (47.83%). A few clams with mature (21.74%) and partially spawned (17.39%) conditions could be seen in the population. Small percentages of clams were in developing and spent stages. During October, males with mature gonad (45.83%) increased in number. Developing (16.66%) and spawning males (25%) were also observed. But in November, the late gametogenesis stage (57.14%) was more. Mature (19.05%) and partially spawned (9.52%) males were also observed in small percentage. During December, 50% of the population was in partially spawned, 31.8% in mature, 9.09 % in spent and 4.55% in early and late gametogenesis.

In the case of females, almost the same trend as in males could be observed. In January, 50% of the females in the population were in late gametogenesis phase. Individuals with mature (22.7%) and partially spawned gonads (13.64%) were also observed. A small percentage of early gametogenesis (9.09%), partially spawned (13.64%) and spent stage (4.55%) were also encountered in this month. During February and March, there was a rise in number of spawning females (50%) in the population while mature females registered only 31.8% and 20% respectively. In April, majority of the female clams were in spent condition (54.16%) in the population. This was followed by partially spawned (20.83%) and mature (12.5%) stages. Small percentage (4.16%) of developing and indeterminate clams was also encountered along with other maturity stages.

After the break of four months, during September most of the clams were in indeterminate condition (50%). Besides, 25% of the clams showed mature, 12.5% partially spawned state of gonad. About 4.16% of the clams were in developing (early and late gametogenesis) and post-spawned stages. During October, clams of mature stage constituted 42.86%. Clams with all the other stages of gonad could be seen in this month. During November, most of the clams were in late gametogenesis stage (48.14%). A few animals with mature (18.51%) and partially spawned (14.81%) gonads could be seen.

A small percentage of indeterminate and late gametogenesis stages could also be observed during the period. All the four stages were observed during December. Individuals with partially spawned (50%) followed by mature (33.33%) condition predominated in the population.

DISCUSSION

Bivalves are the group characterised by gonochorism and hermaphroditism. According to Coe (1943), about 96% of the species included in the Class Bivalvia have separate sexes. In the present study, the wedge clam *Donax incarnatus* is found to be gonochoristic and showed no signs of sex reversal and hermaphroditism. Similar observations were made by Nagabhushanam and Talikhedkar (1977a) in *Donax cuneatus* and in *Crassostrea madrasensis* (Stephen, 1980b; Joseph and Madhyasta, 1984).

The result of the test of variance for homogeneity revealed that the Chi-square value is found to be insignificant.

The classification of different stages in the reproductive cycle in *D. incarnatus* was very similar to the allied species of the bivalves. The maturation process of both male and female gonad involved five maturity stages, namely, early gametogenesis, late gametogenesis, mature, spawning and spent. This is in agreement with the classification of maturity stages

described by Nagabhushanam and Talikhedkar (1977a) and Victor and Subramonium (1988) in *D. cuneatus.*

The breeding habits and seasonal changes of many species of pelecypods from different parts of the world have been recorded: *Meretrix casta* by Abraham (1953); Durve (1964); Salih (1977); *Musculista arculata* Leela and Balakrishnan (1973); *Paphia laterisulca* Nagabhushanam and Dhamne (1977); *Crassostrea madrasensis* Joseph and Madhyastha (1984); *Anadara granosa* Narasimham (1988a); *Villorita cyprinoides* Modassir (1991) and *Marcia opima* by Maqbool (1993).

In the present investigation, *Donax incarnatus* showed a continuous breeding pattern with two spawning peaks in February-March and in December (Fig. 3.11 and 3.12). In tropical species, continuous breeding may not of the same intensity throughout the period and the fluctuations seen may probably be due to the environmental variations. The present findings closely agrees with Alagarswami (1966) in the species *Donax faba* from Mandapam area of the Southwest coast of India, but this species shows a prolonged breeding period with two peaks. The nature of reproductive cycle of a population of one locality is found to differ from that of another population of the same species occurring at a different locality

(Nagabhushanam and Talikhedkar, 1977a; Victor and Subramonium, 1988) in *D. cuneatus.*

Narasimham (1988b) demonstrated two to four spawning peaks in *Anadara granosa* and the peaks were also noticed in *Mercenaria mercenaria* by Hefferman *et al.* (1988). Single extended annual reproductive cycle is shown by some bivalves like *Pinctada albina* (Tranter, 1958b), *Crassostrea gryphoides* (Durve, 1965), *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977a), *Saccostrea cucullata* (Morton, 1990) and *Anadara sopha* (Baron, 1992).

The gonad of bivalve usually is seen in a resting stage after spawning (Loosanoff, 1962). But during the course of this study, germ cells of different developmental stages could be seen in the follicle of the gonad, showing apparently no resting or neutral stage. Similar observations were also made in *Donax faba* (Alagarswami, 1966), *Paphia laterisulca* (Nagabhushanam and Dhamne, 1977), *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977a). In the present study, gametogenesis is initiated soon after spawning and in some females even before the follicles are cleared of cell debris. Braley (1982) attributed this because of a stable food supply, which restores depleted food reserves quickly. However, Victor and

Subramonium (1988) observed an inactive period of three months after spawning in *Donax cuneatus* along the Madras Coast.

The sequence and timing of events in the reproductive cycles of marine invertebrates are influenced by complex physico-chemical variables in the environment. The factors inducing spawning may be quite different from those inducing annual reproductive cycle (Giese, 1959). Under tropical conditions of the Indian Coasts, temperature is relatively high throughout the year and generally does not fall below the optimum requirements of many bivalves. Thus, it may be suggested that temperature may not play a direct role in the spawning of marine bivalves of Indian waters. But rapid changes in salinity are known to stimulate the spawning activity in bivalves of the Indian Coasts and this is dealt in detail in one of the following chapters. Some notable works on this aspect are those of Nagabhushanam and Talikhedkar (1977a) in *Donax cuneatus*, Joseph and Madhyastha (1984) in *Crassostrea madrasensis*, Victor and Subramoniam (1988) in *Donax cuneatus*, Narasimham (1988a) in *Anadara granosa* and Maqbool (1993) in *Marcia opima*.

In the present investigation, during February, March and April, the salinity is found to be relatively higher (Table 1.1) and this promotes intense spawning activity during the period. After a break during monsoon season,

the gradual increase in salinity from September triggers the gametogenesis and during October-December, the increased salinity provides another congenial condition for intense spawning resulting in the second peak. Thus one of the main environmental factors inducing spawning is found to be the salinity of the ecosystem in which *D. incarnatus* inhabits. Increase in salinity has been found to stimulate spawning in many bivalves, viz. *Donax cuneatus* (Rao, 1967; Nagabhushanam and Talikhedkar, 1977b); *Paphia laterisulca* (Nagabhushanam and Dhamne, 1977); *Donax cuneatus* (Victor and Subramonium, 1988) and *Marcia opima* (Maqbool, 1993). However, in *Crassostrea madrasensis* (Stephen, 1980a) and *Saccostrea cucullata* (Sukumar and Joseph, 1988), the peak spawning was observed with the decline in salinity.

The present study on the reproductive cycle has revealed that the wedge clam *Donax incarnatus* is gonochoristic and shows five stages of gonad development in both male and female. Gametogenesis is initiated soon after spawning with no resting stage. The size at which the clam attains first sexual maturity is found to be 13-14 mm. It shows a continuous breeding pattern with two spawning peaks in February-March and December.

CHAPTER IV

Chapter IV

BIOCHEMICAL COMPOSITION

Introduction

The marine molluscs store large quantities of protein, fat and carbohydrate, which render them highly nutritious human food. Among the molluscs, bivalves are considered as valuable food item, because they provide many of the mineral substances, which are essential for a balanced diet. They have, therefore, been the subject matter of several biochemical investigations. Due to the ignorance of bivalve's nutritive value, a lion share of its resource in nature is unexploited. Studies on the nutritive value of edible bivalves and its seasonal variation have a significant role in fulfilling the nutritive demands of the growing population in our country. Understanding the importance of these molluscs, culture of edible molluscs particularly the oysters, mussels, clams, has been taken up in our coastal waters.

Seasonal cycles of energy storage and utilisation in marine bivalves are generally attributed to the complex interaction between environmental parameters, food availability, growth and reproductive activity (Bayne, 1976; Sastry, 1975, 1979; Gabbot, 1976, 1983). Among these, reproductive activity and food availability are found to be of utmost significance. In general,

energy storage in the form of protein, lipid and glycogen occurs during nutrient abundance prior to gametogenesis and is subsequently utilised in the production of gametes when metabolic demand is high (Gabbot, 1975; Bayne, 1976). The relative importance of the different substrates, their sites of storage and timing of utilisation in relation to season vary between species as well as between populations of the same species (Giese, 1969; Barber and Blake, 1981). The vastly different conditions of the tropics give rise to varying metabolic strategies in organisms.

The clam meat is gaining importance as a proteinaceous and nutritive food enriched with better assimilated forms of protein and glycogen. Therefore, in the present part of the investigation, an attempt has been made to evaluate protein, glycogen and lipid level of the clam, their relationships with the reproductive activity of the clam, and its nutritive value.

REVIEW OF LITERATURE

Studies on the biochemical composition of different species of bivalves have been done by many scientific workers in view of their importance as proteinaceous and nutritive food and their role in the overall economy. Joshi and Bal (1965) studied the seasonal variations in the biochemical composition of the calm, *Katelysia marmorata* inorder to find out its nutritive value. Ansell (1972) reported that seasonal changes in body weight and

biochemical composition of *Donax vittatus* were associated with the process of reproduction, growth and storage and utilisation of reserves. The objective of the analysis made by Ansell (1974a, b, c) was to provide information on the extent of seasonal fluctuation in the mean biochemical composition of the bivalves *Nucula sulcata*, *Abra alba* and *Chlamys septerradiata*. Nair and Shynamma (1975a) estimated the seasonal variation in the lipids and caloric values of *Villorita cyprinoides*. Seasonal changes in the caloric content, organic carbon and lipids were studied by Wafer *et al.* (1980) in *Mytilus viridis* and found that these were closely associated with the spawning cycle. Nagabhushanam and Talikhedkar (1977b) commented on the seasonal variations in protein, fat and glycogen of the wedge clam *Donax cuneatus*. No distinction was made between sexes in the chemical analysis. Comparison of events in the seasonal cycle of *Donax vittatus* and *D. trunculus* in European waters by Ansell and Bodoy (1978) showed only smaller seasonal fluctuations in rates of change of tissue weight, reflecting a reduced dependence on reserve storage to seasonal cycle. Pieters *et al.* (1978) described the biochemical composition of *Mytilus viridis* in relation to environmental parameters and spawning. Lakshmanan and Nambisan (1980) opined that the biochemical components in *Meretrix casta* and *Villorita cyprinoides* varied with season and species. Ansari *et al.* (1981) revealed

that seasonal changes in meat weight and biochemical variations in black clam *Villorita cyprinoides* were associated with reproduction, storage and utilisation of reserves. Seasonal changes in tissue weight and biochemical composition of the bivalve *Donax trunculus* on the Algerian Coast were reported by Ansell *et al.* (1980). A comparative study of the gross chemical composition of two species of clams, *Tapes decussatus* and *Tapes philippinarum* were made by Beringer and Lucas (1984). Bressan and Marin (1985) discussed the seasonal variations of biochemical composition and condition index of cultured mussel *Mytilus galloprovincialis* in connection with the reproductive cycles, temperature and phytoplankton availability. Jayabal and Kalyani (1986) estimated the variations in protein, carbohydrate and lipid of the hard clam *Meretrix meretrix* in relation to sex, age and seasons to assess its nutritive value. Seasonal changes in lipid, glycogen, protein and ash content of the meat of *Macoma baltica* from the Southern Baltic were studied by Wenn and Styezy (1987). Maqbool (1993) evaluated the nutritive status of *Marcia opima,* its seasonal variation and the relation of biochemical composition to reproductive cycle of the species. A comparison in relation to sexes was also made.

Biochemical variations in the different body tissues of oysters and clams include those of Nagabhushanam and Deshmukh (1974) in *Meretrix*

meretrix, of Salih (1977) in *M. casta,* Thangavelu and Sanjeevaraj (1988) in *Crassostrea madrasensis* and Shiny (1991) in *Musculista senhausia.* Rivonker and Parulekar (1995) opined that, major biochemical constituents of the raft-grown green mussel, *Perna viridis* were influenced by phytoplankton abundance and also vary with maturation and spawning cycle. Shiny and Radhakrishnan (1995) studied on the energy storage and utilisation to gametogenesis in the mussel *Musculista senhausia* from Cochin backwaters, west coast of India. Biochemical composition of whole body of the clam *Paphia malabarica* from Ashtamudi estuary, was taken up to understand the seasonal changes in the nutritive value of two size groups (Appukuttan and Aravindan, 1995). Southgate (1996) analysed the chemical composition of giant clam *Tridacna gigas* for their water, protein, fat, fibre, cholesterol and ash contents. Sebastian (1997) opined that the chief biochemical constituents, protein, glycogen and fat, of the black clam *Villorita cyprinoides* showed fluctuations in response to the seasons and physiological rhythms.

As information on biochemical composition of *D. incarnatus* is not available, an attempt is made here to evaluate the nutritive status of *Donax incarnatus* in terms of protein, glycogen and lipid content; its seasonal variation and the relation of biochemical composition to the reproductive

cycles of the species. A comparison of the biochemical composition in relation to sex is also made.

MATERIAL AND METHODS

Collections were taken monthly for a period of one year (1991). Clams within the size range of 15 - 18 mm were selected for the study. Individuals of two sexes were separated by the examination of gonad smears microscopically. The soft tissues were washed with minimum quantity of distilled water and blotted dry. It was then weighed and oven dried at 60°-80°C to constancy. Percentage of water content of soft tissues was calculated from the wet weight and dry weight. The protein, glycogen and lipid were estimated using the finely powdered dry soft tissues.

Protein level in the soft tissue was determined by the method of Lowry *et al.* (1951). A weighed sample of the dried soft tissue was extracted with alkali and warmed in a water bath. The extract was treated first with an alkaline solution of copper sulphate and then with Folin Cieocalteu reagent. The intensity of blue colour of the resulting solution was measured spectrophotometrically at 750 nm. The concentration was calculated from the absorbance values with a standard curve prepared using Bovin's serum albumin.

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

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

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

RESULTS

Protein content in male and female clams was at a relatively high level throughout the year exhibiting more or less a similar pattern of accumulation (Fig. 4.1 and 4.2). The monthly variation of protein values expressed in $\mu g.mg^{-1}$ of protein in the males during the one year of study is provided in Table 4.1. Higher values of 337.91 $\mu g.mg^{-1}$ were obtained in January, followed by 319.36 $\mu g.mg^{-1}$ in November, and lower values of 257.88, 275.99 and 282.67$\mu g.mg^{-1}$ were seen in April, March and February respectively.

Highest protein content in the females was observed in January (333.14 $\mu g.mg^{-1}$) and lowest value of 237.31 $\mu g.mg^{-1}$ in April Fig. 4.1 and Table 4.2.

The trend of glycogen values in different months in males and females of the clam *D. incarnatus* showed variations with the breeding behaviour and development of the gonad. In general, both males and females exhibited uniform variation in glycogen concentration. It was at a low level in April (51.54 $\mu g.mg^{-1}$) and high level in October (104.7 $\mu g.mg^{-1}$) in females. In males, the highest value recorded were in October (102.15 $\mu g.mg^{-1}$) and lowest value in April (49.22 $\mu g.mg^{-1}$) as shown in Fig. 4.2 and Table 4.1. The

Table 4.1. Monthly Variation in the protein, glycogen, lipid (μg.mg dry wt^{-1}) and water content (%) of the total tissue (pooled) of male *D. incarnatus*
(Samples not available from May to August)

Parameter	January	February	March	April	September	October	November	December
Protein	337.91 ± 69.44	282.67 ± 69.44	275.99 ± 74.00	257.88 ± 22.93	306.29 ± 70.30	318.94 ± 58.36	319.36 ± 16.60	305.61 ± 23.00
Glycogen	91.92 ± 11.45	62.62 ± 5.26	56.62 ± 0.78	49.22 ± 3.05	79.15 ± 5.66	102.15 ± 2.76	71.59 ± 7.39	61.97 ± 5.87
Lipid	43 ± 1.41	39.33 ± 0.52	38.33 ± 1.86	33.33 ± 1.03	50 ± 1.41	52.33 ± 0.874	36.33 ± 5.00	35 ± 2
% Water content	57.47	70.73	69.85	74.94	70.63	68.13	69.05	72.70

Table 4.2. Monthly Variation in the protein, glycogen, lipid (μg.mg dry wt^{-1}) and water content (%) of the total tissue (pooled) of female *D. incarnatus.*
(Samples not available from May to August)

Parameter	January	February	March	April	September	October	November	December
Protein	331.14 ± 5.01	265.33 ± 5.37	246.08 ± 2.33	237.31± 6.61	316.88 ± 2.15	316.88 ± 2.15	306.42± 32.04	285.94 ± 6.04 .
Glycogen	97.43 ± 7.79	81.7 ± 2.55	63.54 ± 3.04	51.54 ± 5.44	79.62 ± 5.19	104.7 ± 5.91	74.91 ± 8.54	64.81 ± 2.56
Lipid	52 ± 0.89	49 ± 3.03	41.33 ± 0.82	36 ± 0.40	87.17 ± 5.04	92.5 ± 7.37	66.33 ± 3.50	52.33 ± 7.00
% Water content	59.56	68.41	71.23	76.98	72.18	62.91	74.12	75.62

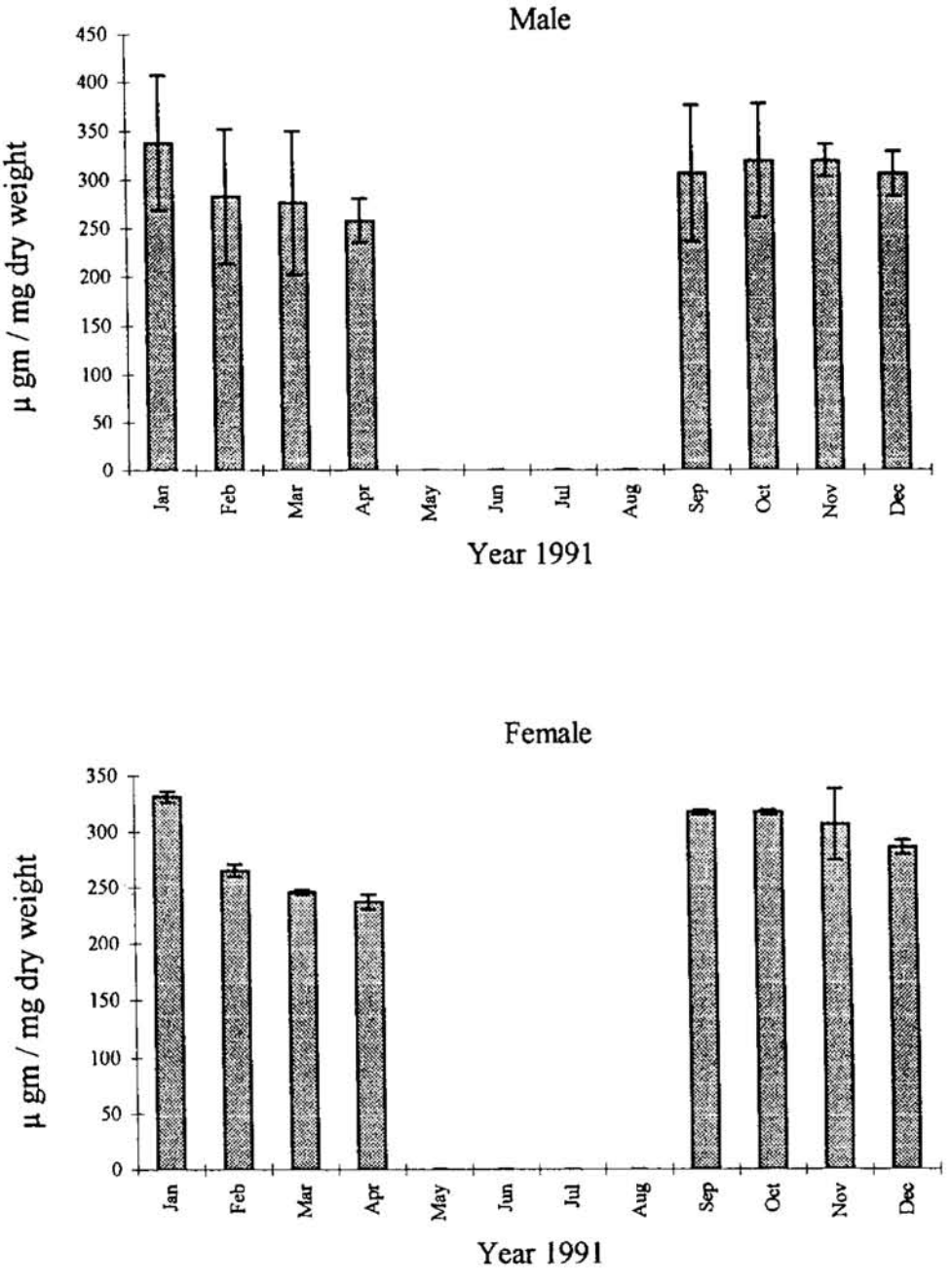


Figure 4.1. Monthly variation of protein in male and female of *D. incarnatus* (Samples not available from May to August)

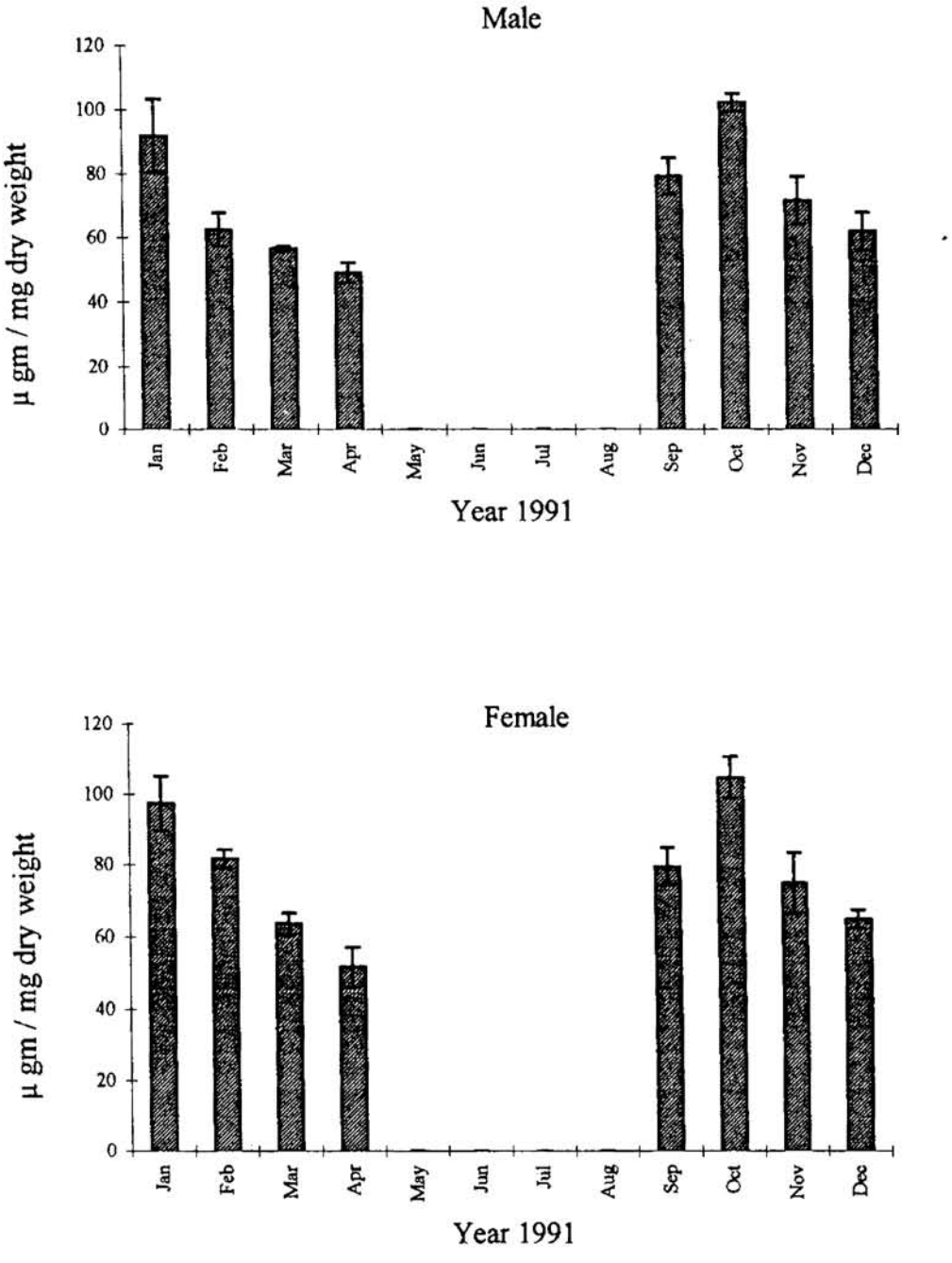


Figure 4.2. Monthly variation of glycogen in male and female of *D. incarnatus* (Samples not available from May to August)

lowest and highest values recorded in both males and females were during spawning and gametogenic period.

Lipid levels showed a clear seasonal variation in both males and females. Males recorded a minimum concentration of lipid in April (33.33 $\mu g.mg^{-1}$) and the maximum value (52.33 $\mu g.mg^{-1}$) in October. Males recorded a low concentration of lipid when compared to females. In females the maximum value of 92.5 $\mu g.mg^{-1}$ was recorded during October and minimum value of 36.0 $\mu g.mg^{-1}$ in April (Tables 4.1, 4.2; Fig. 4.3).

It is evident from Tables 4.1and 4.2 that the water content varied from 57.47% to 74.94% in males and from 59.56% to 76.98% in females of *Donax incarnatus*. In males, the highest value of 74.94% was obtained in April followed by 72.70% in December and lower values of 57.47% and 68.13% in January and October respectively. Similarly, in females the maximum value of 76.98% was recorded in April and minimum values of 59.56% in January as given in Tables 4.1 & 4.2 and Fig. 4.4.

DISCUSSION

Seasonal changes in the biochemical composition are the characteristics of the seasonal activities of bivalves. Variations in the biochemical composition are influenced by different factors such as

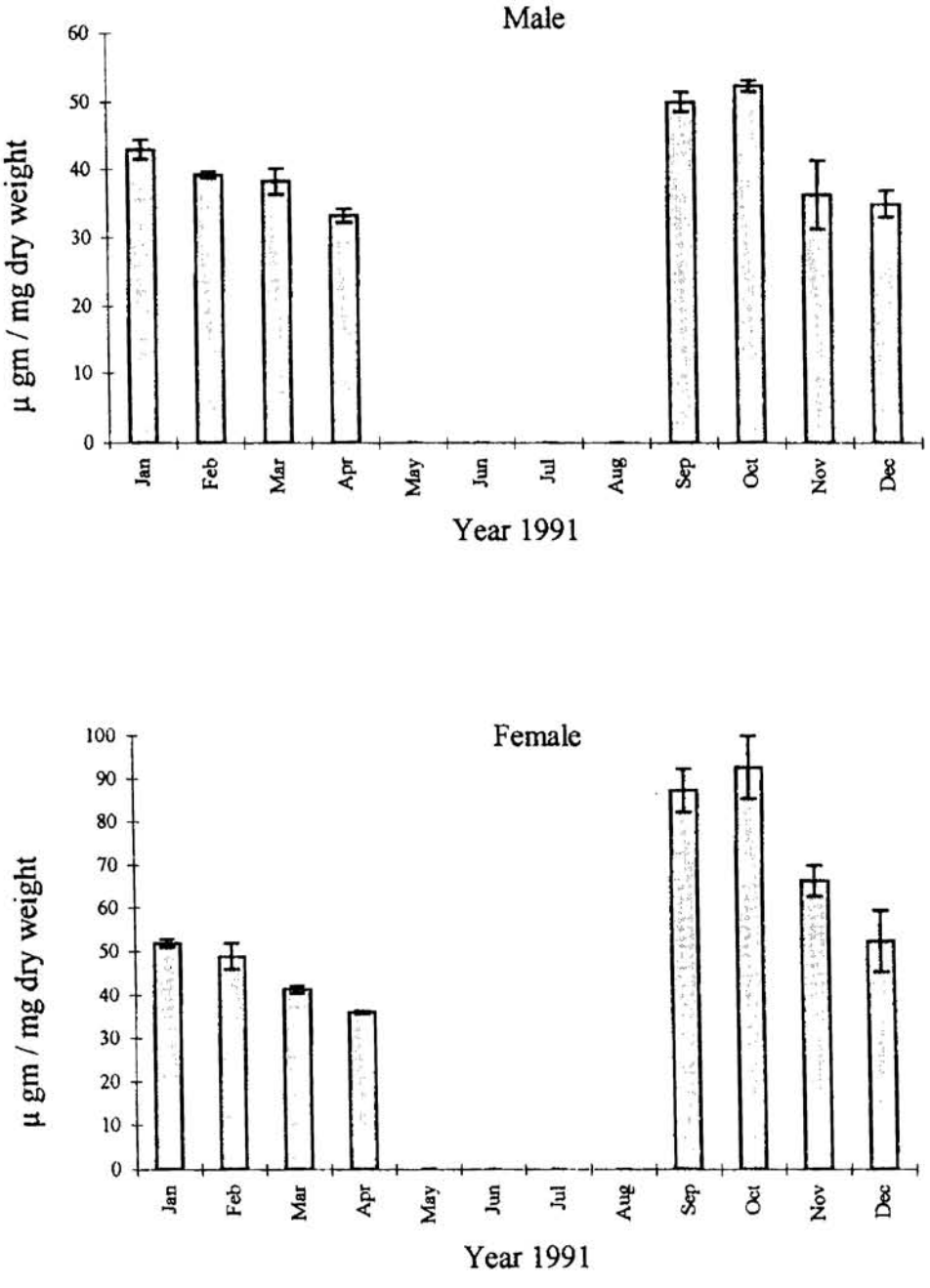


Figure 4.3. Monthly variation of lipid in male and female of *D. incarnatus* (Samples not available from May to August)

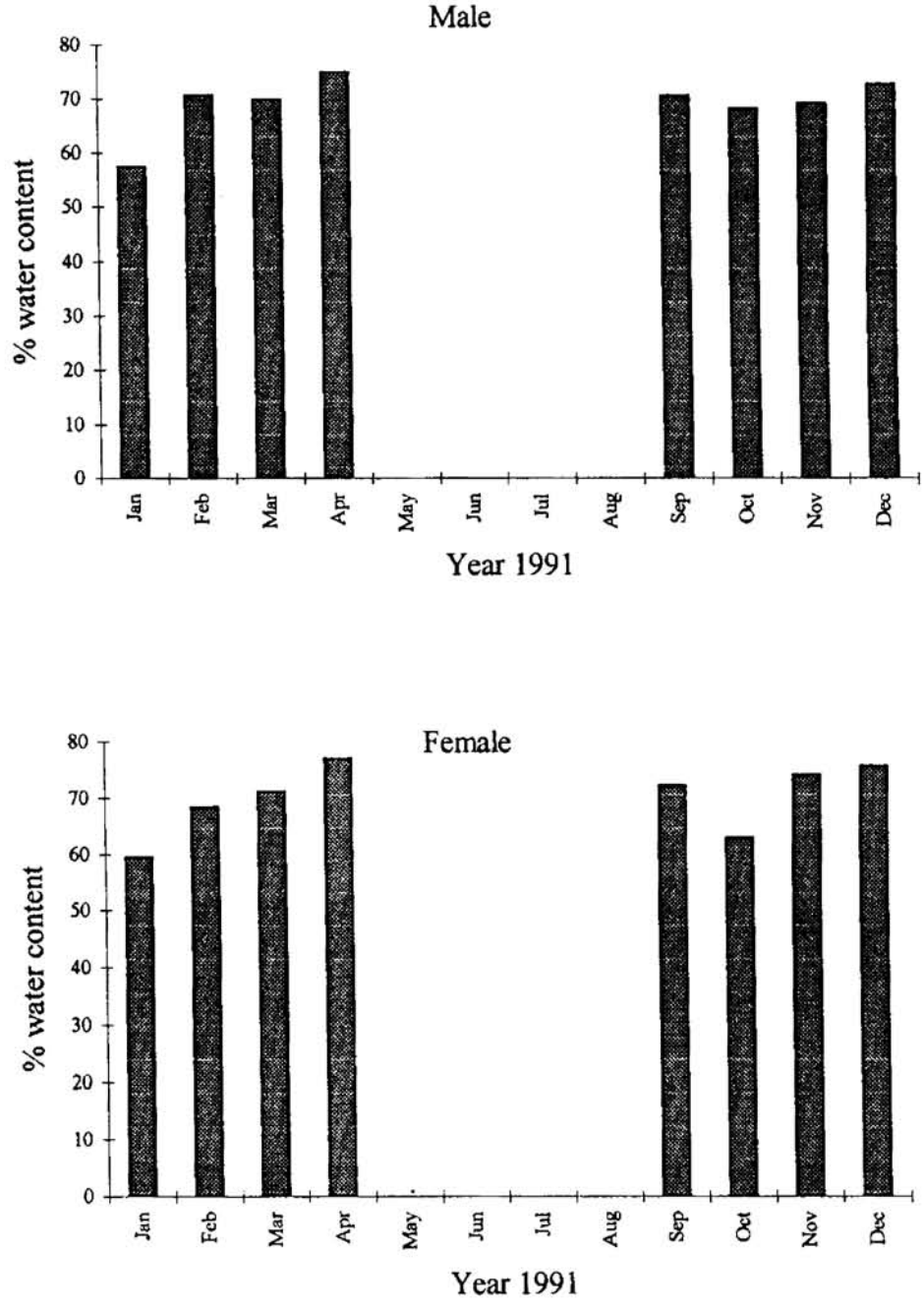


Figure 4.4. Monthly variation of percentage water content in male and female of *D. incarnatus*
(Samples not available from May to August)

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).

From the present findings, it could be seen that the protein content remained relatively high throughout the year except a slight decline during the spawning period in both the sexes of clams. The level of protein build up during gamatogenesis in *Donax incarnatus* is utilised during the peak breeding season by the animal. Likewise an increase in protein content occurs again after the monsoon, which may be utilised for subsequent spawning. An almost uniform level of protein content in the present investigation indicated that sex difference has no influence in the level of protein. In almost all lamellibranchs, the protein content remains at a relatively high level throughout the year and decreased during the period of gametogenic activity and spawning. In *D. cuneatus*, Nagabhushanam and Talikhedhar (1977b) observed a high protein value followed by a decrease during spawning and an increase during maturation period. Nagabhushanam and Deshmukh (1974) noticed an increase in protein content of *Meritrix meritrix* during the gonad development and then it remained steady when gonads matured.

Ansari *et al.* (1981) stated that the level of protein build up in *Villorita cyprinoides* decreased during the breeding season and increased again to a secondary peak before the second spawning. Seasonal changes in protein content associated with the annual reproduction cycle have been reported in *Meretrix casta* of Vellar Estuary (Balasubramanyan and Natarajan, 1988) and *Crassostrea madrasensis* (Thangavelu and Sanjeevaraj, 1988) from Ennore Estuary. Maqbool (1993) opined an increasing trend of protein accumulation in the stages of reabsorption of residual gametes of first spawning, sexually indeterminate resting period and gametogenic stages in the clam *Marcia opima* of Kayamkulam waters.

Glycogen has long been considered to be the principal energy reserve of marine bivalves (Giese, 1969). In *Donax incarnatus*, glycogen content showed great variation in relation to its reproductive cycle. Both males and females showed almost similar pattern of glycogen concentration. Little Wood and Gordon (1986) also noticed that glycogen concentration in males and females did not show significant variation in the oyster *Crassostrea rizophorae*. The glycogen level in *D. incarnatus* increased during early gametogenic period and then its value showed decreasing trend with the advancement of gametogenesis. This may be ascribed to its utilisation in the development of gametes. The glycogen level decreased during mature

conditions. Giese *et al.* (1967) noticed that least carbohydrate storage was present, when mature gametes were present in *Tivela stultorum*. Nagabhushanam and Deshmukh(1974) also noticed high level of glycogen content during the period of gonad development and a fall during mature condition in *Meretrix meretrix*. Similar results were also observed in *Donax cuneatus* (Nagabhushanam and Talikhedhar, 1977b) and in edible oyster *Crassostrea madrasensis* (Thangavelu and Sanjeevaraj, 1988).

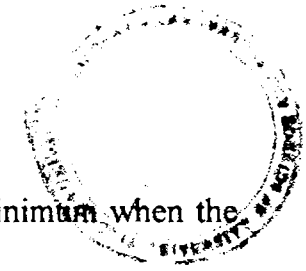
The role of lipid as an energy reserve has been well documented. The lipid in *D. incarnatus* exhibits a clear variation, with the reproductive cycle. Fat accumulates in the developing gonads and depletes during spawning. Mature stage showed considerable amount of fat, which decreased during the spawning time. In *D. cuneatus*, Nagabhushanam and Talikhedhar (1977a) observed a moderate level during the gametogenic period and increased level during the active formation of gametes, which decreased during the spawning period. Nagabhushanam and Deshmukh (1974) noticed low value of fat during spawning, and the mature stage, contained considerable amount of fat in *Meretrix meretrix*. Similar observations were made in *Villorita cyprinoides* var *cochinensis* (Nair and Shynamma, 1975a); *Meretrix meretrix* (Jayabal and Kalyani, 1986); *Donax trunculus*, (Ansell *et al.* 1980); *Marcia opima* (Maqbool, 1993).

Both male and female specimens of *D. incarnatus* recorded a clear difference in lipid accumulation, with higher accumulation in females. This might be attributed to a higher biochemical budget required for egg production. The advantages of storing fat in the eggs and larvae are that, it could be assimilated in more concentrated form and secondly, it favours increased buoyancy due to its lower density than carbohydrate and protein (Gabbot, 1975). A slight increase in lipid values during postmonsoon is seen. This might probably be due to fluctuations in conditions affecting the nutrition of the animal in addition to that arising from spawning.

Seasonal variations in the biochemical constituents seem to be mainly influenced by reproductive cycle and food availability. Jayabal and Kalyani (1986) have noticed an increase in biochemical constituents in relation to peak phytoplankton production in *Meretrix meretrix.*

Further, abundance of lipid-rich phytoplanktonic food (especially diatoms) seem to be a most probable cause of the lipid-dominated pattern of energy storage as observed in *Macoma balthica* by Wenne and Styezy (1987) from the Southern Baltic waters.

The water content of the tissue of bivalves usually gives an indication of the time of spawning. Increase in water content took place in *Donax incarnatus* in April and December and this period coincided with the



spawning season. It was observed that water content is minimum when the gonads are fully developed and increased during the spawning season. This seasonal change in water content seems to be associated with the changes in the physiological state, reproductive cycle and nutritional condition of the organism.

It was further noted that variation in water content showed a clear correspondence with other biochemical constituents and this relation was more conspicuous with lipids. When the water content in both the sexes was at the minimum in January, the lipid was at the maximum value. From a broad general trend of distribution of different biochemical constituents, it may be suggested that an inverse relationship exist between the water content and other body constituents. This indicates the presence of a mechanism for homeostasis and tissue volume. Seasonal variation on water content and its inverse relationship with other organic constituents have also been shown by Durve and Bal (1961) in *Crassostrea gryphoides*; Salih (1977) in *Meretrix casta*; and Stephen (1980 b) in *Crassostrea madrasensis*. Ansell (1972) found the body weight of the clam *Donax vittatus* to be inversely proportional to the water content and that spawning was accompanied by an increase in body water content.

Since the clam meat is gaining much importance as a nutritive food, the present investigation has elucidated the importance of the bivalve *Donax incarnatus* from the point of view of human nutrition. It has been clearly observed that, animals with mature gonads occurring in October-November and January-February are found to offer maximum nutritive value. However, over-exploitation should be checked during this period in order to maintain production at sustainable levels.

CHAPTER V

Chapter V

STUDIES ON SALINITY TOLERANCE AND FILTRATION RATE

Introduction

Salinity is considered as a master factor initiating functional responses and plays an important role in the physiology of marine and estuarine organisms. Salinity affects the organisms through changes in the total osmoconcentration, relative proportion of solutes, coefficients of absorption and saturation of dissolved gases, density and viscosity of the medium (Kinne, 1971). In general, it is well known that the estuaries and adjoining marine realms are subjected to wide variations in salinity under the impact of seasonal changes. In addition to seasonal variations, there is also diurnal variations on salinity due to tides. Animals inhabiting such habitat adopt different mechanisms for their survival. Clams and oysters subsist mainly on particles filtered from the surrounding water, which they pump through the gills. The amount of water that passes over the gills of Lamellibranchs is of considerable interest in the study of the nutritional, respiratory, excretory and the overall performance of these animals. Suspension feeding organisms in the sea, obtain their food by filtering finely dispersed organic matter from the surrounding water. Filtration is a function of (i) amount of water transported

across the feeding surface (ctenidia), (ii) amount of food present in the surrounding water and (iii) retention ability of ctenidia (Owen, 1966).

Filtration rate is influenced by a number of environmental factors like temperature, salinity, dissolved oxygen, flow rate, tidal cycle and suspension matter. The clams like any other bivalves close their shell valves and isolate tissues during unfavourable conditions. This is the first response of many bivalves to an environmental stress (Bayne, 1973a, c).

REVIEW OF LITERATURE

Salinity tolerance studies

The effect of salinity variations in bivalves has been investigated by many workers. Abraham (1953) studied the influence of salinity on the survival of clam *Meretrix casta*. The capacity to tolerate different salinities was worked out in *Martesia striata* by Nagabuhshanam (1955), in *Nausitora hedleyi* by Cheriyan (1966), in *Katelysia opima* by Mane (1974b). Studies were conducted on different bivalves, namely, *Crassostrea madrasensis, Meretrix meretrix* and *Mytilus edulis* by Sundaram and Shafee (1989) and *Crassostrea cucullata* by Nagabushanam and Bidaskar (1975) to find out their tolerance to different salinities. The lethal salinity on the basis of 50% survival was determined in *Donax cuneatus* by Talikhedkar and Mane (1976). The rate of mortality in different salinity level was observed by

Alagarswami and Victor (1976) in the pearl oyster *Pinctada fucata*. The tolerance range in the different size groups were noticed by Nair and Shynamma (1975 b) in *Villorita cyprinoides* and Salih (1978) on *Meretrix casta*. Mohan (1979) worked on the salinity tolerance of *Nausitora hedleyi* to sub and supra normal salinities.

Thampuran *et al.* (1982) opined that the tolerance limit of the clam *Sunetta scripta* decreased as its age increased. Akberali and Davenport (1981) reported the effect of gradual changes in salinity on the behaviour of *Scrobicularia plana*. 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).

Studies on filtration rate

Lamellibranchs have been extensively studied for their rate of water transport and filtration activity under different conditions. Basically two types of methods have been adopted by different workers, the direct method (Galtsoff, 1928; Winter, 1969; De Bruin and Davids, 1970; Hildreth, 1976) which measures the rate of flow of exhalent water entering the tube into the exhalent siphon and estimating the rate of filtered water, and the indirect method (Cole and Hepper, 1954; Durve, 1963; Badman, 1975; Alagarswami and Victor, 1976; Matthews *et al.* 1989) that estimates the filtration rate of

particles removed from the water as measured by changes in optical density or particle counts. Cole and Hepper (1954) employed the rate of removal of neutral red from solution as a means of comparing the amount of water pumped under different environmental conditions, but the majority of indirect methods are based on the rate of removal of suspended particles from the water by the filtering activities of animals.

It is found that filtration rate is strongly affected by temperature changes (Ballantine and Morton, 1956; Winter, 1969; Widdows, 1973; Bayne *et al.,* 1976). Walne (1971) opined that *Crassostrea gigas* and *Mytilus edulis* were the least affected by temperature, *Ostrea* was intermediate while *Venerupis decussata* and *Mercenaria mercenaria* showed a very marked reduction in filtration rate as the temperature was lowered. According to Schulte (1975), the filtration rate of *Mytilus edulis* was directly proportional to temperature upto an optimum level and decreased drastically with further increase in temperature.

Effects of varying salinities on filtration rate of lamellibranchs have been studied by Cole and Hepper (1954); Nagabhushanam (1956) and Alagarswami and Victor (1976). Blake (1961) observed that filtration rate was independent of salinity in *Mya arenaria.* Durve (1963) studied the rate of filtration in *Meretrix casta* in different salinities and noted that it

decreased in extreme low and high salinities. Similar trend was also noticed in *Mytilus edulis* in lower salinities (Bohle, 1972). Mane (1975) opined that the rate of water filtration increased with decreasing salinity in the clam *Katelysia opima*. Likewise, a low filtration rate in higher salinities was observed by Holley and Foltz (1987) in *Rangia cuneata*. Supriya (1992) observed that the rate of water filtration increased with increasing salinity in *sunetta scripta*. However, no significant variation in filtration rate in different salinities was observed by Shiny and Radhakrishnan (1994) in the mussel *Musculista senhausia* indicating their wide tolerance to salinity changes.

Walne (1971) opined that the filtration rate of five species of bivalves increased with increasing flow rate upto a threshold range, above which the flow rate had no further effect. Wildish *et al.* (1987) explained the relationship between ambient seawater, flow velocity and filtration rate in *Placopecten magellanicus.*

Filtration rate have been related to body dimensions like shell length (Winter; 1969, 1978), wet and dry weights (Palmer, 1980; Holley and Foltz, 1987; Supriya, 1992; Shiny and Radhakrishnan, 1994) and total weight (Ali, 1970).

The main objectives of this study were to determine the optimum salinity and the lethal level of lower salinity range for *D. incarnatus*. An attempt was also made to delineate the effect of salinity and size on the filtration rate of this wedge clam.

MATERIAL AND METHODS

Salinity tolerance studies

The animals were brought to the laboratory in plastic containers and immediately transferred to seawater of salinity 30 ± 2 ppt, temperature 29 ± 1°C and acclimatised for a period of one week. During acclimatisation, seawater was changed every 24 hr, keeping the salinity at 30 ± 2 ppt. During this period, the animals were fed with *Cynacocystis salina.*

To study the salinity tolerance, healthy and selected animals were grouped into three size groups, 10 ± 2 mm (small), 17 ± 2 mm (medium) and 25 ± 2 mm (large) in length. Clams were subjected to the salinities 5, 10, 15, 20, 25, 30, 35 and 40 ppt. The experiments were carried out in glass beakers containing one litre experimental solution of different salinites. Desired salinities were prepared either by diluting seawater with distilled water or by evaporation of seawater. Salinity of the seawater was determined by titration with silver nitrate using potassium chromate as indicator.

After the acclimatisation, ten healthy clams from small size group and six clams each from intermediate and large size groups were exposed to various experimental salinities. The experiments were run in triplicate at a given time. The animals were kept under observation for ten days and mortality rate was recorded every 8 hr. The condition of individual clam was noted and it was considered dead when it failed to respond by closing its valves or respond to external stimuli. The rate of mortality is considered as the criterion of tolerance. The salinities which caused death of more than 50% of organisms during the period of the experiment, was considered beyond the range of tolerance of the species (Salih, 1977).

Another set of experiments was conducted to determine accurately the lower limit of salinity tolerance at 1 ppt difference level. Clams of small size and medium were exposed to 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 ppt salinities, and large clams to 20, 21, 22, 23, 24, 25 ppt salinities.

Experiments on filtration rate

For filtration rate experiments, clams of three size groups same as grouped for the salinity tolerance experiments were selected and acclimatised in different experimental salinities (5, 10, 15, 20, 25, 30 and 35 ppt). Filtered seawater was used to prepare the experimental solutions. To evaluate the effect of varying salinities on filtration rate, the method employed were dye

clearance technique (Cole and Hepper, 1954) using a homogeneous solution of neutral red. Ten specimens (clams) of uniform size were transferred to a beaker of one litre capacity containing solution of the respective experimental salinities, with 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. Filtration (clearance) rate was calculated using Quayle's equation (1948)

$$m = \frac{M}{nt} \log_e \frac{Co}{Ct}$$

where

m = Filtration rate ($ml.h^{-1}$)

M = Volume of solution in ml.

n = Number of animals in the test vessel

Co = Dye concentration in initial sample

Ct = Dye concentration in final sample.

t = Time interval between sampling (hr)

After the experiment, soft tissues were removed and dried at 70-80^{0}C to estimate the dry weight. Filtration rate was expressed as $ml.hr^{-1}$. mg dry $^{-1}$.

RESULTS

Salinity tolerance

When exposed to salinity of 5 ppt, the small and medium size groups showed 100% mortality within 72 hr. and the large size group within 48 hr. as shown in Table 5.1. Subjected to 10 ppt salinity, the large clams remained with closed valves until death which occurred within three days. For medium and small sized clams, 100% mortality was noticed within five and seven days, respectively. Irregular opening and closure of valves and reduced extension of siphons were also observed in the medium and small size groups during this period.

In 15 ppt salinity, large clams exhibited 100% mortality within 72 hr. The small and medium size groups were more active and a few of them opened after one and a half hour, some of them opened the second day, while the remaining clams did not open their valves throughout the tenure of the experiment in 15 ppt salinity. In this salinity, medium size group showed 80% mortality by the end of the sixth day, whereas 40% of the small size survived up to the 10th day.

Table 5.1. Rate of mortality in *D. incarnatus* (small, medium and large size) in salinities ranging from 5 ppt to 40 ppt (small 10 ± 2mm; medium 17 ± 2mm; large 25 ±2mm)

Salinity	Size groups	Days of exposure										Mortality (%)
		1	2	3	4	5	6	7	8	9	10	
5	Small	30	40	30	0	0	0	0	0	0	0	100
	Medium	40	30	30	0	0	0	0	0	0	0	100
	Large	80	20	0	0	0	0	0	0	0	0	100
10	Small	20	10	20	10	20	10	10	0	0	0	100
	Medium	0	10	20	60	10	0	0	0	0	0	100
	Large	70	20	10	0	0	0	0	0	0	0	100
15	Small	0	0	0	10	0	10	20	0	0	20	60
	Medium	0	0	40	10	10	20	0	0	0	0	80
	Large	70	20	10	0	0	0	0	0	0	0	100
20	Small	0	0	0	0	0	0	10	0	0	0	10
	Medium	0	0	0	10	15	10	5	0	0	0	40
	Large	50	0	20	10	0	0	0	0	0	0	80
25	Small	0	0	0	0	0	0	0	0	0	10	10
	Medium	0	0	0	0	0	0	10	0	0	0	10
	Large	0	30	10	0	0	0	0	0	0	0	40
30	Small	0	0	0	0	0	0	0	0	0	0	0
	Medium	0	0	0	0	0	0	0	0	0	0	0
	Large	0	0	0	0	0	0	0	0	0	0	0
35	Small	0	0	0	0	0	0	0	0	0	0	0
	Medium	0	0	0	0	0	0	0	10	0	0	10
	Large	0	0	0	0	0	0	0	0	10	0	10
40	Small	10	30	40	10	0	0	0	0	0	0	90
	Medium	0	10	20	10	20	20	10	10	0	0	100
	Large	0	0	40	30	20	10	0	0	0	0	100

In 20 ppt salinity, 80% mortality was shown by large size group within four days. There was no regular sequence in the time of valve opening and extension of siphons. 40% mortality occurred for medium size clams by seven days. These animals were less active when compared to the small ones. The length to which the siphons were extended was also gradually reduced and very little quantity of faeces was observed. 90% survival of small clams was observed in 20 and 25 ppt salinities. All the clams opened their valves within 20 minutes after their immersion in the respective salinities. All of them extended their siphons and the large quantities of faeces and pseudofaeces were seen. Large sized clams were active in 25 ppt and survival was 60%. In the case of medium sized animals, 90% survival was observed in 25 ppt and 35 ppt for all the size groups, 100% survival was noticed in 30 ppt salinity and all animals were active and performed normally. Small and large sized clams continued to be active in 35 ppt and also exhibiting 100% and 90% survival, respectively. In 40 ppt salinity, all the three size groups suddenly became inactive, only a few opened their valves showing erratic behaviour which resulted in 100% mortality for the large and medium ones within six and eight days respectively. Although the smaller ones also showed similar behaviour, 10% survival was observed.

The salinity tolerance range of small clams under the experimental conditions thus ranged between 16 and 35 ppt; for medium sized clams 18-35 ppt; and for large sized clams 24 -35 ppt.

Results of the determination of lethal salinity are given in Tables 5.2, 5.3 and 5.4. The lethal tolerance limit of small and medium sized clams was found to be 15-18 ppt and large ones were obtained as 24 -25 ppt. By the end of tenth day, 60% mortality was seen in 15 ppt salinity in small sized clams. In 16 ppt, the mortality was 40%. It revealed that the lower lethal limit is 16 ppt. The siphon and foot were slightly protruding out and they were withdrawn into the shell cavity at the slightest disturbance. In 17,18 and 19 ppt , survival rate was between 70% and 90%. The lower lethal limit for medium sized animals was 18 ppt in which they showed 45% mortality. They showed erratic behaviour and faecal discharge was less. In the salinities below the lethal level (17-15 ppt), 60-80% mortality was observed. The lower level in large size group was found to be 24 ppt salinity which showed 50% survival of the animals. At lower salinities (23-20 ppt) mortality ranged from 60 to 80%.

Filtration rate

To compare the filtration rate in different salinities and size groups, data were analysed using two way Analysis of Variance. The filtration rate

Table 5.2. Lower lethal limit of *D. incarnatus* (small size) in salinities ranging from 15 ppt to 19 ppt

Salinity	1	2	3	4	5	6	7	8	9	10	Mortality (%)
15	0	0	0	10	0	10	20	0	0	20	60
16	0	0	0	0	20	20	0	0	0	0	40
17	0	0	0	0	20	10	0	0	0	0	30
18	0	0	0	0	0	0	0	0	10	0	10
19	0	0	0	0	0	0	10	0	0	0	10

Table 5.3. Lower lethal limit of *D. incarnatus* (medium size) in salinities ranging from 15 ppt to 20 ppt

Salinity	1	2	3	4	5	6	7	8	9	10	Mortality (%)
15	0	0	40	10	10	20	0	0	0	0	80
16	0	0	0	30	20	10	10	0	0	0	70
17	0	0	0	20	30	10	0	0	0	0	60
18	0	0	0	20	20	5	0	0	0	0	45
19	0	0	0	20	0	10	10	0	0	0	40
20	0	0	0	10	15	10	5	0	0	0	40

Table 5.4. Lower lethal limit of *D. incarnatus* (large size) in salinities ranging from 20 ppt to 25 ppt

Salinity	1	2	3	4	5	6	7	8	9	10	Mortality (%)
20	50	0	20	10	0	0	0	0	0	0	80
21	0	30	10	20	0	10	0	0	0	0	70
22	0	0	20	20	10	10	10	0	0	0	70
23	0	0	20	0	20	10	10	0	0	0	60
24	0	0	0	20	10	10	10	0	0	0	50
25	0	30	10	0	0	0	0	0	0	0	40

was converted to logarithm values for analysis. The ANOVA table is given in Table 5.5. It could be seen from the table that there is significant difference ($P< 0.05$) between salinities and there is significant difference between size groups ($P<0.01$). The least significant difference was calculated at 5% level and the salinity mean and the mean size groups were arranged. Significantly lower filtration rate was observed in salinities 15 and 20 ppt and higher filtration rate was observed in 25, 30 and 35 ppt, with maximum in 30 ppt (Table 5.6).

Among size groups, maximum filtration rate was observed in small size groups followed by medium and very low filtration rate was observed in large size groups (Fig. 5.1).

DISCUSSION

Salinity tolerance

Donax incarnatus occurs in the inter-tidal zone of Malippuram in Cochin region on the Southwest coast of India. This area is subjected to wide range of salinity with a deep fall in salinity during monsoon season.

From the present study, it is clear that the small sized animals can survive in salinities ranging from 16-35 ppt. In the medium sized ones, the lower tolerance limit was 18 ppt. In the case of large clams, tolerance range

Table 5.5. ANOVA of filteration rate ($ml.hr^{-1}.mg\ dry^{wt-1}$) of small, medium and large *D. incarnatus* at different salinities

Source of Variations	Sum of Squares	Df	Mean Square	F
Total	0.3480	14	-	-
Between Salinity	0.0807	4	0.0202	8.08 *
Between Size	0.2473	2	0.1237	49.48 *
Error	0.0200	8	0.0025	

LSD for Salinity	0.092			
Salinity	15	20	25	30	35
Mean	0.1806	0.1853	0.2596	0.2674	0.2368

LSD for Size	0.069		
Size	S	M	L
Mean	0.393	0.204	0.081

* $P < 0.05$

** $P < 0.01$

Table 5.6. Details on the filtration rate (ml. hr^{-1}. mg dry wt^{-1}) of small, medium and large sized *D. Incarnatus* at different salinities. [X ± SD]

Salinity	Small	Medium	Large
5	0.0000	0.0000	0.0000
10	0.0000	0.0000	0.0000
15	0.2715 ± 0.0118	0.1000 ± 0.0176	0.0739 ± 0.0148
20	0.2788 ± 0.0470	0.1591 ± 0.0225	0.0864 ± 0.0166
25	0.3311 ± 0.0546	0.2073 ± 0.0193	0.0912 ± 0.0189
30	0.4116 ± 0.1379	0.2923 ± 0.0107	0.1128 ± 0.0205
35	0.4018 ± 0.0341	0.2136 ± 0.0243	0.1389 ± 0.0257

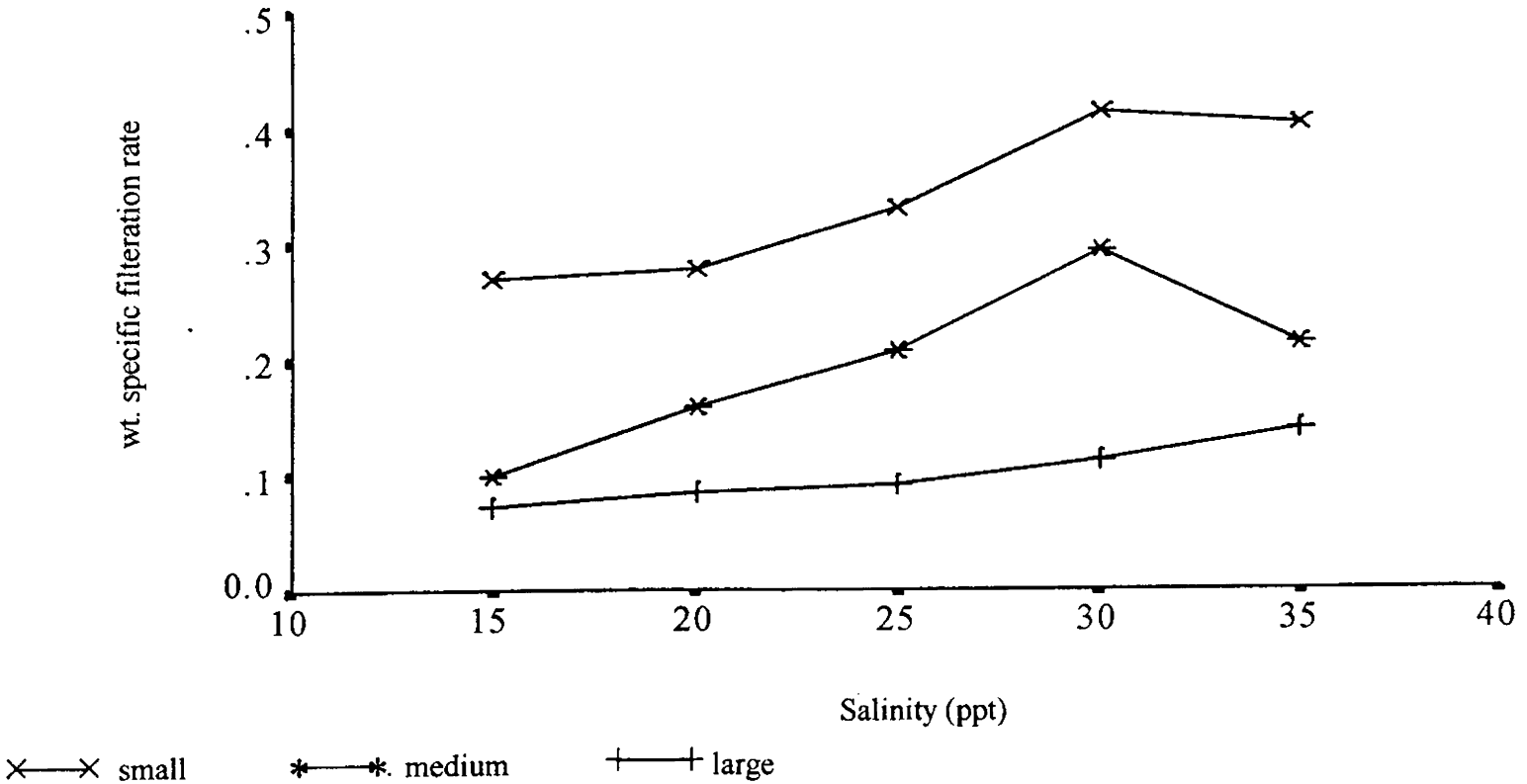


Fig. 5.1 Filtration rate (ml. hr^{-1}. mg dry wt^{-1}) of small, medium and large sized *D. incarnatus* at different salinities.

was found to be between 24 and 35 ppt (Table 5.2, 5.3 and 5.4). Thus, it is found that the smaller ones are more capable of withstanding wider fluctuations in salinity than the larger ones. Thampuran (1986) have recorded 100% survival of small clams in 15-40 ppt, medium clams in 20-35 ppt and large sized clams in 25-35 ppt salinities. The author concluded that even though the clams were seen to tolerate lower salinities, 20-30 ppt can be considered as the range of tolerance, below and beyond this range they were in resistance zone. In accordance with the studies of Thampuran (1986) on *Sunetta scripta*, Salih (1978) on *Meretrix casta* and Nair and Shynamma (1975b) in *Villorita cyprinoides*, the present study also showed that the smaller clams tolerate low salinities better than larger ones.

It is also recorded that the mortality rate is more in the lower salinities than higher salinities. Salih (1978) has reported that decrease in salinity is more disastrous than an increase in salinity. Likewise, in *S. scripta*, the animals are more capable of withstanding higher concentrations than dilutions as observed by Thampuran (1986). He has attributed this to the ability of clams to resist in a better way through loss of water and a gain of ions which occur on higher concentration than a loss of ions and gain of water which happen in the case of dilutions.

The gradual acclimatisation of the clams may again reduce the tolerance limits. But this may have limited applicability to coastal and estuarine littoral situations where changes in salinity are short-term (Davenport and Fletcher, 1978). In the present study, *D. incarnatus* is subjected to sudden acclimatisation. Gradual acclimatisation was found to have no significant effect on conditioning time in *Donax cuneatus* (Talikhedkar and Mane, 1976); *Meretrix meretrix* and *Katelysia opima* (Ranade and Kulkarni, 1973; Mane 1974b). Kinne (1964) has pointed out that the salinity ranges occupied in the sea are not necessarily the same as those tolerated for prolonged period in the laboratory. In the natural habitat, the effects of salinity may be modified by the influence of other environmental parameters. So the clams are found to be more tolerant to natural variation in salinity than in the laboratory.

It is well known that estuaries and the adjoining marine realms in general are subjected to wide variations in salinity under the impact of tides and seasonal changes and monsoon. Kinne (1967) reported that such salinity fluctuations play a paramount role in limiting the distribution of animal population in marine and brackishwater environments. In the present study, dead shells of medium and large clams were noticed during monsoon months. Shiny (1991) has also recorded the occurrence of dead shells of *Musculista*

senhausia in late premonsoon and monsoon periods. The influence of the changes in salinity, temperature, substratum and food availability in the sea may affect the growth and survival of a species in an ecosystem (Bohle, 1972). Mane (1974b) has opined that the retardation of growth in clams during the monsoon is attributed to the behaviour of clams in low salinities. The closure of valves to overcome the unfavourable condition resulted in non-circulation of feeding current and animals were unable to feed. The species abundance - salinity relationship in molluscs is also worked by Gainey and Greenberg (1977). Several authors have also related the high mortality of *Donax* and other bivalves to the condition during the rainy season (Wade, 1967; Ansell and Sivadas, 1973; de Mahieu and Gamba, 1980).

The functional capacity of an organism to withstand salinity stress can be evaluated by the study of its physiological responses such as behaviour, metabolic rate, etc. The prominent behavioural mechanism by which bivalves respond to changes in salinity is valve closure. Several authors have reported the tendency of bivalves to close the valves at lower salinity concentration (Clarke and Finely, 1974; de Mahieu *et al.*, 1988; George, 1993). The valve closure mechanism helps to prevent sudden exposure of the tissue to osmotic shock and offers time for the cell to gradually adjust to the altered environment. When different size groups are concerned, large size group

close their valves much faster than the small and medium size groups. This may be due to the fact that cellular adjustments in response to changes in salinity may be slower in large size groups (George, 1993). Similar observations are noted in the present study also in *D. incarnatus*. In lower salinities, *i.e.* 5 and 10 ppt, these clams exhibited indefinite closure of valves and in 15 and 20 ppt, large clams remained with closed valves for a long duration.

It has been demonstrated that the osmotic pressure of the external medium acts as the principal stimulus controlling the initial opening of the valves and a small amount of salt in the external medium is necessary for the complete closure of valves. (Ranade and Kulkarni, 1973; Mane, 1974 b). Ram Mohan and Velayudhan (1995) have stated that the clams are unable to ensure complete closure in nature and prolonged immersion in low salinities would be detrimental. In agreement with this, Supriya (1992) and George (1993) have found that the tenure of temporary exclusion from the ambient environment by valve closure is maximum in 5 ppt salinity for *S. scripta* and it is the most unfavourable salinity for this species. The present study also reveals similar results indicating lower salinities like 5 and 10 ppt are more detrimental than higher extremes of salinity. This might be the reason why specimens of *D. incarnatus* were not found in the study area and in the

sampling during the low saline waters of the monsoon months. Valve closing, therefore, constitutes a very effective behavioural avoidance mechanism, but of short duration to protect the clams against unfavourable salinity fluctuations seasonally encountered in their environment.

In the case of *D. denticulatus* (de Mahieu *et al.*, 1988) and *Sunetta scripta* (George 1993) correlation was noticed between salinity decrease and decrease in metabolic rate which help them to evoke intracellular adjustment faster than large size group. Further, Newell (1979) indicated that increase in stress conditions can cause an increase in metabolic activity resulting in high energy expenditure which may be cited as the reason for the reduction in the amount of faeces discharged and reduced extension of the siphons as noticed in the present study on *Donax incarnatus*.

From the above experiments, it is concluded that eventhough *D. incarnatus* tolerates low salinities, it appears to have optimum range of 25-35 ppt.

Filtration rate

An indirect method for measuring filtration rate was used employing a homogenous solution of neutral red. 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 (Cole and Hepper, 1954).

According to Owen (1966) feeding is a function of the efficiency of the filter, the food particles present in the ambient water and the pumping rate. 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). The duration of the present experiment was two hours. Bayne *et al.* (1976) opined that the static system of filtration rate measurement results in the accumulation of ammonia and other excreted compounds and reduction of PO_2 in the surrounding water which may in turn inhibit the normal filtration behaviour. 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. The method also assumes that the filtration rate is constant throughout the period of the experiment.

In the present study, the higher filtration rate per body weight for small animals indicate high rate of growth and active metabolism. Similar observations were observed in *Musculista senhausia* (Shiny and Radhakrishnan, 1994), *Katelysia opima* (Mane, 1975) and *Sunetta scripta*

Supriya (1992). Durve (1963) also noticed rapid filtration rate per minute in the case of large, *Meretrix casta*. Thalikhedkar 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 gm body weight.

Lowering of filtration rate in low salinities *i.e.*, 15 and 20 ppt have been observed in the present study. Similar results were obtained by Cole and Hepper (1954); Nagabhushanam (1956).

Higher weight specific filtration rate were observed in 25, 30 and 35 ppt., maximum being in 30 ppt in all the small and medium size groups and 35 ppt in large size group (Table 5.6). This may be due to their greater tolerance to higher salinities when compared to low salinities. Similar adaptation was observed in *M. edulis* (Widdows, 1985) where the rate of adaptation to an abrupt rise in salinity from 15 to 30 ppt is more rapid than the rate of adaptation to a decline in salinity from 30 to 15 ppt. In the seven different salinities studied, at 5 and 10 ppt, not even a single bivalve filtered which was due to indefinite valve closure.

In the present investigation, *D. incarnatus* were observed to filter with maximum efficiency around 30 ppt salinity which falls within the optimum salinity tolerance range (25 - 35 ppt) observed for this species. Similar results

were reported by Supriya (1992) in *Sunetta scripta*. It is interesting to note that *S. scripta* and *D. incarnatus* share similar habitat.

GENERAL DISCUSSION

GENERAL DISCUSSION

About 77% of the living species of the Genus *Donax* (Bivalvia:Donacidae) are distributed in tropical waters, 22% in the subtropical, and 5% extend their distribution to the cold temperate regions. They are typical inhabitants of brackishwater bodies along the coast, estuaries, intertidal areas of the sandy beaches, shallow sub-littoral zone and occasionally, the littoral zone where coarse sediments occur. They enjoy high energy environment with strong wave action and strong currents. The characteristic wedge shape of the shell (hence a common name, wedge clam) helps the animal to live in such dynamic environment, and to burrow rapidly into the soil when adverse conditions are encountered and to regain to surface as the adverse condition returns to normal. Most of the species of *Donax* exhibit tidal migration or movement which benefits the clam in feeding and minimising the environmental stress (Ansell, 1983).

D. incarnatus forms one of the commercial species of clams occurring in the Cochin region. Malippuram beach in the Vypeen Island, near Kochi is an important centre from where *D. incarnatus* is gathered seasonally in appreciable quantity. During October-April it occurs in dense patches along the beach. However, data on the landings of the clam from the region are not available as the fishery is in an unorganised state.

An appraisal of the ecological features of the Malippuram beach has shown that it affords a congenial habitat for *D. incarnatus*, particularly during the post monsoon and premonsoon months (September – April). Moderately exposed beach with fine sand dominated substratum and moderate tidal range prevailing in this period enables the species to colonise the ground immediately after the monsoon. This is further aided by the ideal temperature range between 25^0 C and 31^0 C and salinity range of 25 – 35 ppt of the near shore waters. The nutrient rich inshore waters and the biological productivity provide a base to the animal to feed and grow fast. However, during the monsoon when the erosion of the beach and decline of salinity of the inshore waters occur, the species disappear from the area. Although some mortality of clams on the basis of the occurrence of dead shells is suggested during this period, it is conjectured that at least a part of the population might migrate to the adjoining littoral zone where the environmental factors promote the survival of the clams. However, further studies on the bathymetric distribution of the species in space and time are necessary to confirm this observation.

As in the case of tropical fishes, crustaceans and molluscs, several species of *Donax* living in the tropical and subtropical ecosystems have a short life of span of 1 to 2 years (Ansell, 1983), although certain species from

higher latitudes live for a longer period of 5 to 7 years. The growth pattern in the majority of tropical species of *Donax* is similar and as shown in the present study, the growth is fast initially and slows down on the attainment of sexual maturity. The initial rate of growth in *D. incarnatus* living in the Malippuram beach is found to be about 4mm per month. The maximum size recorded at 28.2 mm is attained in a year. The allometric growth relationship between shell length and breadth and shell length and depth is found to be stable. Although the growth rate recorded in *D.incarnatus* in the present study is comparable with those of other species of *Donax* studied from other regions of the coast by other workers, it may be necessary to confirm this observation as the present study was restricted to the data collected during the postmonsoon and premonsoon months.

As in all species of *Donax*, the sexes are separate in *D. incarnatus*. The maturation process of male and female gonads of *D. incarnatus* is similar and passes through five maturity stages. It attains sexual maturity as the clam grows to 13-14 mm size. The species has a prolonged breeding period with two peaks, one in February–March and the other in December. Salinity is found to be the main environmental mediatory factor limiting the reproductive activity. Thus, the basic reproductive cycle of the species shows no significant difference from those of other species of the genus.

It is well known that the seasonal fluctuations in food availability, quality of the food, environmental factors, and reproductive activity bring about changes in the biochemical composition of body tissues and weight. These changes also determine the quality of the meat. The seasonal changes of major nutrients such as protein, carbohydrate (represented by the stored reserve of glycogen) and lipid, and water content observed in *D. incarnatus* generally agree with those recorded for other tropical species of *Donax*. While the protein level remains high throughout the year in both the sexes, it declines during spawning indicating its utilisation for this activity. Similarly, the glycogen and lipid reserves built up and stored during the early growth and gametogenic period are utilised as the gonads mature and spawning takes place. However, the water content which is observed at a relatively low level during the gonad development, increases during the spawning season. Although these observations on the changes of the biochemical composition of *D. incarnatus* is essentially related to the reproductive cycle of the clam, it is essential to undertake a detailed study of the environmental factors, environmental stress, quality and quantity of the food available and the metabolic demands which greatly influence the seasonal biochemical composition of the clam.

It is natural that the clams living in a dynamic ecosystem of ever changing external physical and chemical factors, are excellently adapted to these conditions and could tolerate wide ranges of fluctuation in these factors. Nevertheless, the major environmental factors such as temperature and salinity influence the ecology and distribution of these clams, and therefore, formed the subject matter of several environmental tolerance studies. In the present thesis, salinity tolerance of *D. incarnatus* is studied. A size related tolerance to salinity variation is observed for *D. incarnatus* . It is found that while the smaller clams (10-12 mm) could survive a wide range of salinity from 16 to 35 ppt, medium size clams (17-19mm) tolerate salinity limit of 18-35 ppt and large sized (25-27mm) clams, relatively narrow limit of 24-35 ppt. This observation is based on the experiments conducted in the laboratory. It is increasingly realised that the animals in nature, and subjected to a combined effect of several environmental factors, would show different performance and hence comprehensive studies on the combined effect of environmental factors on tolerance and behaviour of clams to better appreciate the ecological adaptation are necessary.

Donax species are principally filter feeders on suspended particles. While the functional morphology of feeding and filtering apparatus have been described for several species of *Donax* (Atkins, 1937a,b; Yonge, 1949;

Wade, 1967,1967a; Moueza, 1975, 1976; Ansell, 1981), studies on rates of filtration is generally limited (Ansell, 1983). The filtration rate measured for *D.incarnatus* (10 – 25 mm in length) in different salinities (5 to 35 ppt) showed higher rate for small clams (10 –12 mm), maximum being at 30 ppt for small and medium size clams.

The foregoing brief discussion, the review of literature given in the thesis and the results of investigations presented reveal that although certain information on the biology and reproduction of species of *Donax* is available, many gaps exist in our knowledge. Data on spatial distribution of the species, population characteristics/structure, mortalities, physiological and biochemical changes during maturation and spawning; information on feeding, respiration, metabolic adaptation and the role of *Donax* in intertidal beach ecology and as an indicator of coastal environment are inadequate. Directed research to elucidate the life activities of the species belonging to this group is, therefore, imperative to exploit these resources through capture and culture fisheries and to improve the rural economy.

SUMMARY

SUMMARY

Donax incarnatus (Gmelin) is found in abundance along the intertidal region of Malippuram beach of Kerala Coast. The shell of *D. incarnatus* is triangular, more or less elongated and compressed. It is characterised by its borrowing habit and its migratory behaviour. This wedge clam is found in the lower mid-littoral zone and even extended to sub-littoral zone during monsoon season. The results of the investigations on *D. incarnatus* are presented in this thesis which is divided into five chapters preceded by a general introduction and preface.

The first Chapter includes species description and the hydrological and soil features of the study area. The genus *Donax* belongs to the Family Donacidae within the Superfamily Tellinacea of the Phylum Mollusca. The study area is vulnerable to beach erosion in large scale during active southwest monsoon months (late May-August) when the sea becomes rough. Due to this and the low saline conditions resulting from heavy rain and land drainage, *D. incarnatus* is not available during the late premonsoon and monsoon months. The wedge clam prefers a substratum having fine sand particles.

In the second Chapter the growth rate of *D. incarnatus* was explained on the basis of the examination of random samples collected at fortnightly intervals from February 1990 to February 1991. From the population structure, it is observed that in February, March and April the modal length of the population decreases. This is related to the mortality of young clams due to overcrowding. During September, fresh recruits merge with the adult population. The growth was faster during the succeeding months indicating that in the early phase of life growth rate was faster. The food availability and nutrient rich water during the postmonsoon season facilitates the clam to grow at a faster rate. The retardation of growth occurs as age increases. The young clams observed during January and February 1991 appeared to be the product of the second spawning peak seen in December. During the premonsoon season, the growth rate was moderate.

Growth parameters determined employing von Bertalanffy growth equation have also indicated a similar result. The 'k' values calculated do not show much variation for the estimated three sets of growth curves suggesting a similar growth pattern. Based on the data collected, it can be concluded that the life span of *D. incarnatus* is hardly one year.

Biometric relationships (between breadth and length, depth and length, wet flesh weight and length and dry flesh weight and length) have been

studied in different months using the least square method. It is apparent from these accounts that while shell length - shell breadth and shell length - shell depth relationships tended to be stable in *D. incarnatus* population, some difference occurred in other allometric relationships which could be attributed to physiological and ecological variations.

In the present study, biological data were not available during monsoon season due to extensive erosion of the Malippuram beach, and disappearance of clams from the area. Mortality of animals during monsoon season confirmed by presence of dead shells in the habitat is attributed to low salinity caused by freshwater influx into the coastal zone.

A detailed account on the reproductive cycle of *D. incarnatus* was given in the third Chapter based on the study of histological preparations of the developmental stages of gonad. Five main maturity stages - early gametogenesis, late gametogenesis, mature, partial spent and spent - were recognised in the annual reproductive cycle of both the sexes. The breeding period of the clam is continuous with two spawning peaks, major peak in February-March and minor peak in December. No resting condition was observed in the reproductive cycle. Salinity was found to have influence on the reproductive cycle of *D.incarnatus*. Spawning was observed to be associated with high and relatively stable salinity.

Observation on the variation in water content, protein, glycogen and lipid level in the entire body are explained in relation to reproductive cycle. In the case of water content, total tissue (male and female) showed a decrease in mature condition and later in spawning stage it showed an increase. An almost uniform level of protein content indicated no sex difference in the protein level, though a slight decrease was seen during the gametogenic activity and spawning. The glycogen level in *D. incarnatus* increased during gametogenic period and progressively decreased with the advancement of gametogenesis and in the mature condition. The lipid level exhibited clear variation with the reproductive cycle both in males and females. It is observed that clams with mature gonad occurring during October-November and January-February had maximum nutritive value.

Salinity tolerance and filtration rate of *D. incarnatus* are explained in the fifth Chapter. Experiments on salinity tolerance were carried out with three size groups of the clam viz., 10 ± 2 mm (small), 17 ± 2 mm (medium) and 25 ± 2 mm (large) in length. The animals were subjected to the salinities 5, 10, 15, 20, 25, 30, 35 and 40 ppt. Clams showed difference in valve closure, erratic behaviour and faecal production in different salinities. Studies revealed that small sized animal can survive in salinities ranging from 16 to 35 ppt, medium sized clams, 18 to 35 ppt and large sized clams, 24 to

35 ppt. Small size groups exhibited greater tolerance range than larger clams.

The rate of filtration was studied by adopting neutral red dye clearance technique. Studies on filtration rate were conducted in different salinities (5, 10, 15, 20, 25, 30 and 35 ppt) and in different size groups (10-25 mm in length). Statistical analyses of the results revealed higher filtration rate per body weight for small animals and this was related to high rate of growth and active metabolism. The higher weight specific filtration rate (ml.hr.mg. dry wt^{-1}) were observed in 25, 30 and 35 ppt the maximum being in 30 ppt for small and medium size groups.

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