STUDIES ON ECOBIOLOGY OF *PAPHIA MALABARICA* (CHEMNITZ) FROM ESTUARINE HABITATS OF GOA

A Thesis submitted to Goa University for the Award of the Degree of
DOCTOR OF PHILOSOPHY
in
(Marine Sciences)

BY

SMITA SURESH NAGVENKAR, M.Sc.

CSIR-National Institute of Oceanography
Dona Paula, Goa-403004
INDIA
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Research Guide

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2014

CERTIFICATE

Certified that the work incorporated in this thesis entitled "Studies on ecobiology of *Paphia malabarica* (Chemnitz) from estuarine habitats of Goa", submitted by Smita Suresh Nagvenkar, for the award of the Degree of Doctor of Philosophy in Marine Sciences is based on original studies carried out by the candidate under my supervision. The entire research work was carried out in the National Institute of Oceanography, CSIR. The thesis or part thereof has not been submitted for degree of any University on any previous occasion.

Place: Dona Paula

Date:

Research Guide

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DECLARATION

As required under the University Ordinance OB.9.9 (iv), I state that the present thesis entitled "Studies on ecobiology of *Paphia malabarica* (Chemnitz) from estuarine habitats of Goa" is my original contribution and the same has not been submitted elsewhere for award of any degree to any other university on any previous occasion. To the best of my knowledge, the present work is the first comprehensive work of its kind from the area mentioned. The literature related to the problems analyzed and investigated has been appropriately cited. Due acknowledgement has been made wherever facilities and suggestions has been availed.

Place: Dona Paula, Goa (Smita S. Nagvenkar)

Date: Research Student

Dedicated to my parents

STATEMENT

As required under the University Ordinance OB.9.9 (vi), I state that the present thesis entitled "Studies on ecobiology of *Paphia malabarica* (Chemnitz) from estuarine habitats of Goa" is my original contribution and the same has not been submitted on any previous occasion. For the best of my knowledge, the present work is the first comprehensive work of its kind from the area mentioned.

The literature related to the problems analyzed and investigated has been appropriately cited. Due acknowledgements has been made wherever facilities and suggestions has been availed of.

Place: Goa, India.

Date: **(Candidate)**

Smita Suresh Nagvenkar

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LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance.

CI: Condition Index.

DO: Dissolved Oxygen.

WC: Water Content.

CSIR-NIO: Council of Scientific and Industrial Research-National Institute of Oceanography.

PE: Percentage Edibility.

POC: Particulate Organic Carbon.

DPPH: 2,2-diphenyl-2-picryl-hydrazyl.

FRAP: Ferric Reducing Antioxidant Power.

HNO_3: Nitric Acid.

TBA: Thiobarbituric Acid.

PMF: Post Mitochondrial Fractions.

KCL: Potassium Chloride.

HCL: Hydrochloric Acid.

TCA: Trichloro Acitic Acid.

H_2SO_4: Sulphuric Acid.

LPX: Lipid Peroxidation.

BHT: Butlylated Hydroxytoluene.

TOC: Total Organic Carbon

GENERAL INTRODUCTION Chapter-I

1.1 Marine realm

The marine realm constitutes the most important habitats for the growth and sustenance of various aquatic life forms. The following paragraphs offer a brief overview of some of the aspects of the marine realm and its relevance to biodiversity and fishery. Over 71% of the earth surface is covered with marine waters with average depth of ~ 3.8 km and volume of about 1370 x 10^6 km^3, providing habitat to several species of flora and fauna. Oceanic water is divided into the Pacific Ocean, the Atlantic Ocean, the Indian Ocean, the Artic Ocean and the Southern Ocean. The Indian Ocean is the warmest ocean in the world with an area of 68.556 million sq km and includes Andaman Sea, Arabian Sea, Bay of Bengal, Flores Sea, Great Australian Bight, Gulf of Aden, Gulf of Oman, Java Sea, Mozambique Channel, Persian Gulf, Red Sea, Savu Sea, Strait of Malacca, Timor Sea, and other tributary water bodies. Open marine waters can be broadly divided into boreal, temperate and sub-tropical waters. On the depth scale, it can be classified as epipelagic, mesopelagic, bathypelagic and abyssopelagic (Speight and Henderson, 2010). Relative to the boreal waters, temperate waters sustain a relatively larger suite of ecosystems, such as stratified coastal and oceanic waters, permanently well-mixed waters, temperate rocky and sandy beaches, seagrass meadows, estuaries and saltmarsh habitats. However, the degree of diversity remains relatively greater in the tropical productive coastal waters and also in the monsoonal estuaries.

1.2 Coastal Zone - India

The coastline of India is approximately 6100 km long, bounded by the Arabian Sea in the west, the Bay of Bengal in the east and the Indian Ocean to the south (Mukherjee and Mukherjee, 2012). Its extensive geomorphological stretches contain rich diversity of inland and coastal wetland habitats accounting to ~ 4.1 million hectares of natural and ~ 2.6 million hectares of man-made respectively (Mukerji and Mandeep, 1998). Deltas and estuaries are quite significant among the different physical features of the Indian coastline, with deltaic regions being more predominant in the eastern coast (Ahmad, 1972). The west coast extends from the Rann of Kutuch (Gujarat) in the north to Kanyakumari (Tamil Nadu) in the south, with a length of about 3287 km, and is characterized by cliffs, promontories, lakes, lagoons, mangrove swamps and back waters (Wagle and Vora, 1980).

1.3 Coastal Zone - Goa

The coastal plain of Goa comprise an intricate system of wetlands, tidal marshes and cultivated paddy fields, all intersected by canals, inland lakes, bays, lagoons and creeks (Wagle, 1982; Rao *et al*., 1985). Fringing and patchy mangrove swamps commonly exist along the water bodies of mainland and islands in the estuaries (Jagtap, 1985). The ~120 km coastline of Goa is traversed by seven rivers (Figure 1.1). Water level of the rivers and backwaters are governed by tides by 2 or 3 meters daily. The prominent lowlands adjacent to most of the estuaries are locally known as ‘khazan lands’, a term which denotes land reclaimed by gradual filling of the shallow seas and back water regions. Most of these lowlands are situated almost at or below the sea level (Mascarenhas, 1999).

1.4 Estuaries

An estuary is a partially enclosed coastal body of water which is permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage (Day, 1981). Estuaries form a transition zone between river and ocean environments and are subjected to both marine influences, such as tides, waves, influx of saline/ fresh water and sediment. The inflows from both sea water and fresh water from hinterlands/rivers contribute high levels of nutrients rendering the estuaries among the most productive natural habitats in the world (Pritchard, 1967; McLusky and Elliott, 2004).

Estuaries from the tropics represent one of the most exploited ecosystems in the world (Blaber, 2000). They are rich in biodiversity and may have the highest economic value per hectare relative to any other aquatic environment (Costanza, *et al*., 1997). Therefore, conservation and protection of these water bodies should be on top priority in order to maintain their biodiversity on one hand and viability of fisheries out put on the other. Estuaries are typically characterized as physically controlled, unstable or unpredictable habitats (Schaffner, 1990). Within estuaries, abiotic elements like sediment composition and salinity mainly influence benthic community composition (Henley and Rauschuber, 1981; Kennish, 1986). Freshwater inflow regulates the distribution of salinity and sediment transport within estuaries (Bowden, 1967; Kennish, 1986; Jones *et al*., 1990). The salinity gradient acts as a physiological barrier to organism in stenohaline, marine and freshwater causing environmental stress on euryhaline organisms. Wider fluctuations in salinity concentrations within the midstream region of estuary heighten physiological stress and can result in reduction

in the number of species (Sanders *et al*., 1965). Species diversity has been reported to increase from freshwater (at the mouth of the river) to seawater (Remane and Schleiper, 1971). The changes in sediment characteristics, such as sand and organic matter contents also influence benthic communities across the estuarine gradient (Chester *et al*., 1983; Flint and Kalke, 1985).

Estuaries act as conduits for dissolved and particulate domestic and industrial effluents carried through land drainage to the adjacent coastal environment. Some components of various effluents are consumed by different biota or retained within the estuarine environment. Most existing estuaries were formed during the Holocene epoch by the flooding of river-eroded or glacially scoured valleys during the sea level rise between ~18 to 7 ky BP. Estuaries are typically classified by their geomorphological features or by water circulation patterns. Two of the main challenges of estuarine life are the variability in salinity and sedimentation. Many species of fish and invertebrates are tolerant to the shifts in salt concentrations and are termed osmoconformers and osmoregulators.

Phytoplanktons viz., diatoms and dinoflagellates constitute the primary producers in the estuaries and can be flushed in and out with tides. The primary food source for many organisms in estuaries including bacteria is the detritus in the sediment and suspended load. Some notable estuaries in the world are Albemarle Sound (USA), Amazon River (South America), The Golden Horn (Turkey), Chesapeake Bay (USA), Delaware Bay (USA), Drake's Estero (USA), Gippsland Lakes (Australia), Gironde (France), Great Bay(USA), Gulf of Saint Lawrence (Quebec), Hampton Roads (USA), Humber (England), Laguna Madre (Gulf of Mexico), Lake Borgne (Gulf of Mexico), Lake Pontchartrain (USA), Long Island Sound (USA),Mobile Bay(USA),

Narragansett Bay (England), New York-New Jersey Harbor (USA), Ob River (Russia), Puget Sound (USA), Pamlico Sound (USA), Port Jackson (Sydney Harbour), Rio de la Plata (South America), San Francisco Bay (USA), Shannon Estuary (Atlantic Ocean), Thames Estuary (England), St Lucia Estuary (South Africa), Yantze (China), Yellow river (China).

Total estuarine habitat in India is estimated to be around 2,14,500 hectares (Gouda and Panigrahy, 1999). The highly productive estuaries in India are located at the mouth of the river Hooghly, Godavari, Krishna, and Vellar on the East coast and Mandovi, Zuari, Netravati Garupur, Cochin backwater and Asthamudi on the West coast (Nair *et al*., 1984; Appukuttan, 1993). Goa is drained by seven (Figure 1.1) major rivers (Tiracol, Chapora, Mandovi, Zuari, Sal, Talpona and Galgibag) of which the Mandovi and Zuari with the Cumbarjua Canal form the largest estuarine complex. The total area covered by the estuaries in Goa including the major Mandovi Zuari estuarine complex is approximately 12000 hectares (Norohna, 2012).

1.5 Mandovi and Zuari estuaries - Goa

Mandovi-Zuari forms major estuaries of Goa having confluence regions adjacent to each other (Figure 1.1). Both originate in the Western Ghats and drain through a narrow coastal plain. Both rivers drain through rocks belonging to the Dharwar Super Group of the Archaean Proterozoic age (Gokul *et al*., 1985).

1.5.1 Hydrological features of estuaries

The estuaries of Mandovi and Zuari rivers are classified as “monsoonal estuaries” (Shetye *et al*., 2007; Vijith *et al*., 2009), which receive abundant river discharge only during the monsoon (June – September) and negligible discharge in the remaining

period. The river runoff of Mandovi measured at the head during June–October is ~ 258 $m^3 s^{-1}$ and during November – May is ~ 6$m^3 s^{-1}$ (Vijith *et al*., 2009).

In Zuari River the runoff measured at Sanguem and Kushavati tributaries during the monsoon (June–September), post-monsoon (October–January), and pre-monsoon (February–May) are ~ 147, 7.3, and 0.8 $m^3 s^{-1}$, respectively. These estuaries are mesotidal and the tidal ranges are ~ 2.3 and 1.5 m during the spring and neap tides, respectively (Manoj and Unnikrishnan, 2009). Saline waters penetrate ~ 45 km upstream from the river mouth into the main channels of both rivers during the dry season, i.e., October to May (Shetye *et al*., 2007).

1.5.2 Ore transport and Navigation

The Mandovi-Zuari rivers forms an inexpensive and efficient means of ore transport. Several big open cast iron and manganese ore mines operate in the drainage basins of the rivers. Iron and Mn ores are mainly stored on the shore along upstream regions of the estuary, loaded on to barges at various loading points and transported through these estuaries to the Mormugao port or midstream point from where the ore is exported in ships (Kessarkar *et al*., 2013). Mandovi has 37 loading points with 1500 trips of barges per year, while Zuari has 20 loading points with 1800 trips per year. Ore transport through rivers have been reported to increase annually from 14 million tonnes (mt) in 1980 to 30.7 mt in 2004 (GMOEA, 2004). Of this, 19.1 mt of ore are transported through the Mandovi and 11.6 mt transported through Zuari. Part of the ore (11 mt) carried through the Mandovi estuary is diverted to the port through Cumbarjua canal during the monsoon (Rangaraj and Raghuram, 2005). During heavy monsoon rains, abundant ore materials are being flushed into the estuaries. During the entire handling, loading, transportation and unloading on ships from barges, abundant

ore materials get released into the estuaries which may lead to serious environmental problems for the aquatic ecosystem.

1.6 Marine biodiversity

Marine biodiversity means the variety of life in genes, species and habitats (Heip, 2012). Currently the World‘s biodiversity is estimated at ~1.75 million species, excluding microbial species (Heywood and Watson, 1996). However, Reaka-Kudla (1997) estimates the same in the range from 5 to 120 million. The two biggest repositories of marine biodiversity are coral reefs (because of the high number of species per unit area) and the deep sea (because of its enormous area). Estimates for coral reefs range from 1 to 9 million species (Reaka-Kudla, 1997), but they are very indirect as they are based on a partial count of organisms in a large tropical aquarium or on extrapolations stemming from terrestrial diversity estimates (Erwin, 1982; Small *et al*., 1998). The largest estimate of 10 million benthic species (Grassle and Maciolek, 1992) was based on an extrapolation of benthic macrofauna, although others (Poore and Wilson, 1993; Snelgrove, 1998) suggested the same to be 5 million species as a more appropriate number. India is among one of the 12 mega-biodiversity countries with 25 hot spots of the richest and highly endangered eco-regions of the world (Myers *et al*., 2000). Of the 0.2 million species available in the marine realm, 80000 are identified, of which 15000 are known from seas around India (Venkataraman *et al*., 2012). Around 665 different marine species have been reported from Goa (Shastri, 1977; Agadi and Untawale, 1978; Parulekar *et al*., 1980; Dhargalkar, 1982; Goswami, 1985; Jagtap, 1985; Santhakumaran, 1985; Achuthankutty and Parulekar, 1986; Harkantra and Parulekar, 1986; Parulekar, 1990; Devassy, 1992; Chinnaraj, 1993).

1.7 Fisheries

Oceans are the primary source of global fish catch. Pacific ocean contributes 84.234 million metric ton of fish, Atlantic Ocean with 24.045 million ton, Indian Ocean with 10.197 million ton and Southern Ocean contributes 0.147 million ton of fish (Mood and Brooke, 2010). India reported 3.94 million metric tonnes marine fish landing during the year 2012 with Kerala being the highest contributor towards production with 8.4 lakh tones (Rao, 2013). Except West Bengal and Odisha, all maritime states and union territories reported increase in fisheries production during 2012. The annual marine fish catch in Goa was estimated at 72307 tonnes during 2012 with pelagic fishes contributing 86.1%, demersal fishes 7.2%, crustacean 5.5% and molluscs contributing 1% (Rao, 2013).

A coastal environment like estuaries show abundance of benthic communities, amongst which Mollusca forms the major group and forms the second largest phylum of invertebrates (Apte, 1998). Sediments are enriched with nutrients in the estuarine zones, which provide favorable organic beds to burrowing and filter feeders. The commercial species of molluscs are more abundant on the West coast than on the east coast of India (Jhingran, 1975). Molluscs play important role in marine fisheries resource and have high demand in the seafood markets. Coastal fishing communities all over the world exploit molluscan groups.

The phylum mollusca constitutes dominant group of benthic macrofauna belonging to seven classes namely Aplacophora, Monoplacophora, Polyplacophora, Gastropoda, Bivalvia, Scaphopoda and Cephalopoda. In India, till the date, 5070 *spp*. of molluscs have been reported, of which 3370 *spp*. are from marine habitats (Subba Rao, 1991, 1998) and are affiliated to 220 families and 591 genera. These include 1990 *spp*. of

gastropods, 1100 *spp*. of bivalves, 210 *spp*. of cephalopods, 41 *spp*. of polyplacophorans and 20 *spp.* of scaphopods (Subha Rao, 1991, 1998; Appukuttan, 1996). Andaman and Nicabar Islands are also reported to have rich molluscan diversity, of which over 1000 *spp*. are from marine region (Subba Rao and Dey, 2000). Gulf of Mannar and Lakshadweep accounts for 428 and 424 *spp*. respectively (Venkataraman *et al*., 2004). Eight species of oyster, two *spp*. of mussels, 17 *spp*. of clams, 6 *spp*. of pearl oysters, 4 *spp*. of giant clams, 1 *sp*. of window-pane oyster and other gastropods such as sacred chank, Trochus, Turbo as well as 15 *spp*. of cephalopods are exploited from the maritime state of India (Venkataraman and Wafar, 2005). List of bivalve *spp*. present along west and east coast of India are given in Table 1.1 while edible forms are depicted in plates 1.1-1.3.

Clams are most widely distributed and found abundantly in India. They are exploited for their meat and shell. There is high demand for clam *spp*. especially venerid clams, out of which three genera namely, *Meretrix, Katelysia* and *Paphia* are most important. Out of 15 *spp*. of *Paphi*a, five *spp*. are distributed along the Indian coast (Appukuttan, 1993). In Goa, four *spp*. of clams are commercially exploited viz., *Meretrix casta*, *Paphia malabarica, Villorita cyprinoids* and *Katelysia opima* (Plate 1.1). In addition to these, several other species of clams (Table 1.1) are also consumed by the local people but are not commercially exploited. These include *Mactra* sp., *Sunetta scripta*, *Meretrix meretrix*, *Donax scortum*, *Placuna placenta*, *Solen kempi*, *Anadara rhomdea* and *Polymedosa erosa*.

1.8 *Paphia malabarica* – an edible bivalve

Paphia malabarica is a traditional staple food, rich in protein, for the local population and are available round the year with variable abundance depending on the production

at different season. A large section of fisherman depends on the sale of *P. malabarica*. Inspite of its commercial importance hardly any required data such as total quantum harvested and income from such fisheries exist from the Goa for detailed economic analyses. Information on *P. malabarica* from coastal Karnataka, Kerala and Maharashtra showed variation in its productivity, average shell size of individuals and nutritional content of *P. malabarica* (Rao, 1988; Appukuttan and Aravindan, 1995; Appukuttan *et al*., 1996a,b; Thomas *et al*., 2003; Thomas and Nasser, 2009; Mohite, 2010). Such kind of information does not exist for *Paphia* population from the Goa.

Temperature, salinity, food etc. are known to influence the growth of *Paphia* at the larval stage (Rajesh, *et al*., 2001; Gireesh and Gopinathan, 2004, 2008). However, the extent to which natural as well as anthropogenic parameters influence the growth and spread of *P. malabarica* is unknown from Indian. Detailed studies on anthropogenic input in estuarine regions is required in order to demarcate the pollution free zone where contaminant free assemblage of *P. malabarica* can be grown for consumption. Influence of metal pollutants on the productivity of *P. malabarica* as well as nutritive values has to be studied. Metal contamination and its influence on nutritional status in *Paphia* need to be evaluated for the management purpose.

Environmental parameters in ambience of *Paphia* beds and allometry, biochemical composition, antioxidant properties and metal composition of *P. malabarica* at two major locations of Goa coast were investigated. The data presented in the present document may be helpful in improving knowledge gap and assist the fisheries as well as the research and consumer community to develop sustainable management stratergies relevant to clam, particularly *Paphia* fisheries.

1.9 Taxonomic Position

Kingdom: Animalia

Phylum: Mollusca

Class: Bivalvia

Order: Veneroida

Family: Veneridae

Genus: *Paphia*

Species: *malabarica* (Chemnitz)

2.0 Morphological features

Anterior and posterior margins of *P. malabarica* shell are rounded and the dorsal margin bears a very slight indentation towards the anterior end (Plate 1.4). The surface of the shell is sculptured with close set of raised and rounded concentric ridges. The separating interstitial grooves are deeper. As the concentric ridges and grooves are strictly parallel to the margin of the shell, they are slightly flexed posteriorly, in conformity with the slight indentation of the ventral margin towards the anterior end. The hinge bears three short thick cardinal teeth. The tooth in front of the cardinals in the left valve and the hollow in the right are rudimentary. The pallial sinus is very deep and "U" shaped. The inner surface is quite smooth throughout and its margin is not denticulated. The lunule is short and broad. The shell is of a pale yellowish brown colour with greyish brown bands. Sometimes the surface is more elaborately mottled with brownish angular markings all over (Rao *et al*., 1989; Shanmugam *et al*., 1990; Apte, 1998).

2.1 Review of literature

Coastal communities all over the world exploit various molluscan resources among which bivalves are abundant in the estuarine regions of India (Jones, 1970). They mainly consist of clams, mussels and oysters and green mussel (*Perna viridis*), estuarine oysters (*Crassostrea madrasensis* and giant oyster *C. gryphoides*) and clams (*P. malabarica, Meretrix casta, M. meretrix, Villorita cyprinoids*) are of commercial value. The Mandovi-Zuari estuarine system forms one of the largest riverine networks on the west coast of India. These rivers supply considerable amount of organic matter to the near shore coastal waters, thus influencing the primary productivity and trophic dynamics of the coastal ecosystem (Rodrigues, 2003). Several studies have been done on the physico-chemical and biological characteristics of estuarine ecosystems of the Mandovi and Zuari (Dehadrai, 1970; Dehadrai and Bhargava, 1972; Das *et al*., 1972; Bhargava *et al*., 1973; Dwivedi, 1974; Goswami and Singbal, 1974; Rao, 1974; Parulekar and Dwivedi, 1974; Dalal, 1976; Singbal, 1976; Parulekar *et al*., 1980; De Sousa *et al*., 1981; Qasim and Sen Gupta, 1981; Ansari, 1988; Rivonkar, 1991; Rattan, 1994; Sarma *et al*., 2001; Nayak, 2002; Bhosle *et al*., 2004; Qasim, 2004; Shetye *et al*., 2007). The above studies showed that hydrological parameters are highly influenced by the South-West monsoon exhibiting a rhythmic seasonal pattern. Various environmental factors affecting growth, biology and overall production of bivalves have been studied by Seed (1968); Walne (1972); Rao *et al*. (1975); Qasim *et al*. (1977); Mahadevan and Nayar (1987); Siddique and Ahmed (2002); Bergquist *et al*. (2006) and Turner (2006).

Fifty-five species of bivalve molluscan species have been reported from the Gulf of Mannar (Hamid and Somasundaram, 1998). Distribution of clams from several

estuaries and bays along the Maharashtra coast has been investigated by Patil *et al*. (2002). Clam resources of coastal Kerala have been studied by Narasimham (1988; 1991) and James (1990). Fishery and biology of *M. casta* in the Pulikat lake, Tamil Nadu, has been reported by Thangavelu and Sanjeevaraj (1985). A number of researchers such as Algarswami (1966) and Achary (1979) have reported clam resources from different estuarine areas of India. Biological and fisheries aspects of *P. malabarica* from other regions of India have been reported by Rao (1987), Appukuttan *et al*. (1996a) and Thomas *et al*. (2003). Length range of 18-50 mm contributed maximum to the catch during peak fishing in October. *Paphia malabarica* showed higher growth rate, as a result, it attains a marketable size in less time (Mohite and Mohite, 2009a). Comparison of the growth and age of bivalves from temperate and tropical estuarine ecosystems have been carried out by Parulekar *et al*. (1984). Appukuttan *et al*. (1996b) have described population dynamics of *P. malabarica* from Astamudi estuary, South India. It showed that *P. malabarica* attains 30 mm length in one year, 38 mm in two years and 41 mm in three years. Nair *et al*. (1984) reported clam resources of Kali river in Karnataka, where *P. malabarica* was observed to have an average density of 120 no./m^2. Rao *et al*. (1989) recorded the density of *P. malabarica* to 10 no./m^2 and 85 no./m^2 in two beds in the Mulky estuary and 122 clams/m^2 in the Gurupur estuary. Appukuttan *et al*. (2002) reported that about 1200.78 ha. in Ashtamudi Lake have rich clam resources harbouring about 61255 tonnes of commercially important clams. Galstoff (1931) attempted studies on allometric relationships of the pearl oyster, *Pinctada* sp. Similar studies have also been attempted on Indian bivalves (Rao and Nayar, 1956; Parulekar *et al*., 1973; Durve, 1973; Alagarswami and Chellam, 1977; Ansari *et al*., 1978; Nair *et al*., 1978; Hickman, 1979; Mohan, 1980; Chatterji *et al*., 1984; 1985; Parulekar *et al*., 1984;

Schaefer *et al*., 1985; Rivonkar, 1991; Blaber, 2000). In Gurupur estuary in Karnataka, *P. malabarica* recolonised and formed thick beds after a gap of ten years (Rao *et al*., 1989). Mohite and Mohite (2009b) studied gonadal development in *P. malabarica* in relation to hydrographic parameters in Kalbadevi estuary. Histological studies showed that the clam had an extended spawning period from September to January.

Effects of various environmental parameters such as density, microalgal diets, effect of salinity and pH on spat and larval develoment of *P. malabarica* have also been reported from estuarine regions of India (Sivlingam *et al*., 1994; Gireesh and Gopinathan, 2004; 2008). Mohan and Velayudhan (1998) found that 20-30 psu (practical salinity unit) is optimum (no mortality) salinity for *P. malabarica* to grow but can tolerate a salinity of 12-40 psu with 50% mortality. Rajesh *et al*. (2001) reported the effect of varying algal cell concentration, salinity and body size on the filtration and ingestion rates of *P. malabarica*. It was inferred that increasing algal cell concentrations resulted in increasing filtration and ingestion rate. Liong (1979) observed mortality in cockle *A. granosa* due to fluctuation in salinity. Higher salinity is known to retard the rate of filtration of water in the clam *M. casta* and consequently the feeding (Durve, 1970). Ranade and Kulkarni (1972) observed that the opening of the shell valves in *M. meretrix* and *Katelysia opima* is delayed as the salinity decreases. Growth rate of the black clam, *V. cyprinodes* was found to be more in higher concentration of salinity (Panikkar, 2004).

Seasonal variations in biochemical composition of marine bivalves have been evaluated for their nutritive potentials (Williams, 1969; Ansell, 1972; Dame, 1972; Gabbott and Bayne, 1973; Seed, 1973; Holland and Spencer, 1973; Gabbott and

Stephenson, 1974; Dare and Edwards, 1975; Lubet, 1976; Mann, 1979; Jones *et al*., 1979; Zandee *et al*., 1980; Ruiz *et al*., 1992; Páez-Osuna *et al*., 1993; Robert *et al*., 1993; Patrick *et al*., 2006; Dridi *et al*., 2007). Indian bivalves too were studied for their nutritional contents by Venkataraman and Chari (1951); Durve and Bal (1961); Durve (1964); Saraswathy and Nair (1969); Wafar *et al*. (1976); Kumari *et al*. (1977); Shafee (1978); Nagabhushanam and Bidarkar (1978); Lakshmanan and Nambisan (1980); Stephen (1980a); (1980b); Ansari *et al*. (1981); Joseph and Madhyastha (1984); Chatterji *et al*. (1985); Ponniah (1988); Balasubrahmanyan and Natarajan (1988); Rivonkar and Parulekar (1995). Appukuttan and Aravindan (1995) reported seasonal changes in biochemical composition of *P. malabarica* from Ashatamudi Lake, however, no marked variation was reported in the percentage in meat content, water content and biochemical composition between size groups. Mohite *et al*. (2009) observed seasonal changes in condition index (CI) and percentage edibility (PE) of *P. malabarica* from estuarine regions. Biochemical compositions studied for eggs, D-shaped larvae, umbo larvae and pediveliger of *P. malabarica* show that the protein and lipid provide similar amount of energy required for metamorphosis compared to carbohydrate (Gireesh *et al*., 2009). Meat and mantle fluid extracts of *P. malabarica* were found to possess high antiviral activity when tested with influenza virus (Chatterji *et al*., 2002). Modassir and Ansari (2000) reported an adverse effect of diesel oil on the growth, CI and biochemical constituents of *P. malabarica*. Radical scavenging potentials of bivalves from India have been very poorly understood. Jena *et al*. (2010) reported antioxidant activity of *P. viridis* and its protective role against ROS induced lipid peroxidation and protein carbonyl.

Studies have been carried out on the concentrations of trace metals in sediment of Mandovi and Zuari estuaries of Goa (Alagarsamy, 2006; Singh *et al*., 2009). Wilfred

et al. (1994) reported that *P. malabarica* serve as a bioindicator of copper pollution. Minor differences in the accumulation of metals in the whole soft tissue of *P. malabarica* between small size (25-35 mm) and big size groups (35-40 mm) were reported (Kumari *et al*., 2006). Copper and Zinc were within permissible limit, whereas, lead concentrations were found to be beyond permissible level. Studies on the effect of copper uptake on development and survival rate of *P. malabarica* larvae under low salinity conditions have been carried out by Gireesh and Gopinathan (2009). The survival rates in *P. malabarica* were found to decrease considerably with low salinities. Trace metal concentrations in different species of marine bivalves, particularly mussels *Mytilus* spp., were used as indicator parameter for evaluating and monitoring the coastal pollution (Talbot *et al*., 1976; Bourget and Cossa, 1976; Phillips, 1976a; 1976b; 1977a; 1977b; 1978; Nair *et al*., 1977; D'Silva and Kureishy, 1978; Bhosle and Matondkar, 1978; Popham *et al*., 1980; Gordon *et al*., 1980; Talbot, 1985; 1987; Borchardt *et al*., 1988; Fang and Wang, 2006). Similarly, *Pecten maximus* and *Modiolus modiolus* were also studied by Segar *et al*. (1971), *P. viridis* by Rivonkar (1998) for their trace metal concentrations.

Clam cultures are practiced in several Southeast Asian countries, USA, France and Tunisia (Pannikkar, 2004). Bivalve culture (edible and pearl oysters and mussel) on commercial basis has gained momentum in India. Panikkar (2004) carried out the culture of *V. cyprinoides* (Gray) in laboratory conditions and in pens. Higher growth rate was observed in the pen culture, indicating the influence of ecological factors like salinity. Sivlingam *et al*. (1994) studied the effect of population density on the growth of the spat of *P. malabarica* in the hatchery. Girkar (2003) reported high average survival (69%) of *P. malabarica* at stocking density of 50 no./m^2 at Kalbadevi estuary during the culture period of 6 months. Parulekar *et al*. (1984) dealt with ecology and

culturing of various bivalves including *P. malabarica* in Goa and reported higher yield than naturally occurring species. Results showed considerably increase in the yield, suggesting technical feasibility for the commercial production of bivalves.

2.2 Objectives

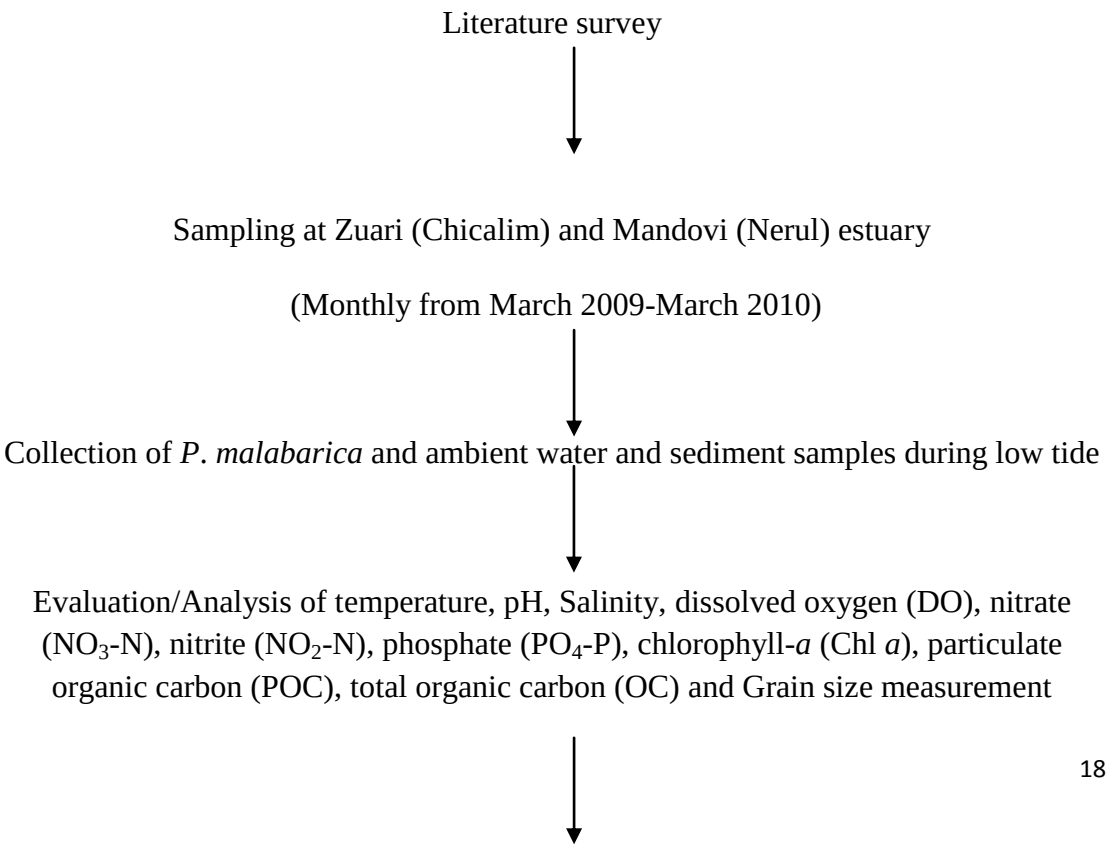
- Seasonal variation in the abundance of *Paphia malabarica* in two habitats
- Allometric relationship in *P. malabarica*
- Impact of environmental anomalies on *P. malabarica*
- Seasonal variations in proximate biochemical composition and calorific value of *P. malabarica*
- Bioaccumulation of selected metals in *P. malabarica*

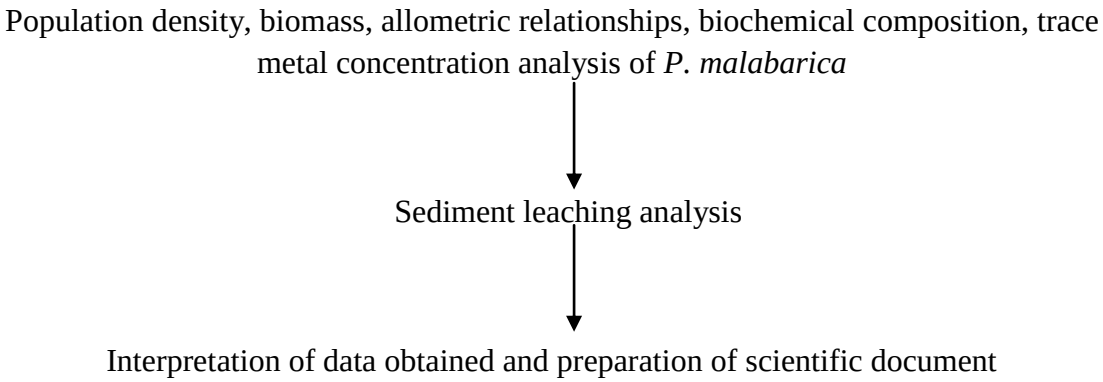
Marine bivalve are exploited from the time immemorial, however, now a days the exploitation rate has increased considerably due to population explosion. The proper assessment and harvesting practices are often not maintained and exploitation is irregular. Such activities may cause severe threats to natural stocks resulting in dwindling of resources. Due to increasing demands from other countries, the bivalve resources are harvested on a large scale from natural habitat and exported. The exploitation of seed clam (juvenile size) before attaining maturity and destruction of habitat are some of the important factors adversely affecting the clam fishery. In addition to this, mining activities, sand extraction and industrial pollution are the main reasons affecting the clam resource (Ingole *et al*., 2006). As a result of this, related estuarine and backwater zones remain under constant threat from various types of

contamination. It is therefore, very necessary to evaluate the health status of edible organisms to understand their suitability for consumption.

Paphia malabarica forms one of the major edible bivalves in Goa occuring round the year along the west coast of India. They are indiscriminately harvested for human consumption in Goa, particularly, during the monsoon season (Plate 1.5). Knowledge of the stock size, fishing season, exploitable resource and food value are essential aspects for commercialization of any species. Data and relevant information discussed in the present document would greatly help in understanding and modeling the ecological and physiological processes of *P. malabarica*. It will also be of beneficial in the aquaculture programmes, in determining the best season for the fishery, spawning season and growth patterns of *P. malabarica*. This would further be utilised to develop effective sustainable management of clam resources in the state which will serve the model for the rest of the maritime states.

2.3 Work plan and brief overview of the present study





2.4 Generic structure

The present thesis is structured into seven chapters. Chapter 1 provides information on the open ocean, estuaries and marine biodiversity of world and India. Information on coastal zones of India, coastal ecosystem of Goa, particularly of the Mandovi and Zuari estuaries, have also been discussed. It also includes ecological, economical importance, taxonomy and morphological features of *P. malabarica*. The literature reveals that very limited information exist on the ecobiology of *P. malabarica* from Goa. The data generated in the present investigation would strengthen the existing knowledge on *P. malabarica* beds from Goa. Objectives of the present work are also given in this chapter.

Chapter 2 deals with materials and methods used for evaluating population structure of *P. malabarica* from selected beds. It also includes methodology adopted for understanding the ambient environmental parameters influencing population. The various experimental protocols in this chapter include physico-chemical analysis of water and sediment, metal concentrations in sediment/tissue, allometric relationships and biochemical parameters of *P. malabarica* during the study period (March 2009 to March 2010).

Chapter 3 includes results presented in graphical and tabulated forms. The results include various environmental parameters viz., temperature, salinity, pH, DO, POC, nutrients (NO_3-N, NO_2-N, PO_4-P), chl *a*, TOC and grain size.

Chapter 4 describes population density, biomass and allometric relationship of *P. malabarica* in relation to its ambient environmental parameters. Morphometric relationships between shell length-shell breadth and shell length-shell depth, shell length-total weight, shell length-shell weight, shell length-wet weight, and shell length-dry weight variables were studied.

Chapter 5 deals with biochemical composition (protein, carbohydrate, lipid, ash), condition index (CI), percentage edibility (PE) and antioxidant properties (2,2-diphenyl-1-picryl-hydrazyl, reducing potential, inhibition of lipid peroxidation levels, hydroxyl radical scavenging activities, ferric reducing antioxidant power) of *P. malabarica*.

Chapter 6 provide concentrations of selective metals (Iron, Manganese, Lead, Cadmium and Zinc) in tissue of *P. malabarica* and sediment leachates

Chapter 7 comprises conservation and management of *P. malabarica*. This include stock assessment, shellfish farming, propagation hatchery, spat collection and relaying, the coastal regulation zone, management of clam beds, overfishing and culture .

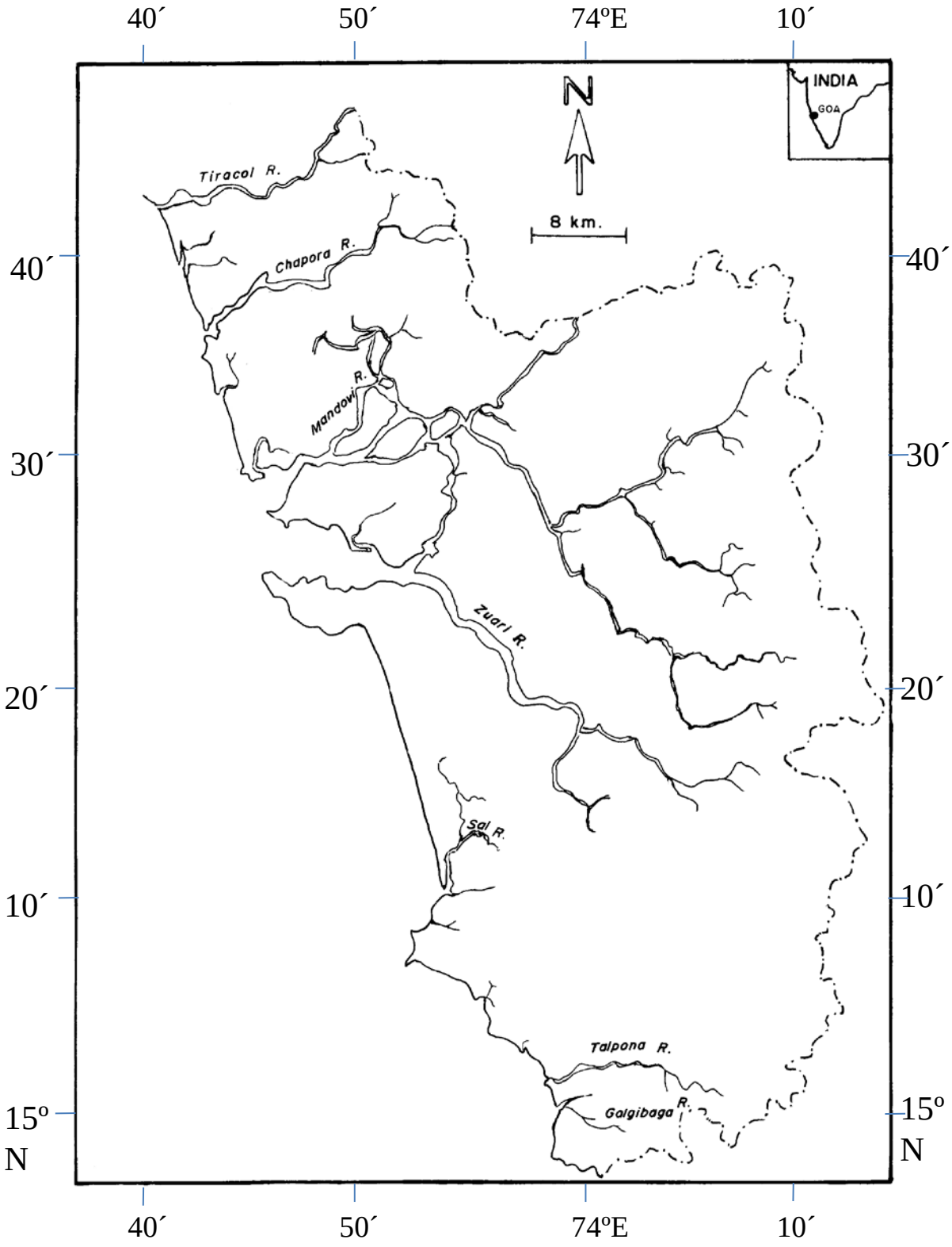


Figure 1.1: Map of Goa showing various river basin.Source: Nagi, 2008.

Meretrix casta

Polymedosa erosa

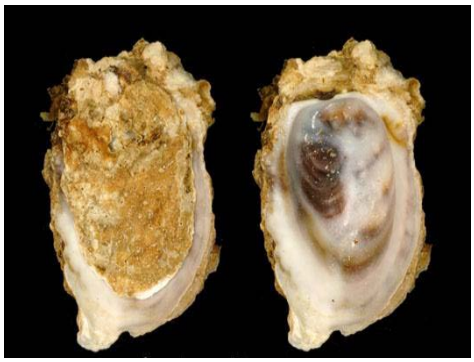
Villorita cyprinoides

Anadara granosa

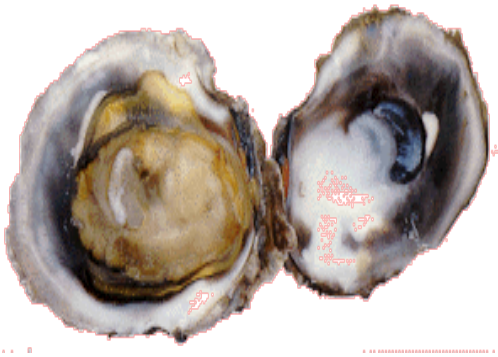
Plate 1.1: Common edible bivalves from marine environment of Asian countries. Source.
http://ranong.myspecies.info/category/clade/flora-and-fauna/mollusca/bivalvia/veneridae/meretrix-casta

Meretrix meretrix

Paphia textile



Saccostrea cucullata

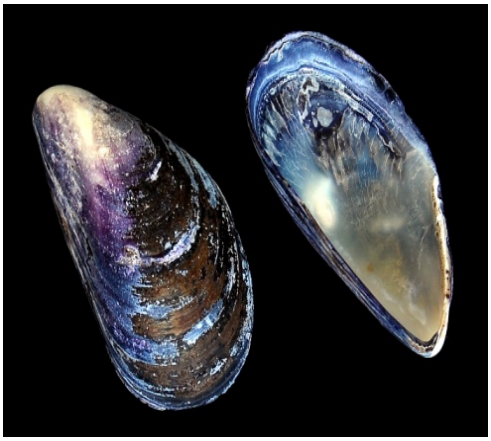


Crassostrea madrasensis

Plate 1.2: Common edible bivalves from marine environment of Asian countries.Source: http://www.celkau.in/Fisheries/CultureFisheries/Edible%20Oysters/species.aspx

Pinctada margaritifera

Placuna placenta



Mytilus edulis

Perna viridis

Plate 1.3: Common edible bivalves from marine environment of Asian countries. Source:
http://www.jaxshells.org/pernazf.htm

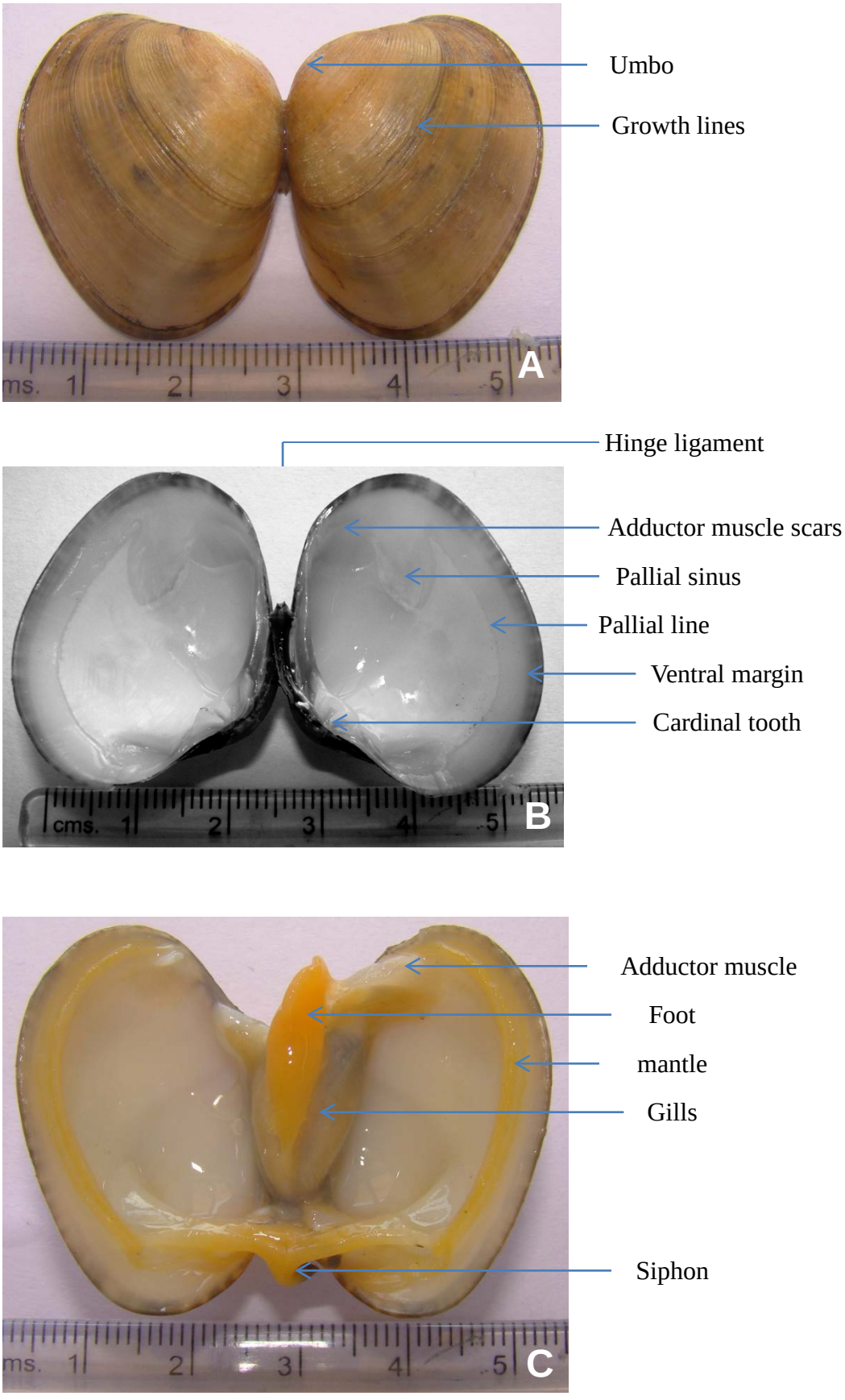


Plate 1.4: (A) External and (B) internal features of the shell valves and (C) internal features of the tissue of *P. malabarica*.

Plate 1.5: *Paphia malabarica in local market - a* commercial source for coastal people.

Table 1.1: List of edible bivalves from Asia.

Name of species	West Coast, India	East Coast, India	Other Asian countries
Anadara granosa (Linnaeus, 1758)	+	+	+
A. rhombea (Born, 1778)	-	+	-
Meretrix casta (Gmelin, 1791)	+	+	-
M. meretrix (Linnaeus, 1758)	+	+	-
M. ovum (Hanley, 1845)	+	-	-
M. lyrata (Sowerby II, 1851)	-	-	+
Paphia malabarica (Chemnitz)	+	-	-
P. textile (Gmelin, 1791)	+	-	-
P. marmorata (Lamarck, 1818)	+	-	-
P. tenerrima (Carpenter, 1857)	-	-	+
P. staminea (Conrad, 1837)	-	-	+
Polymesoda erosa (Solander, 1876)	+	-	-
P. maxima (Lamarck, 1818)	+	-	-
Villorita cyprinoides (Gray, 1825)	+	-	-
V. cornucopia (Prashad, 1921)	+	-	-
Crassostrea madrasensis (Preston)	+	+	-
C. cucullata (Born, 1778)	+	-	-
C. gryphoides (Schlotheim, 1813)	+	-	-
C. discoidea (Gould)	+	-	
C. cristagalli (Linnaeus, 1758)	+	-	-
C. palmipes (Sowerby, 1871)	+	-	-
C. rivularis (Gould, 1861)	+	-	-
C. gigas (Thunberg, 1793)	-	-	+
C. virginica (Gmelin, 1791)	-	-	+
C. gigantissima (Finch, 1824)	-	-	+
C. angulata (Lamarck, 1819)	-	-	+
Saccostrea cucullata (Born, 1778)	+	+	+
S. glomerata (Gould, 1850)	-	-	+
Placuna placenta (Linnaeus,1758)	+	-	+
Pinctada margaritifera (Linnaeus, 1758)	+		-
P. fucata (Gould, 1850)	+	+	-
P. radiata (Leach, 1814)		+	-
Katelysia opima (Gmelin, 1791)	+		-
Perna viridis (Linnaeus, 1758)	+	+	+
P. indica (Linnaeus, 1758)	+	-	
P. canaliculus (Gmelin, 1791)	-	-	+
P. canalicula (Gmelin, 1791)	-	-	+
P. perna (Linnaeus, 1758)	-	-	+
Enigmonia aenigmatica (Holten, 1802)	+	-	-
Gafrarium tumidum (Roding,1798)	+	-	-
G. divaricatum (Gmelin, 1791)	+	-	-
G. pectinatum (Linnaeus, 1758)	+	-	-
Cardita bicolor (Lamarck, 1822)	+	-	-

Mesodesma glabrata (Gmelin, 1791)	+	-	-
Mactra corbiculoides (Reeve, 1854)	+	-	
M. discors (Gray, 1837)	-	-	+
M. stultorum (Linnaeus, 1758)	-	-	+
Cardium asiaticum (Bruguiere, 1789)	+	-	-
C. setosum (Redfield, 1846)	-	+	-
Donax cuneatus (Linnaeus, 1758)	+	+	-
D. scortum (Linnaeus, 1758)	+		-
D. faba (Gmelin, 1791)	+	+	-
D. spiculum (Reeve, 1855)	+	-	-
D. incarnates (Gmelin, 1791)	+	-	-
D. lubricus (Hanley, 1845)	+	-	-
D. trunculus (Linnaeus, 1758)	-	-	+
D. californicus (Conrad, 1837)	-	-	+
D. laevigata (Gmelin, 1791)	-	-	+
Modiolus barbatus (Linnaeus, 1758)	+	-	-
M. metacalfei (Hanley, 1843)	+	+	-
M. striatulus (Hanley, 1844)	+	-	-
M. undulates (Dunker, 1856)	+	-	-
M. tulipa (Lamarck, 1819)	+	-	-
M. rectus (Conrad, 1837)	-	-	+
Acra granosa (Linnaeus, 1758)	+		-
Chlamys senatoria (Gmelin, 1791)	-	-	-
Sanguinolaria diphos (Linnaeus, 1771)	+	-	-
S. atrata (Reeve, 1857)	+	-	-
S. nuttalli (Conrad, 1837)	-	-	+
Solen truncatus (Wood, 1815)	+	-	-
S. brevis (Hanley, 1842)	+	-	-
S. sicarius (Gould, 1850)	-	-	+
S. rosaceus (Carpenter,1864)	-	-	+
S. strictus (Gould, 1861)	-	-	+
S. Kempi (Preston, 1915)			
Placenta placenta (Linnaeus, 1758)	+	+	-
Mesoderma glabratum (Lamarck)	+	-	-
Dosinia prostate (Linnaeus, 1758)	+	-	-
Bactronophorus thoracites (Gould, 1856)	+	-	-
Mercia opima (Gmelin, 1791)	+	-	-
Sunetta scripta (Linnaeus,1758)	+	-	-
S. meroe (Linnaeus,1758)	-	-	+
Geloina bengalensis (Lamarck, 1818)	+	+	-
Tellina pinguis (Hanley, 1844)	+	-	-
T. rhodon (Hanley, 1844)	+	-	-
T. bodegensis (Hinds, 1845)	-	-	+
Geloina bengalensis (Lamarck, 1819)	+	-	-
Barbatia candida (Helbling, 1779)	+	-	-
Barnea candida (Linnaeus, 1758)	+	-	-

Cardites biocolor (Lamarck, 1819)	+	-	-
Codakia tigerina (Linnaeus, 1758)	+	-	-
Nuculana mauritiana (Sowerby, 1833)	+	-	-
Arca lateralis (Reeve, 1844)	+	-	-
A. inaequivalvis (Bruguiere, 1789)	+	-	-
A. tortuosa (Linnaeus, 1758)	+	-	-
A. indica (Gmelin, 1791)	+	-	-
Septifer bilocularis (Linnaeus, 1758)	-	+	-
S. virgatus (Wiegmann, 1837)	-	+	-
Musculus senhousia (Benson in Cantor, 1842)	+	-	-
Lithophaga nigra (d'Orbigny, 1842)	+	-	-
Pteria margaritifera (Linnaeus, 1758)	+	-	-
P. chinensis (Leach, 1814)	-	+	-
Pinna bicolor (Gmelin, 1791)	-	+	-
Pecten tranquebaricus	+	+	-
P. novaezelandiae (Reeve,1852)	-	-	+
P. circularis (Sowerby I, 1835)	-	-	+
P. maximus (Linnaeus, 1758)	-	-	+
P. jacobaeus (Linnaeus, 1758)	-	-	+
Lucina ovum (Reeve, 1850)	+	-	-
Cardium quadragenarium (Conrad, 1837)	-	-	+
C. corbis (Martyn, 1784)	-	-	+
C. elatum (Sowerby I, 1833)	-	-	+
Dosinia modesta (Sowerby, 1835)	+	-	-
D. cretacea (Reeve, 1850)	+	-	-
D. trigona (Reeve, 1850)	+	-	-
D. histrio (Gmelin, 1791)	+	-	-
Venus imbricata (Gmelin, 1791)	+	-	-
V. reticulate (Linnaeus, 1758)	-	+	-
Venerupis macrophylla (Deshayes, 1853)	+	-	-
V. decussate (Linnaeus, 1758)	-	-	+
V. philippinarum (Adams & Reeve, 1850)	-	-	+
V. senegalensis (Gmelin, 1791)	-	-	+
Circe scripta (Linnaeus, 1758)	+	-	-
Chione tiara (Dillwyn)	+	-	-
C. fluctifraga (Sowerby II, 1853)	-	-	+
C. undatella (Sowerby I, 1835)	-	-	+
C. californiensis (Broderip, 1835)	-	-	+
Sunetta scripta (Linnaeus, 1758)	+	-	-
Geloina bengalensis (Lamarck, 1818)	+	-	-
G. galatheae (Morch, 1850)	+	-	-
G. siamica (Prime, 1861)	+	-	-
Standella pellucid (Gmelin, 1791)	+	-	-
Gari psammobia (Lamarck, 1818)	+	-	-
Theora opalina (Hinds, 1843)	+	-	-

Solen lamarckii (Sowerby, 1874)	+	+	-
Gastrachaena gigantean (Deshayes)	+	+	-
G. apertissima (Deshayes, 1855)	-	+	-
Martesia striata (Linnaeus, 1758)	+	-	-
Pholas orientalis (Gmelin, 1791)	+	+	-
Jouanntia cumingii (Sowerby II, 1849)	+	-	-
J. globosa (Quoy & Gaimard, 1835)	+	-	-
Isognomon ephippium (Linnaeus, 1758)	+	-	-
I. nucleus (Lamarck, 1819)	-	+	-
Cyrena ceylonica (Andaman)	+	-	-
Tridacna maxima (Roding, 1798)	+	-	-
Bankia bipennata (Turton, 1819)	+	-	-
B. campanellata (Moll & Roch, 1931)	+	-	-
B. carinata (Gray, 1827)	+	-	-
B. nordi (Moll, 1935)	+	-	-
B. rochi (Moll, 1931)	+	-	-
Dicyathifer manni (Wright, 1866)	+	-	-
Dosinia tumida (Gray, 1838)	+	-	-
Tanysiphon rivalis (Benson, 1858)	-	+	-
Solenaia soleniformis (Benson, 1836)	-	+	-
Parreysia sikkimensis (Lea, 1859)	-	+	-
Chama reflexa (Reeve, 1846)	-	+	-
Mesodesma glabratum (Lamarck)	-	+	-
Gafrarium tumidum (Roding, 1798)	-	+	-
Pinctada margaritifera (Linnaeus, 1758)	-	+	-
Pinna bicolor (Gmelin, 1791)	-	+	-
Atrina pectinata (Linnaeus, 1767)	-	+	-
Venerupis macrophylla (Deshayes,1853)	-	+	-
Marcia opima (Gmelin, 1791)	-	+	-
M. marmorata (Lamarck, 1818)	-	-	+
Clavagella lata (Broderip, 1834)	-	+	-
Brachidontes variabilis (Krauss, 1848)	-	+	-
Irus exoticus (Lamarck, 1818)	-	+	-
I. reflexus (Gray, 1843)	-	-	+
I. elegans (Deshayes, 1854)	-	-	+
Lithophaga teres (Philippi, 1846)	-	+	-
L. gracilis (Philippi, 1847)	-	+	-
L. nigra (d'Orbigny, 1842)	-	+	-
L. stramineus (Dunker)	-	+	-
L. cinnamomea (Lamarck, 1819)	-	+	-
L. lithophaga (Linnaeus, 1758)	-	-	+
Petricola lithophaga (Retzius, 1786)	-	+	-
Ostrea forskalii (Gmelin, 1791)	-	+	-
O. lurida (Carpenter, 1864)	-	-	+
O. virginiana (Roding, 1798)	-	-	+
O. chilensis (Philippi, 1844)	-	-	+

O. edulis (Linnaeus, 1758)	-	-	+
O. angasi (Sowerby, 1871)	-	-	+
Galeomma paucistriata (Deshayes, 1856)	-	+	-
Scintilla hanleyi (Deshayes, 1856)	-	+	-
Lunulicardia retusa (Linnaeus, 1767)	-	+	-
Mercenaria mercenaria (Linnaeus, 1758)	-	-	+
Mya arenaria (Linnaeus, 1758)	-	-	+
M. truncate (Linnaeus, 1758)	-	-	+
Arctica islandica (Linnaeus, 1767)	-	-	+
Spisula solidissima (Dillwyn, 1817)	-	-	+
S. aequilateralis (Deshayes, 1854)	-	-	+
Ruditapes decussates (Linnaeus, 1758)	-	-	+
R. largillierti (Philippi, 1847)	-	-	+
Metis alta (Conrad)	-	-	+
Siliqua patula (Dixon, 1789)	-	-	+
S. lucida (Conrad, 1837)	-	-	+
S. patula (Dixon, 1789)	-	-	+
Tivela stultorum (Mawe, 1823)	-	-	+
Panopea abrupt (Conrad, 1849)	-	-	+
Ensis directus (Conrad, 1843)	-	-	+
E. ensis (Linnaeus, 1758)	-	-	+
E. siliqua (Linnaeus, 1758)	-	-	+
E. arcuatus (Jeffreys, 1865)	-	-	+
Hinnites giganteus (Gray, 1825)	-	-	+
Zygochlamys delicatula (Hutton, 1873)	-	-	+
Amusium pleutonectus (Linnaeus,1758)	-	+	-
Mytilus galloprovincialis (Lamarck,1819)	-	+	+
M. californianus (Conrad, 1837)	-	-	+
M. edulis (Linnaeus, 1758)	-	-	+
M. coruscus (Gould, 1861)	-	-	+
Anomia peruviana (d'Orbigny, 1846)	-	-	+
Monia macroschisma (Deshayes)	-	-	+
Austrovenus stutchburyi (Wood, 1828)	-	-	+
Amiantis callosa (Conrad, 1837)	-	-	+
Circomphalus yatei (Gray, 1835)	-	-	+
Dosina zelandica (Gray, 1835)	-	-	+
Dosinia greyi (Zittel, 1864)	-	-	+
D. anus (Philippi, 1848)	-	-	+
Saxidomus nuttalli (Conrad, 1837)	-	-	+
S. giganteus (Dashayes, 1839)	-	-	+
S. gigantea (Dashayes, 1839)	-	-	+
Senilia senilis (Linnaeus, 1758)	-	-	+
Macoma nasuta (Conrad, 1837)	-	-	+
M. secta (Conrad, 1837)	-	-	+
Semele decisa (Conrad, 1837)	-	-	+
Psammobia californica (Conrad, 1849)	-	-	+

P. edentula (Oldroyd, 1924)	-	-	+
Gomphina maorum (Smith, 1902)	-	-	+
Tagelus californianus (Conrad, 1837)	-	-	+
Cerastoderma edule (Linnaeus, 1758)	-	-	+
Schizothaerus nuttalli (Conrad, 1837)	-	-	+
Argopecten purpuratus (Lamarck, 1819)	-	-	+
A. irradians (Lamarck, 1819)	-	-	+
Platyodon cancellatus (Conrad, 1837)	-	-	+
Panope generosa (Gould, 1850)	-	-	+
Zirfaea gabbi (Adegoke, 1969)	-	-	+
Parapholas californica (Conrad, 1837)	-	-	+
Pholadidea penita (Conrad, 1837)	-	-	+
Diplodonta striatula (Finlay, 1927)	-	-	+
Paphies australis (Gmelin, 1791)	-	-	+
P. subtriangulata (Wood, 1828)	-	-	+
P. ventricosa (Gray, 1843)	-	-	+
Macomona liliana (Iredale, 1915)	-	-	+
Musculium novaezelandiae (Deshayes, 1853)	-	-	+
Tawera marionae (Finlay, 1928)	-	-	+
T. mawsoni (Hedley, 1916)	-		+
T. phenax (Finlay, 1930)		-	+
T. spissa (Deshaye, 1835)	-	-	+
Pholadidae tridens (Gray, 1843)	-	-	+
Cleidothaerus albidus (Lamarck, 1819)	-	-	+
Patinopecten yessoensis (Jay, 1857)		-	+
Placopecten magellanicus (Gmelin, 1791)	-	-	+
Plebidonax deltoids (Lamarck, 1818)	-	-	+
Sinonovacula constricta (Lamarck, 1818)	-	-	+
Callista chione (Linnaeus, 1758)	-	-	+
Tiostrea chilensis (Philippi, 1844)	-	-	+
Tresus allomyax (Scott, 2000)	-	-	+
T. capax (Gould, 1850)	-	-	+
T. keenae (Kuroda & Habe, 1950)	-	-	+
T. nuttallii (Conrad, 1837)	-	-	+
Austrovenus stutchburyi (Wood, 1828)	-	-	+
Chamelea striatula (da Costa, 1778)	-	-	-

+ = Present

- = Absent

Resources: Ansari, 1978; Nair *et al*., 1978; 1984; Morton, 1984; Wells, 1986; Chatterji *et al*., 2002; Macintosh and Ashton, 2002; Kathiresan and Quasim, 2005; Venkararaman and Wafar, 2005; Boominathan, *et al*., 2008; Idris *et al*., 2008; Sundaram and Deshmukh, 2011; Hamli *et*

al., 2012; Khade and Mane, 2012a; 2012b; Pawar and Prabhakar, 2012; Ramachandra *et al*., 2012; Suresh *et al*., 2012

METHODOLOGY Chapter-II

2.1 Study area

State of Goa (15º 44´ 30" and 14º 53´ 30" N Latitude and 73º 45´ and 74º 26´ E Longitude) along the west coast of India, has coastline of ~120 km and bound by Arabian Sea to the West, and the Western Ghats (Sahyadri) on the East. The coast is highly indented with sea cliffs, notches and promontories alternating with rivers and estuaries (Ahmad, 1972). Small islands and shoals occur offshore in close vicinity, while fringing and patchy mangrove swamps are common features along estuaries and their islands (Jagtap, 1985). Rivers originate from the Sahyadri ranges of Goa and flow into the Arabian Sea, and are dominated by diurnal tides. The other rivers run for short distance as Terekhol (22.4 km), Chapora (28.8 km), Baga (5.4 km), Sal (16.1 km), Talpona (11.2 km), and Galgibag (3.8 km) in length (Esteves, 1966; Fonseca, 2001). Goa is divided for administrative purposes into 11 talukas or counties, viz. Ilhas (Tiswadi), Bardez, Salcete, Mormugao, Ponda, Bicholim, Pernem, Quepem, Sanguem, Canacona and Satari.

Mandovi (61.6 km in length) and Zuari (92.4 km in length), with their interconnecting Cumbarjua Canal (15 km in length) forms a major estuarine complex that sustains rich mangrove formations. The mangrove influenced regions towards/in confluence habitats of these habitats sustain major *Paphia malabarica* meadows and harvested round the year. The other estuaries sustaining such beds include Chapora in the North and Talpona and Galgibag in South Goa. For the purpose of present study the two sites, each from Mandovi in the North and Zuari in the South were selected.

2.2 Climate

In Goa normally three periods prevail in a calendar year. They are pre-monsoon (February-May), monsoon (June-September) and post-mosoon (October-January). The pre-monsoon is the warmest period of the year and experiences occasional showers towards the end of May. Followed by, the southwest monsoon during which the state receives 90% of its annual rainfall, with an average ranging from 2,800 to 3,500 mm. About 36% of annual rainfall occurs during the month of July (Esteves, 1966; De Souza, 1979). The post-monsoon season is a fair and stable season. The maximum temperature during the year is about 36 ºC and the minimum is about 18 ºC; the warmest days in the year are generally in May and the coolest mostly in February. The mean daily temperature varies slightly, from around 25 ºC to about 30 ºC, due to the maritime nature of the climate. The average annual temperature remains to be 26 ºC. The Sahayadris range (Western Ghats) prevents the cold, dry winds of the inland from sweeping down Goa and hence the state does not experience a normal winter (De Souza, 1979). However, temperature variation remains wider in the eastern region due to the mountainous topography, particularly in December to January, during which the Indian subcontinent experiences the winter. Overall, the climate in the state of Goa is of equitable nature. All the estuaries in Goa are classified as microtidal estuaries, as the tidal level is below 2 m (Ahmad, 1972). Along the Goa coast the tidal amplitude varies from 0.01 to 2.44 m with a mean sea level of 1.3 m. During the monsoon, the tidal height may reach upto 4 to 5 m.

2.3 Sampling stations

The estuarine habitats are dynamically complex ecosystems due to their constantly changing environment. Two stations sustaining major beds of *Paphia malabarica* (Chemnitz) were selected based upon the preliminary observations and published literature on clam beds from the state (Figure 2.1, 2.2; Plate 2.1). Clam collections at Chicalim (Zuari) and Nerul (Mandovi) villages are very common among the local fishing communities, either as a source of additional income or for local use. While selecting the two stations, the influences of mining (Bauxite and Iron) activities were also taken into account. Selected bivalve beds in the Mandovi and Zuari were relatively more influenced by mining activities as the major ore loading and transport takes place through these estuaries. However, not much work has been done on the ecobiology of *Paphia malabarica* from Goa coast, hence the study of this clam was undertaken. Information about the ecology of clam is essential to understand the various seasonal changes that may result in the different physiological processes in the animal in relation to its environment.

2.3.1 Survey of clam beds

A rapid survey was conducted for mapping and evaluation of *Paphia* beds along the Goa coast during May 2010 (Jagtap *et al*., 2011). The beds get exposed at low tides, and hence observations were carried out at ebb. Population densities were assessed using the standard quadrat (625 cm^2) method. Ten quadrats were placed randomly over the beds so as to cover the maximum possible area of the individual bed at each sampling location. All samples were collected during low tides. Clams were separated by sieving sediments through a 500 µm mesh size. *Paphia malabarica* and other bivalve beds were observed to be under severe exploitation.

Paphia spp. inhabit shallow subtidal (>1m) regions with sandy substratum, mixed with pebbles, gravel, dead shells and silt. Beds of *Paphia* were mainly confined to estuaries like Mandovi, Zuari and Talpan (Figure 2.1). In North Goa, clams were found confined to Sinquerim, Nerul, Verem and Campal locations. While in South Goa *Paphia* beds were located at Madkai, Zuari (Zuary estuary) and Talpan (Talpona estuary). *Paphia malabarica* density ranged from 338–893 nos./m^2 with maximum density (893 nos./m^2) at Campal (Mandovi-towards confluence region) and minimum (338 nos./m^2) at Talpan (Table 2.1). Information on the nature of clam beds and geographic coordinates for sampling stations is provided in table 2.1.

2.3.2 Chicalim bay

Chicalim bay (Lat-15° 24' 35"N, Lon-73° 52' 59"E) is located on the southern bank towards confluence of Zuari (Figure 2.2; Plate 2.1). This area is rich in mangrove vegetation and aquatic resources (shellfish, finfish and other invertebrates). Chicalim bay contains a variety of bivalve resources such as oysters and clams. Among clams, *P. malabarica* forms one of the dominant species (Plate 2.2A).

2.3.3 Nerul creek

The Nerul creek (Lat-15° 30' 55"N, Lon-73° 46' 59"E) a tributary of Mandovi estuary extends inside the land in U-shape up to a length of ~ 8.5 km (Figure 2.2; Plate 2.1). It is navigated by small fishing boats and fringed by patchy mangrove vegetation (Plate 2.2B). This bed contains various types of fauna, including crustaceans and molluscs. Among molluscs, clams belonging to genus *Paphia* are found in large numbers.

2.4 Methodology

2.4.1 Hydrological Parameters

Surface water samples (n = 3) from the study areas were collected monthly, during the period March 2009 to March 2010 for assessing various physico-chemical parameters. Temperatures of surface water were noted immediately after collections. Samples were transported in ice boxes to the laboratory within the shortest possible time periods, after collections, and analyzed for various hydrological features. Salinity, dissolved oxygen (DO), pH, chlorophyll *a* (Chl *a*), particulate organic carbon (POC) and nutrients (NO_3-N, NO_2-N and PO_4-P) were analyzed following standard analytical techniques (Strickland and Parsons, 1977; Parsons *et al*., 1984; Patnaike, 1997 and De Medina, 2000). All the analysis were carried out in triplicate and expressed on an average basis. Monthly rainfall and atmospheric temperature data were obtained from the meteorological station at Altihno (Goa) for the study period.

Temperature

The in situ water temperatures were measured with the help of a mercury thermometer calibrated to 0.1 $^{\circ}$C.

pH

LABINDIA µp (Controlled pH Analyser) was used to measure the pH values of the water samples.

Salinity

Salinity was determined by using hand held refractometer (ATAGO, S/Mill-E) and results were expressed as psu (practical salinity unit).

Dissolved Oxygen (DO)

Dissolved oxygen concentrations were estimated by Winkler's method. This method involves fixation of dissolved oxygen using Winkler reagents A and B, immediately after collection of the water sample in a bottle, followed by titration against standard sodium thiosulfate solution (0.01N) using starch as indicator. The results were expressed in mg/l of water.

For the preparation of Winkler reagent A, 36.4 g of $MnSo_4.H_2o$ was dissolved in 100 ml of distilled water. For the preparation of Winkler reagent B, 50 g of NaoH was dissolved in 100 ml of distilled water containing 15 g of KI followed by addition of 1 g of NaN_3 (1g in 4 ml of distilled water). Dissolved oxygen is fixed by the addition of Mn(II) under basic conditions, resulting in a brown precipitate, manganic hydroxide ($MnO(OH)_2$) and subsequently titrated with winkler reagent B for determination of DO concentration. DO concentration was calculated using the formula:

DO (mg/l) = 0.01 x (V-b) x 5.6 (B/B-2) x 1000/S

V = Volume of $Na_2S_2O_3$ consumed

b = Blank

B = Volume of Winkler samples bottle (ml)

S = volume of sample taken for titration

POC

500 ml of water was filtered through GF/F filter paper (47 mm) and the filtrate was then placed in a 30 ml beaker containing 1 ml of phosphoric acid + 1ml of distilled water. The mixtures were kept in water bath (100 - 110 $^{\circ}$C) for 30 minutes and allowed to cool at room temperature. Later 10 ml of sulphuric acid-dichromate oxidant + 4 ml of distilled water was added to the reaction mixture, heated for 60

minutes at 100 - 110 °C and allowed to cool. The absorbance was measured spectrophotometerically at wavelength of 440 nm. The POC was estimated as described by Parsons *et al*. (1984) and values were expressed in µg C/l.

Chl a

For the estimation of chl *a*, known volume (500 ml) of water was filtered through a GF/F (47 mm) glass fibre filter paper and extracted in 90% acetone overnight. The extracts were used for the estimation of flouroscence before and after acidification using Turner designs fluorometer (10-AU). The values were expressed as µg/l of seawater.

Nutrients

Nutrients like NO_3-N, NO_2-N and PO_4-P were analyzed when the water samples attained room temperature. The samples were filtered to remove high concentration of phytoplankton and suspended matter. Nitrate (NO_3-N) and nitrite (NO_2-N) concentrations were determined by reducing NO_3-N quantitatively to nitrite by passing through a Cadmium (Cd) column. Nitrite thus produced was determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine to form highly coloured azo dye, which was measured spectrophotometrically at wave length 543 nm, using spectrophotometer Shimadzu UV mini-1240 model. Nitrate concentrations were measured by subtracting NO_2-N from the measured NO_3-N + NO_2-N.

Phosphate (PO_4-P) was measured by allowing the water to react with a composite reagent containing Ammonium Molybdate tetrahydrate solution, sulfuric acid, ascorbic acid and Potassium Antimonyl-tartrate solution. The resulting complex was

reduced to give a blue colouration, which was measured spectrophotometerically at wavelength of 885 nm. Results of nutrient concentrations were expressed as µg/l.

2.4.2 Sediment parameters

Sediment samples (n = 3) from both the stations were collected monthly using clean plastic spatulas to avoid contamination. Samples were collected in zip locked polythene bags and transferred in ice boxes to the laboratory, for further laboratory analysis for the evaluation of total organic carbon (TOC), granulometry and trace metal concentration. Analyses were carried out in triplicate and results were expressed on average basis.

TOC

Sediment samples were first desalted by washing repeatedly with deionized water and dried in an oven at 45 $^{\circ}$C. Dried and powdered sediment of 0.5 gm was digested with Chromic acid and the resultant was titrated with Ammonium Ferrous Sulfate using O-phenanthroline as an indicator. The end point was first noted using glucose as a standard and then the samples were titrated. The amount of TOC was then analyzed in triplicate as described by El Wakeel and Riley (1957). The results were expressed on average basis as %.

Grain size analyses

Sediment samples were analyzed for their grain size following Buchanan (1984) by desalting the sediment with deionized water and drying at 45 $^{\circ}$C. Dried sediment of 10 gm was disaggregated in deionized water and treated overnight with 30% H_2O_2 for removal of organic matter. Sodium hexametaphophate (decoagulant) was added in the sediment water mixture prior to wet sieving through a 63 µm sieve (ASTM 230 mesh)

to separate the sediment fraction >63 µm from the bulk sediment. The >63 µm fraction was oven dried at 60 °C and weighed to calculate coarse fraction content (wt%). The sediment suspension contacting <63 µm fraction was thoroughly mixed and the particles were allowed to settled through the water column undisturbed. The clay fraction was removed by pipetting out a fixed volume of suspension from the top after a calculated time period following Tucker (1988). The pipetted out fraction is dried and weighed for calculating the clay and silt contents.

2.4.3 Paphia malabarica sampling

Organisms were handpicked at monthly intervals from each station. Population densities were assessed using standard quadrat (625 cm^2) method. Nine quadrants were placed randomly over each bed in a manner to cover maximum area of the individual bed. Clams were separated by sieving sediment through 500 µm mesh size. Population density was converted into nos./m^2 and biomass (wet tissue weight) was expressed as g/m^2. Animals from the sampling sites were carried to the laboratory in polythene bags filled with ambient water and were cleaned in freshly collected seawater to remove adhered fowlers and were kept in the seawater for an hour in glass tanks with aeration for defecation before extraction of flesh from them for further analysis. Excess water from soft tissues were blotted with filter paper, weighed and then dried in the oven at 60 °C ± 2 until it attained constant weight.

2.4.4 Percentage of Edibility (PE) of P. malabarica

Edible biomass percentage or the PE values in *P. malabarica* was determined by calculating the percentage ratio of the wet meat weight to the whole weight of the individual specimen (Venkataraman and Chari, 1951; Mohite *et al*., 2009):

$$PE = \frac{Weight\ of\ wet\ meat\ (g)}{Total\ Weight\ of\ the\ clam\ (g)} \text{ X } 100$$

Where, Total weight = Shell weight + meat weight

2.4.5 Condition Index (CI) of P. malabarica

Various methods have been described for measuring CI values of bivalves. Condition indices of *P. malabarica* were calculated according to the method proposed by Lucas and Beninger (1985) and Orban *et al*. (2006) using following formula:

$$CI = \frac{Weight\ of\ dry\ meat\ (g)}{Weight\ of\ dry\ shell\ (g)} \text{ X } 1000$$

2.4.6 Allometric Relationships

A total of 1871 and 1338 specimens from Chicalim and Nerul, respectively, were studied for allometry. Individuals were measured for their length, breadth, depth, total weight, shell length, flesh (wet and dry) weight. Shell length (the maximum distance along the long axis of the valves), shell depth (the maximum distance across the two valves when they are closed) and shell breadth (the maximum distance along the short axis of the valves) were measured to the nearest 0.01 mm using Vernier callipers. Total weights, shell weight, flesh (wet and dry) weight of an each specimen were determined by using a mono pan electric balance (Mettler) to an accuracy of 0.01g. The dry weight of tissue was obtained after removing the shell and drying it at constant temperature of 60 $^{\circ}$C for 48 hours.

The estimation of the morphometric relationships between the shell dimensions (length, depth and breadth) were independently evaluated using the linear equation:

$$Y = mX + C$$

where m (slope) and C (intercept) are constants.

The estimation of length-weight relationship was done by power law equation:

$$Y = mX^C$$

$$\text{i.e. } W = mL^C$$

Its logarithmic regression equation is as,

$$\text{Log } Y = C + m \log X$$

where m and C are constants.

The degree of association between the variables was evaluated by the correlation coefficient (r) following the methodology of Pauly, 1983.

2.4.7 Biochemical Composition and Caloric values

Dried meat of *P. malabarica* were pulverized using mortar and pestle and analyzed for its biochemical constituents and energy value (calorific value) following standard techniques described below. All the analysis were carried out in triplicate and results expressed on an average basis.

Water contents

Water contents (moisture content) of *P. malabarica* were determined by calculating the difference between the wet weight of the tissue and its weight after drying to constant weight at 60ºC and the results were expressed in percentage. The formula used for calculation was,

$$WC = \frac{\textit{Wet weight of tissue - dry weight of tissue (g)}}{\textit{Wet weight of tissue (g)}} \text{ X } 100$$

Protein contents

Proteins were estimated following the method of Lowry *et al*. (1951) using Bovine Serum Albumin as a standard. The method is based on the principle of both the reaction between the peptide bonds of protein and Copper under alkaline condition to produce Cu^{+}. Copper thus produced reacts with Folin reagent to result in blue colour complex as phosphomolybdotungstate, which gets reduced to heteropolymolybdenum blue by the Copper-catalyzed oxidation of aromatic amino acids. The intensity of the blue colour complex depends partly on the tyrosine and tryptophan contents.

One ml of NaOH (1N) was added to 10 mg of the dried flesh of *P. malabarica* and kept in water bath (60 $^{\circ}$C) for 30 minutes to extract protein contents. It was cooled to room temperature (27 $^{\circ}$C) and neutralized with 1 ml of 1N HCl. The protein extract thus obtained was centrifuged at 2000 rpm for 10 minutes. One ml of this aliquot was further diluted with distilled water (1:9 v/v). Then, 1 ml of this solution was allowed to react for 20 minutes with 2.5 ml of mixed reagent (Carbonate-Tartarate-Copper) and 0.5 ml of 1N Folin's reagent. The blue colour developed was measured spectrophotometerically at wavelength 750 nm. The results were expressed as percentage of protein on dry weight basis.

Lipid contents

Lipid contents were analyzed by the method of Folch *et al*. (1957). Dry tissue of 10 mg was extracted with Chloroform – Methanol mixture (2:1, v/v), filtered and re-

extracted with chloroform, allowed to evaporate till dryness. The residue thus obtained was weighed and the percent total lipids were calculated.

Dried powder (10 mg) of *P. malabarica* was homogenized in 10 ml of chloroform methanol mixture (2:1 v/v). The homogenate was centrifuged at 2000 rpm and the supernatant than was transferred to a pre-weighed test tube through a filter paper and washed with 0.90% KCL (saline solution) to remove the non-lipid contaminants. The upper layer was discarded by siphoning and the lower phase was allowed to dry in an oven and weighed as lipid contents. The results were expressed as percentage of lipid on dry weight basis.

Carbohydrate contents

Total carbohydrates were estimated by the Phenol Sulfuric acid method (Dubois *et al*., 1956) using glucose as a standard. Phenol and sulfuric acid were added to digested sample. The solution turns to a yellow orange colour as a result of the interaction between the carbohydrate and the phenol. The absorbance was measured at 490 nm, which was proportional to the carbohydrate concentration.

Dried flesh of 10 mg sample was digested with 2 ml of 80% H_2SO_4 for about 20 – 21 hours at room temperature (27 $^{\circ}$C). 2ml of 5% phenol reagent followed by 5 ml of concentrated H_2SO_4 were added to the digested sample and allowed to cool at room temperature. The absorbance of the developed brown colour was measured spectrophotometerically at 490 nm wavelength. The concentrations were expressed as percentage of carbohydrate on dry weight basis.

Calorific value

The calorific value (nutritional or food energy value) were estimated by using conversion factors 4.20 Kcal/g, 5.56 Kcal/g and 9.45 Kcal/g dry weights for carbohydrates, proteins and lipids, respectively (Phillips, 1969).

Ash content

Two grams of dry tissue samples of *P. malabarica* were taken in pre-weighed silica crucible and placed in a furnace at 600 $^{\circ}$C for 4 hours and subsequently cooled in a desiccator and weighed. Ash content values were expressed as percentage of ash content on dry weight basis (Appukuttan and Aravindan, 1995).

2.4.8 Determination of radical scavenging activity

The wet tissue (10% w/v) of *P. malabarica* was homogenized and extracted in methanol (90% v/v) by agitation in rotary shaker for 24 hours. Extraction procedures were carried out at 4 $^{\circ}$C (with crushed ice) in order to maintain the stability of bioactive compounds (Ekanayake *et al*., 2004). The stepwise methanolic extraction procedure included repeated extractions at every 6 hours of time interval. Initially, the whole extract contents were centrifuged (8000 × g for 10 minutes at 4 $^{\circ}$C) and supernatant was collected in separate vials. The tissue pellet obtained in consequent steps was further treated similarly with methanol to achieve maximum extraction and recovery of the bioactive compounds. All the fractions were finally pooled together, filtered through Whatman paper No. 1 (110 mm) and concentrated through Rota evaporator (Buchi Rotavapor R–200).

The yield was estimated (Ekanayake *et al*., 2004) by evaporating 1 ml extract in pre-weighted aluminium dish at room temperature (27 $^{\circ}$C) until complete dryness and was

expressed as mg (crude dry weight extract)/ml. The condensed methanol extracts were adjusted to 10 mg/ml either by diluting or by concentrating with the same solvent. Sample extracts were then preserved at -20 °C until further use. As synthetic antioxidants exhibits higher activities at the same concentration of the sample extract, all standards were maintained at lower concentration (40 µg/ml). All the analysis were carried out in triplicate and expressed on average basis.

DPPH radical scavenging assay

Free radical scavenging potential was measured against 2,2-diphenyl-1-picryl-hydrazyl (DPPH) by colorimetric reduction assay (Blois, 1958). The reaction mixture containing 2.5 ml of DPPH solution (0.1 mM in methanol) and extract (0.1-0.3 ml) was adjusted to 3 ml by adding methanol. The absorbance at 517 nm were measured at 0 and after 30 minutes. Butlylated hydroxytoluene (BHT) was used as the standard. Scavenging effect was calculated using formula given below and expressed in terms of relative percent activity by comparing with standard.

$$[A_0 - A_1 / A_0] \times 100$$

Whereas, A_0 - absorbance at 0 minute and A_1 - absorbance at 30 minutes

Reducing power assay

The reducing power of the extract was determined by using standard protocol (Oyaizu, 1986). The reaction mixture containing 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%) was added to aliquots of extract (0.1-0.3 ml). Ascorbic Acid (AsA) was used as standard solution while blank was maintained with same reaction mixture without sample. The mixtures were incubated at 50 ºC in water bath for 30 minutes and allowed to cool at room temperature. Later 2.5 ml of 10% Trichloro Actetic Acid (TCA) was added to the reaction mixture and centrifuged

at 2000 × g for 10 minutes. 2.5 ml of supernatant was transferred to another test tube, and to this 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1%) were added and allowed to react for 10 minutes at room temperature (27 ºC). The absorbance was measured at 700 nm.

Inhibition of in vitro lipid peroxidation

Lipid peroxidation of tissue samples were assayed as described earlier (Ohkawa *et al*., 1979). The sheep liver was washed with ice-cold potassium chloride (1.15%) and 20% tissue homogenate was prepared in KCl and filtered. The filtrate was centrifuged at 10,000 × g for 10 minutes at 4 °C to get post mitochondrial fractions (PMF) along with most of the other organelle. In vitro lipid peroxidation was induced with $FeSO_4$ (100 μM) in PMF of sheep liver extract. To this, sample extract (0.1-0.3 ml) was added and further treated with Thiobarbituric Acid (TBA) for 60 minutes at 95 °C. The formation of TBA-reactive species (TBARS) was measured at 532 nm. Samples and standard (AsA) were analyzed in triplicates. The inhibition of lipid peroxidation was expressed in relative percentage (%) activity and calculated by following formula,

$$[1- (A_0 - A_1 / A_2)] \times 100$$

Whereas, A_0 - absorbance in the presence of extract (i.e. reaction mixture + sheep liver homogenate + sample extract of *P. malabarica*)

A1 - absorbance without sheep liver homogenate (i.e. reaction mixture + sample extract of *P. malabarica*)

A2 - absorbance of the control without *P. malabarica* extract (i.e. reaction mixture + sheep liver homogenate).

Protein concentrations of ship liver homogenate were estimated by using Folin-Ciocalteu reagent and BSA (Bovine serum albumin) as a standard (Lowry *et al*., 1951).

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging ability of *P. malabarica* extracts were tested by quantifying Fenton reaction (Chung *et al*., 1997). Reaction mixture containing 200 µl each of $FeSO_4.H_2O$ (10 mM), EDTA (10 mM), 2-deoxyribose (10 mM) was added with sample (0.1-0.3 ml), 1 ml of phosphate buffer (0.1 mM, 7.4) and 200 µl of H_2O_2 (10 mM) to initiate the reaction. Later, 1 ml of Trichloroacetic Acid (TCA - 2.8%) and TBA (0.1%) were added after incubation at 37 $^{\circ}$C for 4 hours and placed in boiling water bath for 10 minutes. The absorbance was measured at 532 nm.

The scavenging effect of hydroxyl radical was calculated as follows,

$$[1- (A_{sample} / A_{control})] \times 100$$

Whereas A_{sample} is the absorbance in the presence of the tested samples

$A_{control}$ is the absorbance of the control.

Results were expressed as relative activity (%).

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP was evaluated by the measuring Fe^{2+}/TPTZ-complex by colorimetric method (Benzie and Strain, 1996). The FRAP reagent containing 2.5 ml of 10 mM TPTZ in 40 mM HCl solution, 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 ml of Acetate Buffer (300 mM, pH 3.6) was prepared. Different concentrations of sample extract were added in 3 ml of FRAP reagent and incubated for 15 minutes at 37 ºC. The antioxidant potential or the antioxidant content of the extract was expressed in terms of µg/ml of AsA (ascorbic acid) equivalents.

Data obtained was compared with standard synthetic antioxidant and presented as relative activity in case of DPPH, in vitro inhibition of LPX and hydroxyl radical scavenging assays. Whereas, reducing powers and FRAP values were expressed in terms of increasing absorbance and µg/ml of AsA equivalents, respectively.

2.4.9 Trace Metals Analysis

Tissue samples of *P. malabarica* and sediments in ambience were collected monthly during March 2009 to March 2010 from Chicalim and Nerul beds and analyzed for trace metals using standard methods. Analyses were carried out in triplicate and results were expressed on average basis (ppm/ppb).

Leaching of sediment Samples

Sediment samples from both the stations (Figure 2.2) were collected using plastic spatula to avoid metal contamination. The sediment samples were first desalinated by washing repeatedly with deionized water, centrifuged and dried in an oven for 24 hours at 60 $^{\circ}$C. Dried sediments were pulverized and stored in clean vials. Aliquotes of 0.4 gm were leached in 15 ml of dilute HNO_3 (adjusted to pH 5 [10^{-5}M] through step dilution) by shaking in a rotary shaker for 4 hours. After leaching, the acid/particle mixture was centrifuged at 10,000 rpm for 5 minutes in 50 ml polycarbonate centrifuge tube. A 10 ml aliquot was taken from the supernatant leachate and stored at room temperature in a acid washed vial for trace element analysis using a ICP-MS.

Clam samples

Tissue of *P. malabarica* from fresh samples were dried in an oven for 24 hours at 60 $^{\circ}$C to a constant weight. The dried tissue was pulverized and stored in clean vials. The

known weight (0.5 gm) of the dried tissue powder was moistened with one ml of deionised water. Two ml of 69.5% HNO_3 (Merck, supra pure) was added and the mixture was left for reaction overnight. Reaction mixture was heated for 5-6 hours at 80 $^{\circ}$C on a hot plate by further adding 1 ml of 69.5% HNO3 for achieving complete digestion of the tissue. The digest was allowed to cool. Four ml of 1:1 69.5% HNO3 was further added to the digested solution and diluted to 100 ml with (double distilled) nanopure water. The solution then passed through a acid washed filter paper (Whatman No. 42 mm), and stored at room temperature for further analysis.

For both the sediment and tissue trace metal concentration measurements triplicate sub-samples were analysed using a inductively couple plasma mass spectrometer (ICP-MS: X series 2, Thermo Scientific) to estimate the concentrations of various trace metals (iron, manganese, lead, cadmium and zinc). Results were expressed in ppm or ppb on an average basis. Standard calibration curves were constructed using Merck certified stock solutions.

2.5 Data analysis

Data was statistically analyzed for mean and standard deviation. Significant spatial and temporal variations in environmental and biological parameters were studied by two-way analysis of variance (ANOVA) and Tukey's (honestly significant difference) post hoc analysis. Data was processed using statistica 6 Software.

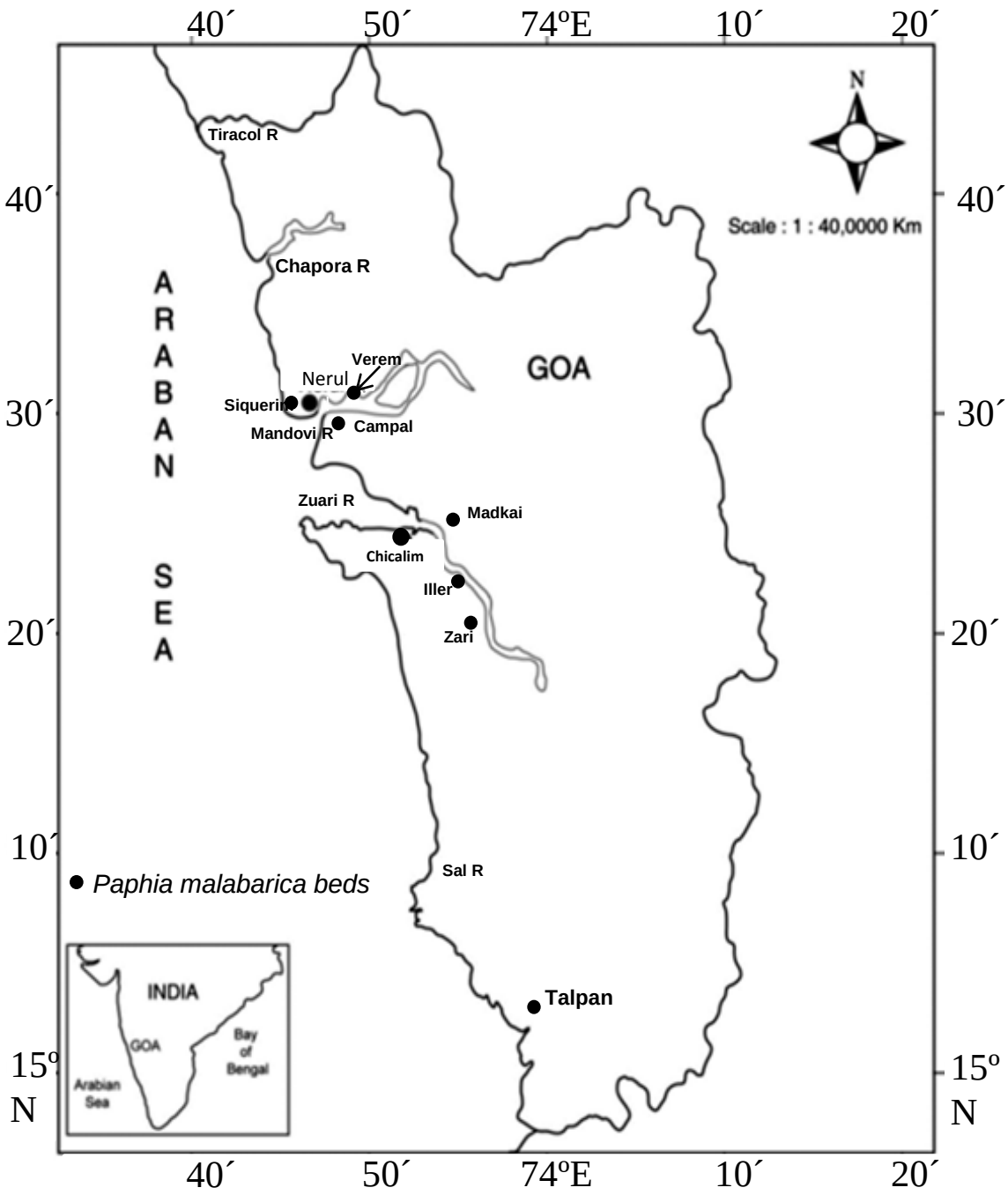


Figure 2.1: Map showing distribution of *Paphia spp*. beds along the coast of Goa. Source. Jagtap *et al.*, 2011

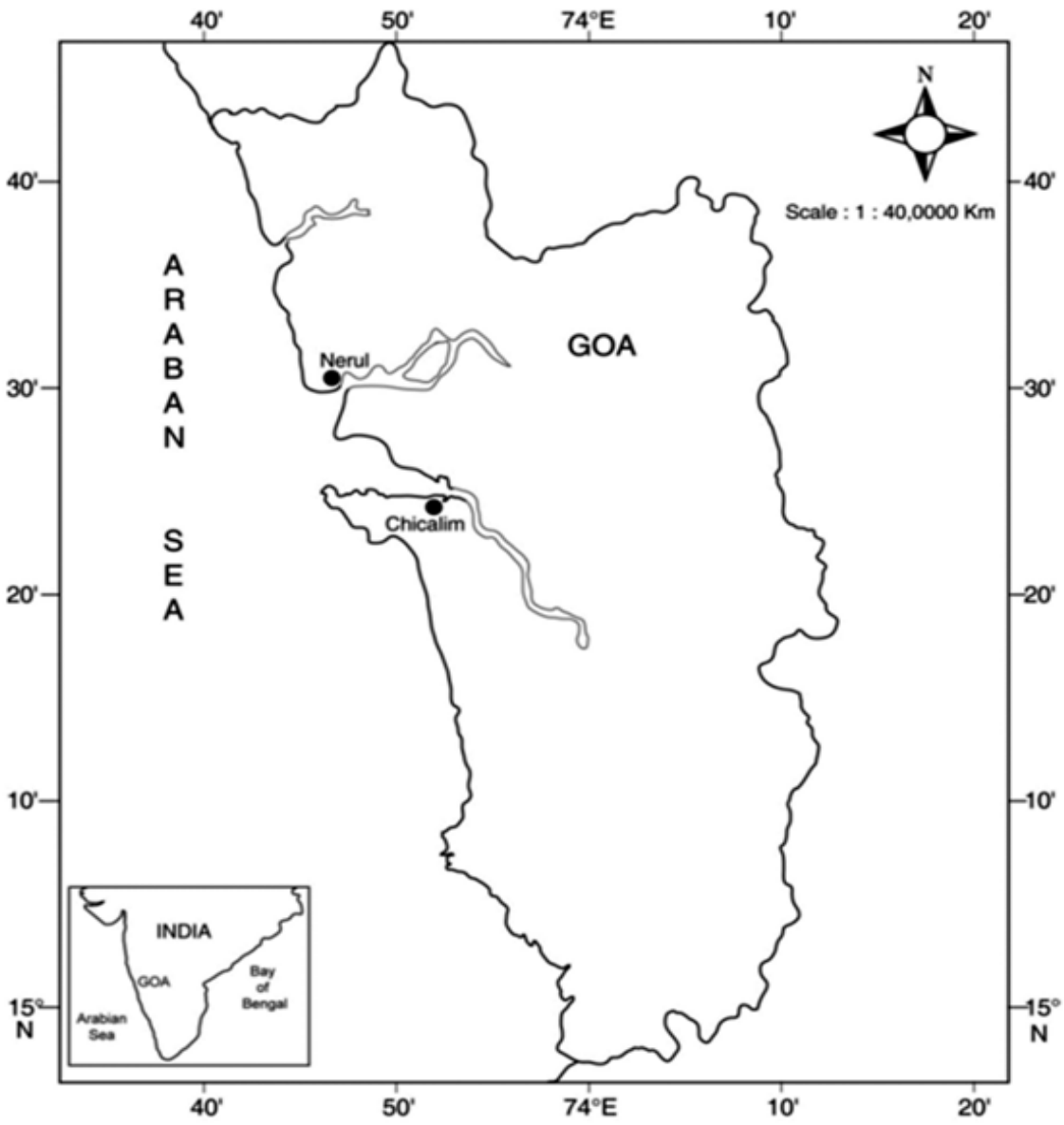


Figure 2.2: Sampling locations in Zuari and Mandovi estuaries, Goa.

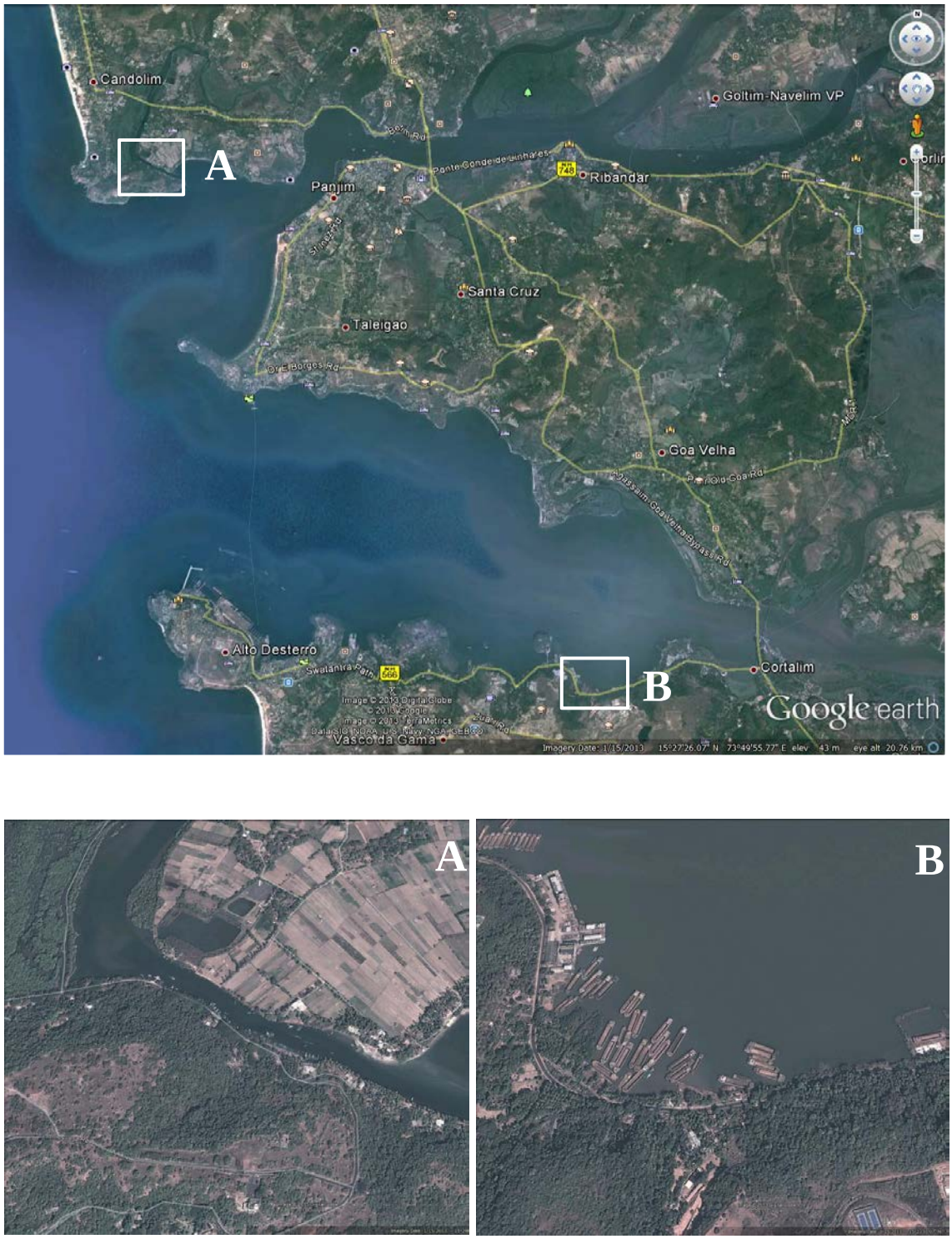


Plate 2.1: Google map of sampling stations at (A) Nerul (Mandovi estuary) and (B) Chicalim (Zuari estuary).

Plate 2.2: Beds of *Paphia malabarica* at (A) Chicalim and (B) Nerul.

Table 2.1: Density of *Paphia spp*. along the Goa coast.

Stations	**Latitude**	**Longitude**	**Density (nos./m^2)**
Verem	15º 30' 34.956"	73º 48' 50.004"	653
Campal	15º 29.473'	73º 48.807'	893
Madkai	15º 24' 28.404"	73º 54' 32.364"	623
Zuari	15º 24' 35"	73º 52' 59"	730
Talpan	14º 59' 7.62"	74º 2' 38.004"	338
Nerul	15º 30' 55"	073º 46' 59"	420
Siquerim	15º 29' 49.02"	73º 45' 53.85"	420

\

Table 2.2: Geographical coordinates and nature of habitat at different stations.

Locality	**Geographical coordination**		**Nature of habitat**	**Species**
	Latitude (N)	**Longitude (E)**		
Nerul	15º 30' 55"	73º 46' 59"	Clay	*P. malabarica*
Campal	15º 29.473'	73º 48.807'	Sandy	*P. malabarica*
Zuari	15º 24' 35"	73º 52' 59"	Sandy and Rocky	*P. malabarica*
Talpan	14º 59' 7.62"	74º 2' 38.004"	Sandy and Rocky	*P. malabarica*

ENVIRONMENTAL PARAMETERS Chapter-III

3.1 Introduction

Environmental and ecological variables in ambience greatly influence population structure of biotic communities. Water characteristics help in understanding the various biological processes (growth, physiology, reproduction etc.) and the general productivity of aquatic ecosystem. The physicochemical parameters such as temperature, salinity, dissolved oxygen and nutrients are of profound biological significance and are used as population indicators (Head, 1985). Temperature and light also play an important role in the gonadal gametogenesis, spawning and initiation of gonadal development (Pandey and Shukla, 2005).

Estuarine ecosystem is characterized by a wide variation in salinity and temperature compared to the Sea. In tropical estuaries seasonal changes in salinity is greater than in temperature (Blaber, 2000). Salinity of estuaries in India vary from near 30-37 psu during dry season i.e. pre-monsoon (Qasim, 2004) however, during monsoon, particularly during months of July-August (heavy rain) reaches to zero due to influx of fresh water from hinterlands. Mandovi - Zuari and Cumbarjua estuarine complex, in Goa, represent unique features. Hydrological and biological characteristics of estuarine complex are subjected to seasonal phenomenons that are induced by the annual cycle of the monsoon. Heavy rainfall (~3000mm/annum) during monsoon (June to September) brings about large changes in physico-chemical properties, flow pattern and sediment/suspended load, when the estuary becomes freshwater dominated (Qasim and Sen Gupta, 1981). These natural phenomena apparently affect the composition and dynamics of the estuarine biota. These speculations could be revealed from earlier studies relevant to the environment of Mandovi-Zuari estuarine

complex (Qasim and Sengupta, 1981; Nasnolkar *et al*., 1996; Wafar *et al*., 1997; kumari and John, 2003). Estuarine habitats are dynamic in nature, as a result the biota within them adapts to changing ambience. For better assessment of ecobiological interactions of *Paphia malabarica* with ambience, it was necessary to understand relevant environment and ecological features associated with beds at Chicalim and Nerul.

3.2 Results

Atmospheric temperature

The atmospheric temperature ranged from 26 ºC to 28.5 ºC. The maximum (28.5 ºC) atmospheric temperature was recorded during May, 2009 and minimum (26 ºC) in the month of September 2009 (Figure 3.1A).

Rainfall

The total rain fall during study period received was about 3026 mm in the state of Goa. The pre and post-monsoon constitute dry season, with occasional pre-monsoon showers. The maximum (1116 mm) average rainfall was recorded during July 2009 (Figure 3.1B).

Tide

The tide ranged from -0.11 to 2.57 M during the sampling period (Figure 3.1C). Average tidal level of high tide water was observed between 2.2 to 2.57 M and during low tide water level was between -0.11 to 0.18 M. High tide water attains its maximum (2.57 M) level during February 2010 whereas, its lowest (2.2 M) tidal water level was observed during October 2009. Lowest water level (-0.11 M) during

low tide was observed during July 2009 whereas, its maximum (0.18 M) water level was observed during March 2010 (Figure 3.1C).

Humidity

The relative humidity ranged from 74% during the month of February 2010 to 86% during October 2009 with an annual average of 79.9% (Figure 3.2A).

Wind speed

Wind speed varied from 4.3 to 7.6 m/s with an annual average of 5 m/s. The area experienced winds during monsoon season with a peak value (7.6 m/s) in July 2009 (Figure 3.2B), whereas low wind speed (4.3 m/s) was reported during April 2009 (Figure 3.2B).

Cloud cover

The cloud cover ranged from 1 to 5 octave with higher values (5octave) during July and August 2009 where as during post-monsoon it showed decreased in value during November and December 2009 (1 octave), later, it increased gradually from January (Figure 3.2C) to March 2009.

Water temperature

At Chicalim maximum temperature of overlying waters on *Paphia* bed recorded was 34 ºC in the month of October 2009, while the lowest surface water temperature was 29 ºC in August 2009. Highest surface water temperature recorded at Nerul was 32.5 ºC in the month of June 2009, while the lowest temperature observed was 27ºC in August 2009 (Figure 3.3A). The average water temperature recorded during the sampling period was 30.7 ± 1.52 ºC and 30.3 ± 1.52 ºC from Chicalim and Nerul,

respectively. The two way ANOVA revealed significant ($p<0.001$) variation in water temperature between months and stations.

Salinity

At Chicalim, salinity of surface water on *Paphia* beds varied from 1.00 ± 0.28 psu in July 2009 to 35 psu in June 2009 and March 2010. While at Nerul, it ranged from 1.00 ± 0.57 psu (July 2009) to 36 ± 1.00 psu (May, June 2009 and February 2010) (Figure 3.3B). Salinity showed a significant ($p<0.001$) variation at both the stations.

pH

pH values were found to be statistically significant ($p<0.001$) among stations with highest value recorded in water column over *Paphia* beds at Chicalim (8.4 ± 0.02) in December 2009. Similarly, pH showed significant ($p<0.001$) temporal variations with the lowest value (7.3 ± 0.45) in the month of March 2009 at Chicalim. Similarly, at Nerul, maximum pH (8.3 ± 0.03) was recorded in December 2009, and minimum (7.5 ± 0.05) during March 2009 (Figure 3.3C).

DO

Dissolved oxygen values ranged between 1.5 ± 0.38 to 5.1 ± 0.57 mg/l at Chicalim with the lowest value (1.5 ± 0.38 mg/l) during March 2009, whilst the highest (5.1 ± 0.57 mg/l) during July 2009. Nerul habitat exhibited the lowest value (1.2 ± 0.10 mg/l) in the month of March 2009, whereas, highest value (4.4 ± 0.04 mg/l) was recorded during January 2010 (Figure 3.4A). Significant spatio–temporal variation was found in overlying waters on *Paphia* beds ($p<0.001$).

Chl a

Chlorophyll *a* concentration varied significantly ($p<0.001$) between the month and stations. The concentrations varied from 0.8 ± 0.01 to 4.9 ± 0.35 µg/l at Chicalim and from Nerul waters the same varied from 0.7 ± 0.00 to 2.5 ± 0.58 µg/l. The highest value of chl *a* (4.9 ± 0.35 µg/l) was observed during January 2010 and lowest of 0.8 ± 0.01 µg/l was recorded in July 2009 at Chicalim. While at Nerul maximum concentration (2.5 ± 0.58 µg/l) was observed during October 2009 and minimum (0.7 ± 0.00 µg/l) during August 2009 (Figure 3.4B).

POC

A significant spatio-temporal variation ($p<0.001$) was also observed in the POC content. The POC values were highest during July 2009 at Chicalim (4992.9 ± 538.3 µg C/l) and Nerul (4589.6 ± 435.64 µg C/l). Low values were observed during February 2010 (1063.9 ± 125.41 µg C/l) in Chicalim and in April 2009 (1771.4 ± 690.64 µg C/l) at Nerul (Figure 3.4C).

Nutrients

Nitrate-Nitrogen concentrations showed a significant spatio-temporal variation ($p<0.001$) with highest value (9.9 ± 0.21 µg at-N/l) during July 2009 at Nerul and Chicalim (9.2 ± 0.34 µg at-N/l). A lowest value (0.27 ± 0.17 µg at-N/l) of NO_3-N was recorded in October 2009 at Chicalim and (0.29 ± 0.11 µg at-N/l) in March 2010 at Nerul (Figure 3.5A). Nitrite-Nitrogen concentration also showed a significant variation ($p<0.001$) among stations and months. Highest value of NO_2-N (1.8 ± 0.09 µg at-N/l) was recorded during July 2009 and lowest of 0.22 ± 0.03 µg at-N/l was observed during August 2009 in waters from Nerul. At Chicalim maximum value (1.7

± 0.08 µg at-N/l) was recorded in July 2009 and minimum (0.17 ± 0.10 µg at-N/l) during August 2009 (Figure 3.5B). A significant variation ($p<0.001$) was also noticed in phosphate-phosphorus concentrations among months and stations. Highest concentration (3.2 ± 1.21 µg at-P/l) of PO_4-P was observed during May 2009 and lowest (0.21 ± 0.04 µg at-P/l) during August 2009 at Chicalim. While at Nerul, maximum values (2.3 ± 0.28 µg at-P/l) were recorded during December 2009 and minimum (0.2 ± 0.08 µg at-P/l) during August 2009 (Figure 3.5C).

TOC

TOC content in the sediment varied significantly ($p<0.001$) between months and stations. Sediment at Chicalim was observed to have highest content (3.2 ± 0.05% dry wt) during September 2009 and lowest (0.3 ± 0.02% dry wt) in August 2009. However, Nerul showed highest content in August (2.4 ± 0.00% dry wt) and lowest (0.3 ± 0.02% dry wt) during June 2009 (Figure 3.6A).

Grain size

Sediments from *Paphia* beds found to be rich in coarser fraction while clay contents were found to be the minimum. Coarse fraction varied from 40.94% (dry wt) during September 2009 to 98.72% (dry wt) in April 2009 at Chicalim (Figure 3.6A). While it ranged from 62.12% (dry wt) in May 2009 to 91.7% (dry wt) in June 2009 in sediments from Nerul. Silt percentage ranged from 0.79% (dry wt) in the month of April 2009 to 33.58% (dry wt) in September 2009 at Chicalim (Figure 3.6B). At Nerul silt percentage varied from 5.46% (dry wt) in March 2010 to 17.85% (dry wt) in May 2009. Clay percentage ranged from 0.48 wt% in April 2009 to 25.48% (dry

wt) in September 2009 at Chicalim. Nerul showed variation in clay percentage from 2.31% (dry wt) in June 2009 to 20.02% (dry wt) in May 2009 (Figure 3.6C).

3.3 Discussion

Earlier record relevant to hydrological regime of Mandovi-Zuari estuarine system revealed that the physical, chemical and biological features within are adapted to a seasonal rhythm, depending upon the monsoonal cycle and the quantum of freshwater received (Qasim and Sen Gupta, 1981). During the sampling period, it was observed that Goa received about 3026 mm of rainfall. The relative humidity (Figure 3.2A) was found to increase during monsoon due to the cloud cover (Figure 3.2C) and heavy precipitation, which act like a blanket to restrict the water vapour near the earth's surface. Heavy rainfall and land runoff during monsoon bring about dynamic changes from typically marine to brackish water condition in the estuaries (Qasim and Sen Gupta, 1981) resulting changes in temperature, salinity, flow pattern, DO and nutrients. The variations in these parameters have been found to be lesser in Zuari compared to the same in Mandovi (Padmavati and Goswami, 1996). Such changes, however, are insignificant except during monsoon. Diurnal variations in the physio-chemical conditions in Mandovi and Zuari estuaries are governed by the tides (Sundar and Shetye, 2005). Qasim and Sen Gupta (1981) stated that the water remains well mixed during the pre-monsoon season and gets stratified during monsoon and exhibit tounge formation i.e. the lighter and less saline water floats at the surface while heavier water remains at the bottom.

The lower temperature (27-29 ºC) observed during monsoon (Figure 3.1A and 3.3A) may be attributed to speedy wind, precipitation (Figure 3.1B; Figure 3.2B) as well as the greater cloud cover (Figure 3.2C), which results in reduced solar radiation. During

monsoon, heavy rainfall and freshwater influx brings cooler water from the upper reaches of the river which results in decrease in water temperature. Similar observations were reported by Dehadrai (1970), Qasim and Sen Gupta (1981) and Rivonkar (1991). Higher temperature recorded during pre and post monsoon could be attributed to higher atmospheric temperature (Figure 3.1A) prevailing during these periods.

In estuarine waters salinity generally undergoes spectacular changes round the year (Qasim, 2004). Salinity over *Paphia* beds at both stations was found to be uniformly high (30-36 psu) with minor variations, except during monsoon (Figure 3.3B). Decreased salinity during monsoon, particularly during July month might be predominantly due to heavy fresh water runoff and intensive precipitation (Figure 3.1B). Intensive cloud cover during monsoon (Figure 3.2C) reduces the solar radiation decreasing the temperature (Figure 3.1A and 3.3A) and rate of evaporation. Salinity gradually begins to rise with onset of post-monsoon during September as a result of extensive reduction in the cloud cover (Figure 3.2C) and rainfall (Figure 3.1B) and hence decrease in fresh water flow as well as increase in temperature (Figure 3.1A) and humidity (Figure 3.2A). High atmospheric temperature also leads to increasing the rate of evaporation, which in turn increase the salinity values (Figure 3.3B).

The lower pH of overlying waters of *Paphia* beds during monsoon (Figure 3.3C) compared to the same during the rest of the period might be due to the reduced abundance of phytoplanktons (chl *a*) and thus photosynthesis, as a result of increased turbidity (Rao and Madhavan, 1964) due to the influx of fresh water. The cloud cover during monsoon season reduces the availability of the sunlight. The increased turbidity also decreases the sunlight penetration in the water column, subsequently

reducing the growth of phytoplankton (chl *a*) and thus primary productivities. The reduced photosynthetic activity leads to the increase in carbon dioxide in the water column lowering the pH values (Sarma *et al*., 2001). pH values of estuarine water tend to be alkaline due to various types of salts in it. However, decrease in pH during monsoon could be attributed to fresh water input that dilutes the salts to a greater extent (Subramanian and Mahadevan, 1999). Low pH values during monsoon have been reported to be very common phenomenon in estuaries (Srinivasan and Pillai, 1973; Singbal, 1985; James and Najmuddin, 1986). Heavy runoff during monsoon contributes a large amount of suspended matter including organic materials forming organic acids, which is responsible for the decreased pH values. The highest values of pH occurred during post-monsoon period have also been reported by Rivonkar (1991).

Dissolved oxygen in the water reached maximum levels in the monsoon (Figure 3.4A) and exhibited an inverse relationship with salinity. Decreased salinity results in increasing solubility of oxygen, which may be the primary reason for the increased dissolved oxygen during the monsoon season (Singbal, 1976; Qasim and Sen Gupta, 1981; De Souza and Sen Gupta, 1986). The increased concentration of DO during monsoon (Figure 3.4A) could also be attributed to precipitation and influx of oxygen rich fresh water from river runoff. High percentage of oxygen saturation has been reported earlier in Mandovi and Zuari waters (Singbal, 1976; Qasim and Sen Gupta, 1981) during the southwest monsoon. Dehadrai and Bhargava (1972) reported DO in the range of 4.1-5.3 ml/l from Mandovi-Zuari waters and decreased concentrations during pre-monsoon were related to high temperature and high salinity of the water.

The maximum concentration of Chl *a* during post-monsoon could be due to the dominance of high temperature and high saline condition (Pednekar *et al*., 2011). Generally, during monsoon season estuaries receive considerable amount of detritus, humus, suspended terrigenious matter through river run-off and the resulting turbidity restricts light penetration in the water column limiting phytoplankton production. Hence inspite of the high nutrient influx (Figure 3.5) into the system during monsoon by land run-off, there is no effective utilization of these nutrients due to less population of phytoplankton (Figure 3.4B). High Chl *a* during post-monsoon in the present study agrees with the results provided earlier for estuarine water in Goa (Krishnakumari *et al*., 2002).

During monsoon and post-monsoon POC values remained high (Figure 3.4C) and could be associated with fresh water runoff (Krishnakumari *et al*., 1978; Goes, 1983). Organic matter is released from the substratum due to associated disturbances in the monsoon (Jagtap, 1985). Organic matter in coastal and shelf environments is both, allochthonous from terrestrial materials and autochthonous from in-situ aquatic production (Degens and Ittekkot, 1985; Nelson *et al*., 1987). The main source of higher POC values during monsoon cannot be due to autochthonous materials since during this period Chl *a* values observed were low. Higher values could be due to the allochthonous materials brought down by riverflow runoff from mangrove swamps (Jagtap, 1985; Nagi, 2008).

The nitrogen and phosphorus compounds in estuaries are mainly derived from land drainage; discharge of effluents and partly from seawater influx. The highest concentration of NO_3-N, from both the study stations, occurred in the monsoon period (Figure 3.5A) may be attributed to heavy rainfall and freshwater runoff (Pai and

Reddy, 1981; Sardessai and Sundar, 2007). Hinterland of Goa possess potential mine zones of ore like Fe, Mn and Bauxite which are being intensively mined and transported through Mandovi and Zuari (Patel *et al*., 1980) for the last five decades. Mining operations in the hinterlands use explosives containing nitrates, which forms a source of nitrate in the estuaries of Goa (De Sousa, 1983). The increased NO_3-N in estuarine waters during monsoon can also be contributed from fertilizers applied in the low lying agricultural land (Sardessai and Sundar, 2007). The Mandovi and Zuari flow over a fertile coastal plain that supports rice cultivation and horticulture. Both these activities involve the use of fertilizers with nitrates as a component and the runoff during the monsoon is expected to carry some of these nitrates to the two estuaries. The Mandovi and Zuari estuarine banks have mangroves, which harbor sediments rich in organic matter (Wafar, 1987). Wafar *et al*. (1997) noted that the dissolved organic nitrates (DON) and dissolved organic phosphates (DOP) released from the mangrove litter are important for sustaining the nutrient budget of the estuaries. Hence, the passage of freshwater through mangroves could supply substantial amounts of nitrogen to the estuaries. Nitrate is removed from estuarine water by phytoplankton and phytobenthos and by bacterial respiration in sediments. Bacteria utilize nitrate as oxidant, in the absence of oxygen, during the decomposition of organic matter. The intensity of this process will be greater in mangrove sediments that harbour very high populations of bacteria as the presence of organic matter is very high. The low values of NO_3-N (Figure 3.5A) during pre-monsoon could be attributed to the removal of it by phytoplanktons viz biological productivity in the area as denitrification process could be ruled out because the sampling sites are shallow and well oxygenated (De Sousa *et al*., 1981).

An inverse relation is observed between salinity and nitrite profiles at both the stations during peak monsoon. It has been observed that low temperature and salinity may inhibit growth of nitrifiers causing decrease nitrification rate leading to low nitrite (Nwankwoala *et al*., 2009). Heredia (2000) reported that in the estuarine systems oxidation of ammonia and reduction of nitrate is a chief source of nitrite. However, in monsoon, the high concentration of NO_3-N corresponding to low salinity indicates that nitrite addition is through the riverine advection. The negative correlation with nitrite-salinity further indicates heavy input of nitrite via fresh water. Nutrients values tend to be higher where effects of land drainage remain more pronounced and depth is less (Goswami and Singbal, 1974). Qasim and Sen Gupta (1981) reported decrease of nitrate concentration in estuarine systems of Goa from head towards mouth during the dry season, due to its utilization for photosynthetic activity. Phosphate concentration is an important component of biogeochemical cycling in an estuary. In estuarine water phosphate are contributed from natural weathering processes and breakdown of polyphosphates used in detergents (De Sousa *et al*., 1981), and could be released to the water by disturbances in substrates (Sankaranarayanan and Qasim, 1969). Low PO_4-P concentration during monsoon (Figure 3.5C) may be attributed to scavenging of dissolved PO_4-P by Fe-oxyhydroxide particulates (Ball, 1992).

Autochthonous (produced in an estuary) and allochthonous (supplied from the sea and rivers) materials form the major source of sediment organic matter in estuaries (Cifuentes *et al*., 1988). A high value of TOC during monsoon (Figure 3.6A) is attributed to terrestrial runoff (allochthonous) which is brought by heavy downpour. Shirodkar (1984) suggested that sediment organic carbon in Mandovi estuary is a contribution from the land drained sources. A low value of organic carbon is due to

oxygenation (Paropkari, 1979). Total organic carbon concentrations were found to be lower as compared with concentrations reported earlier (Shirodkar and Sengupta, 1985; Nasnolkar *et al*., 1996). The seasonal variation of TOC in the sediments could be due to the oxidation and decaying of litter (fauna and flora) during the pre-monsoon season and land drainage during the monsoon season.

Sediment granulometry forms an important feature for the biological constituents of the environment in general and benthos in particular. Sediment characteristics of both the estuaries have been studied in detail earlier (Parulekar and Dwivedi, 1974; Parulekar *et al*., 1975; Parulekar *et al*., 1980; Shirodkar, 1984). Sediment texture analysis revealed coarse particulates as a predominant (75.86 - 99.6% dry wt) component of bivalves beds (Figure 3.6B); the low organic matter in sediment could also be attributed to the sandy nature of bivalve beds. It is recognised that clay rich sediments are enriched in organic carbon content possibly due to high surficial adsorption and preservation potential relative to coarse particulates (Varshney *et al*., 1999).

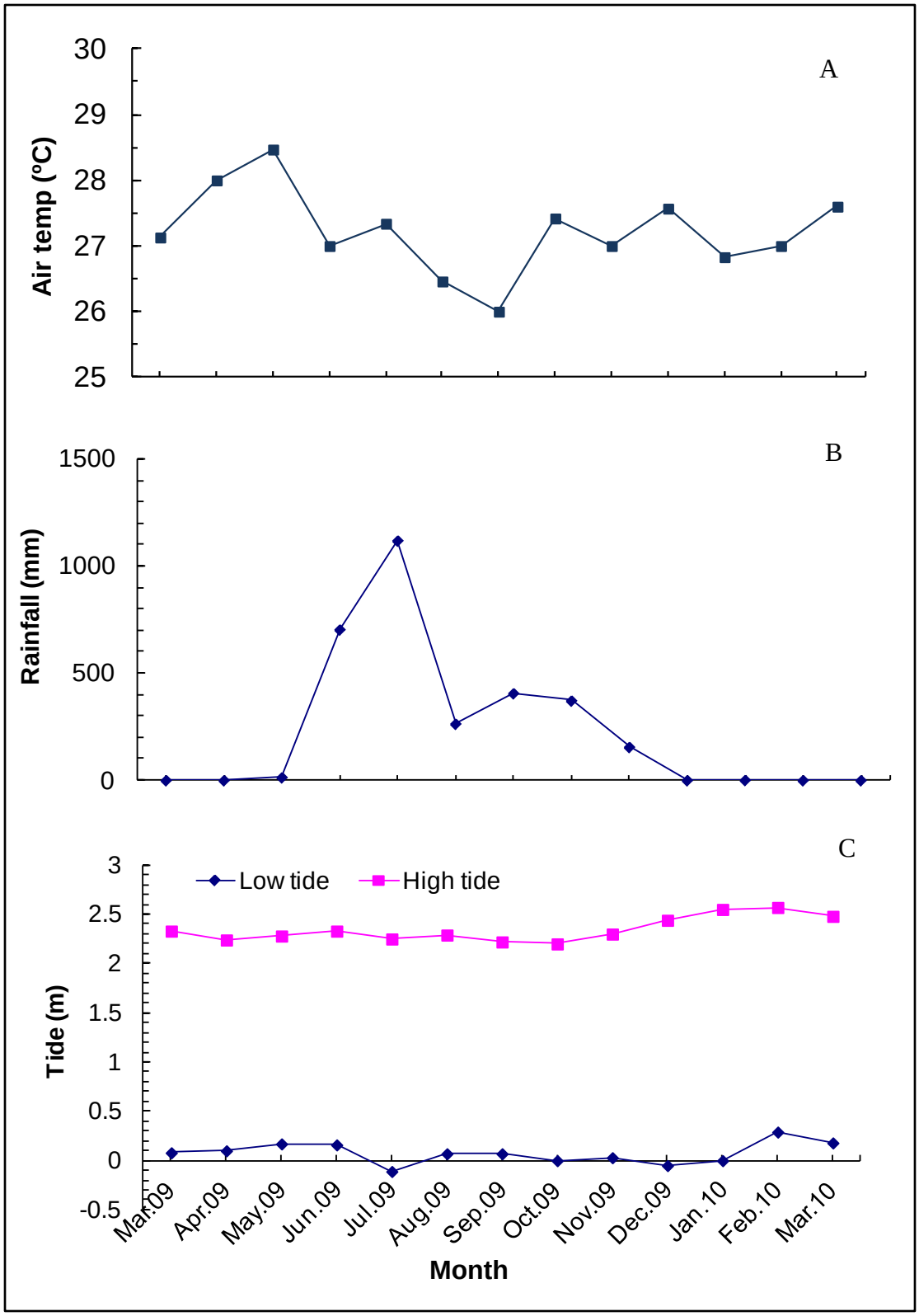


Figure 3.1: Monthly variations in climatological parameters from study area (A) Air temperature (B) Rainfall (C) Tide.

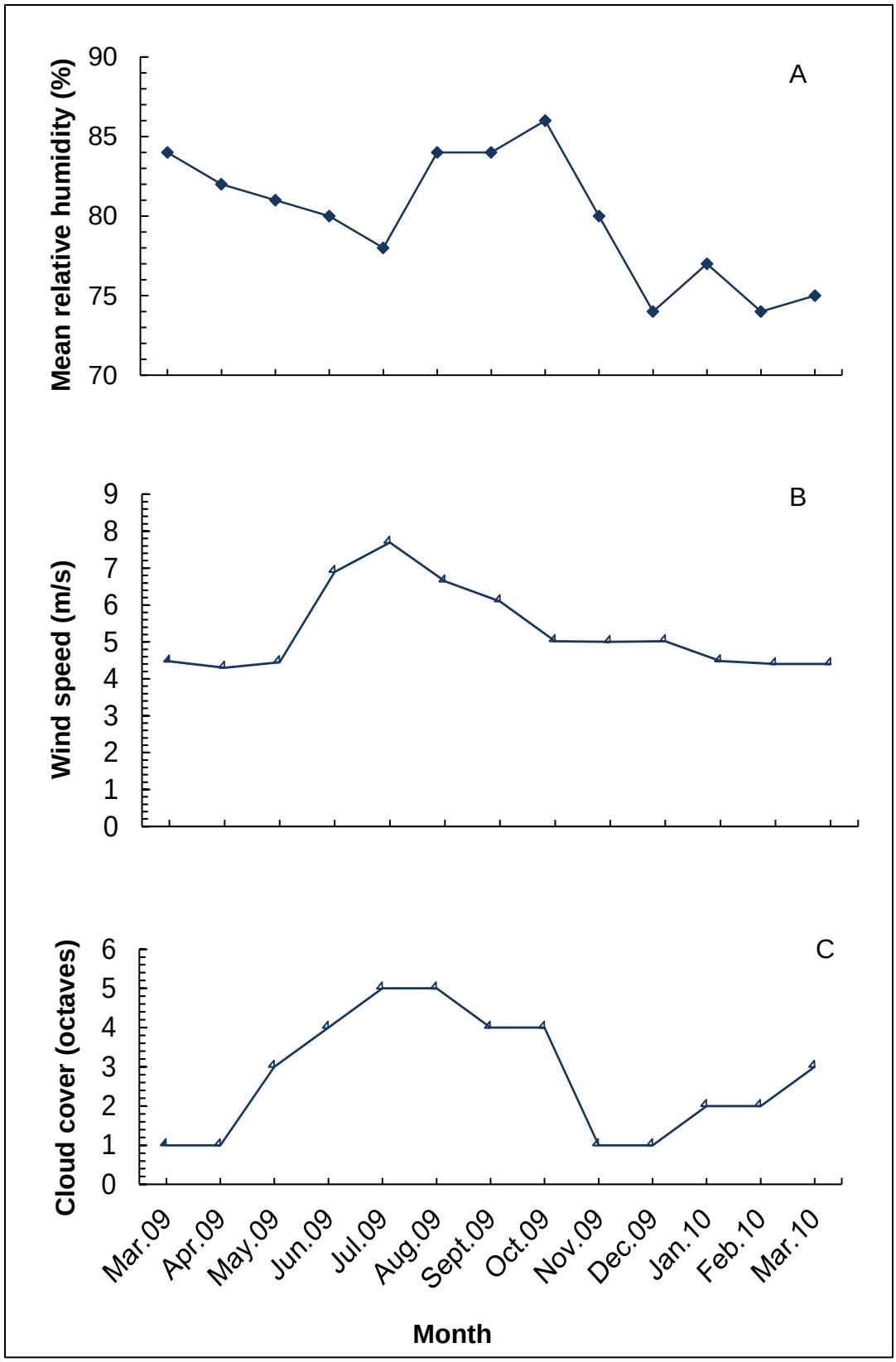


Figure 3.2: Monthly variations in climatological parameters from study area (A) Mean relative humidity (B) Wind speed (C) Cloud cover.

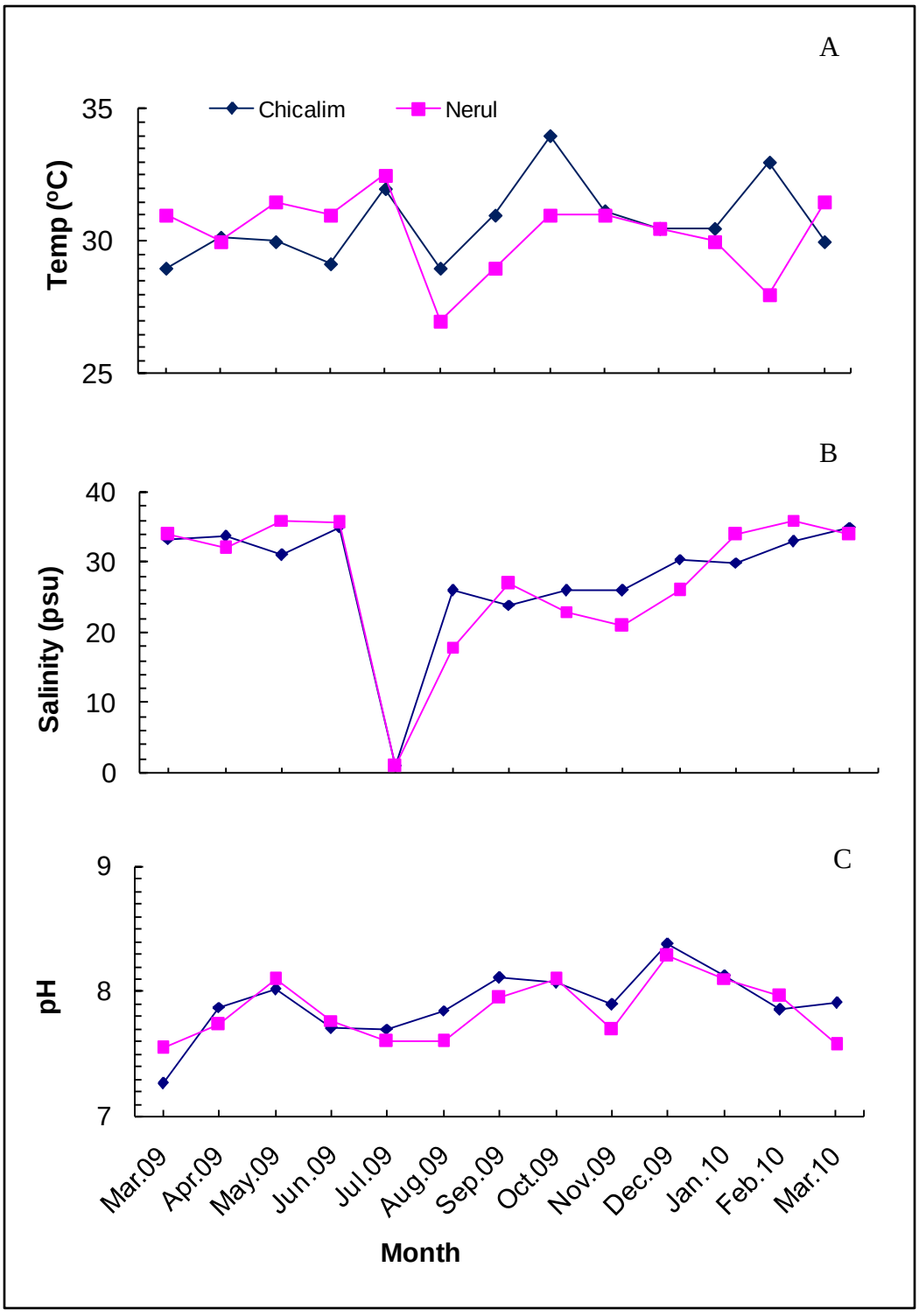


Figure 3.3: Monthly variations in the physico-chemical parameters from study area (A) Water temperature (B) Salinity (C) pH.

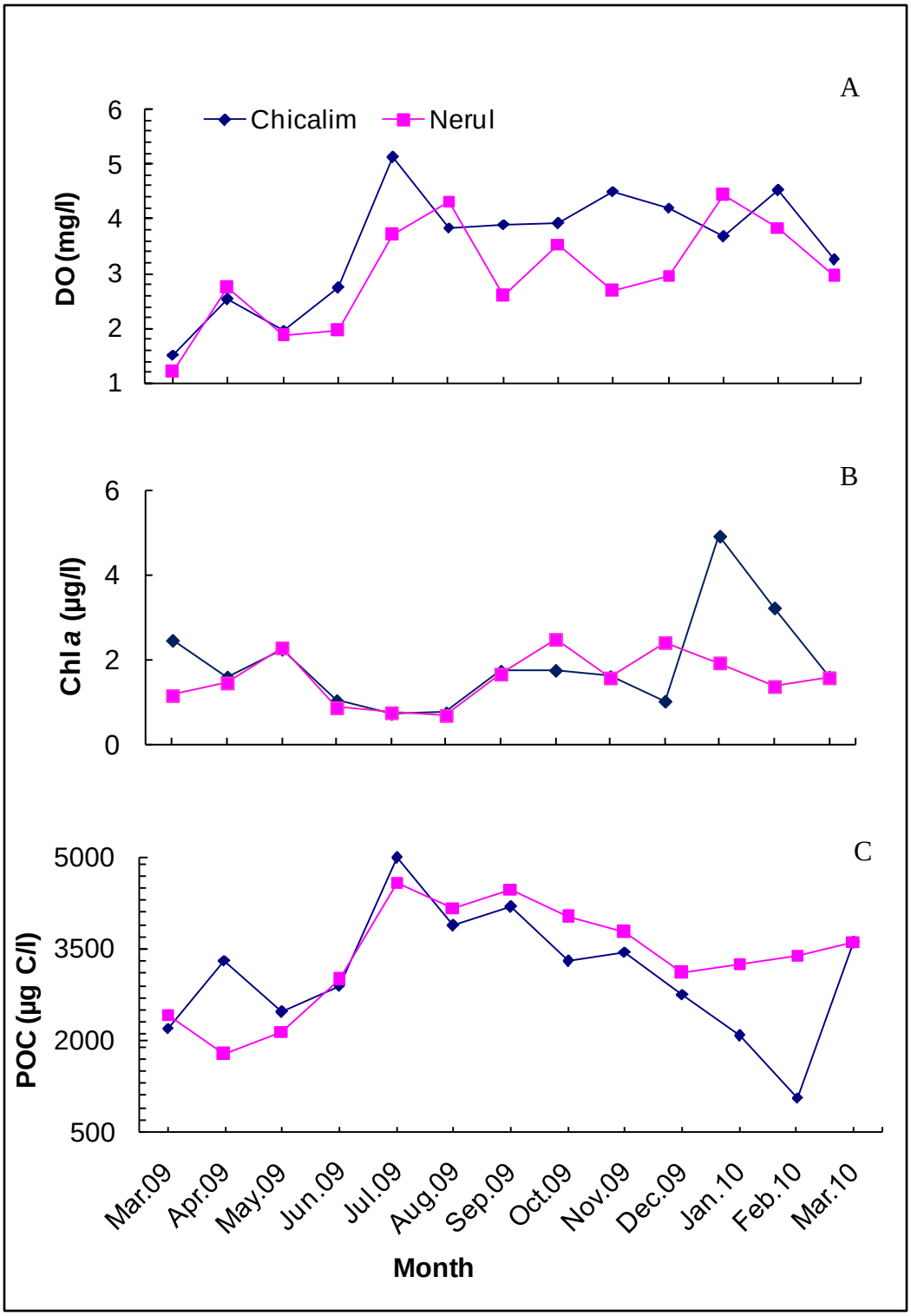


Figure 3.4: Monthly variation in physico-chemical parameters from study area (A) DO (B) Chl *a* (C) POC .

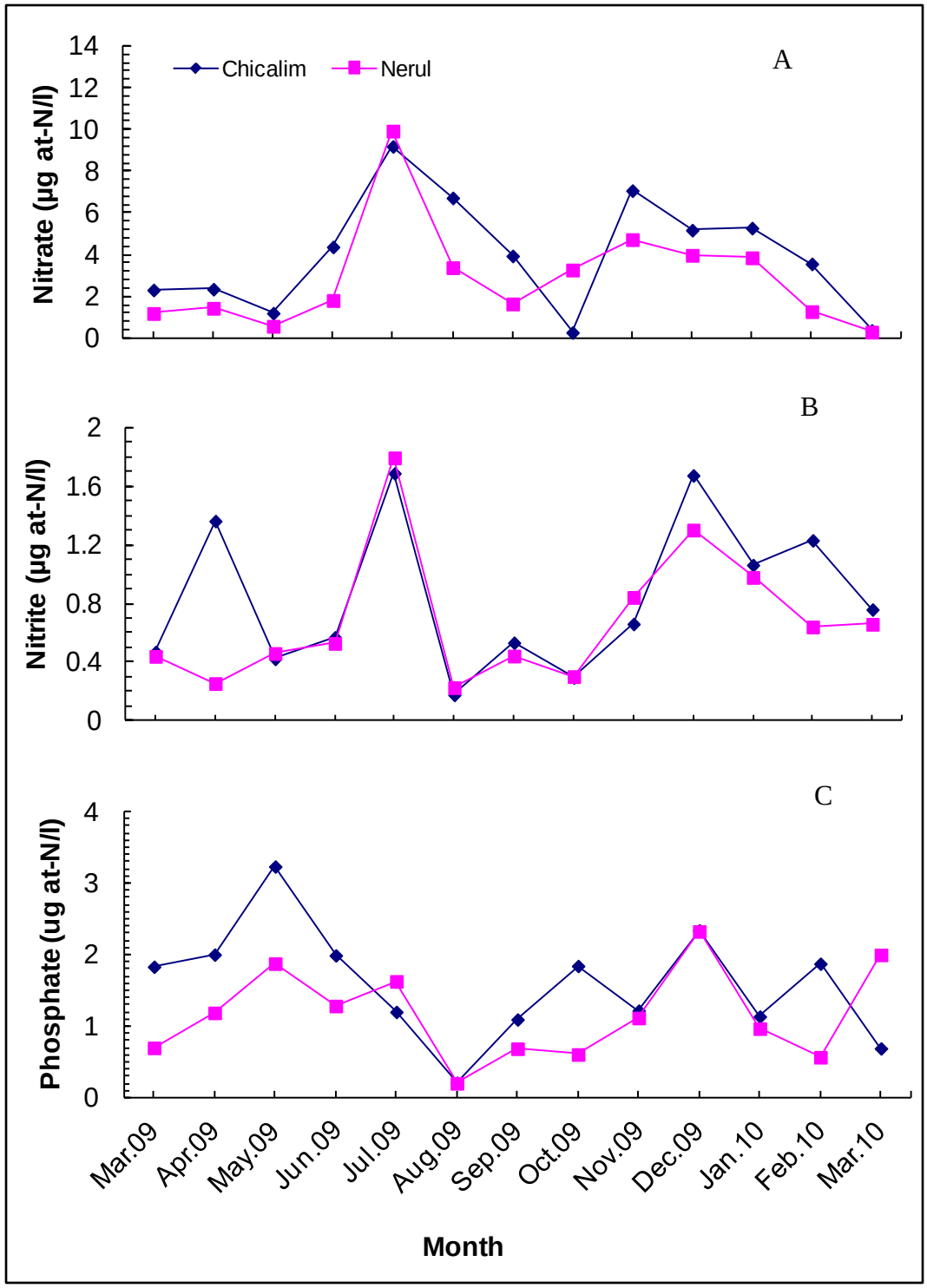


Figure 3.5: Monthly variations in physico-chemical parameters from study area (A) Nitrate (B) Nitrite (C) Phosphate.

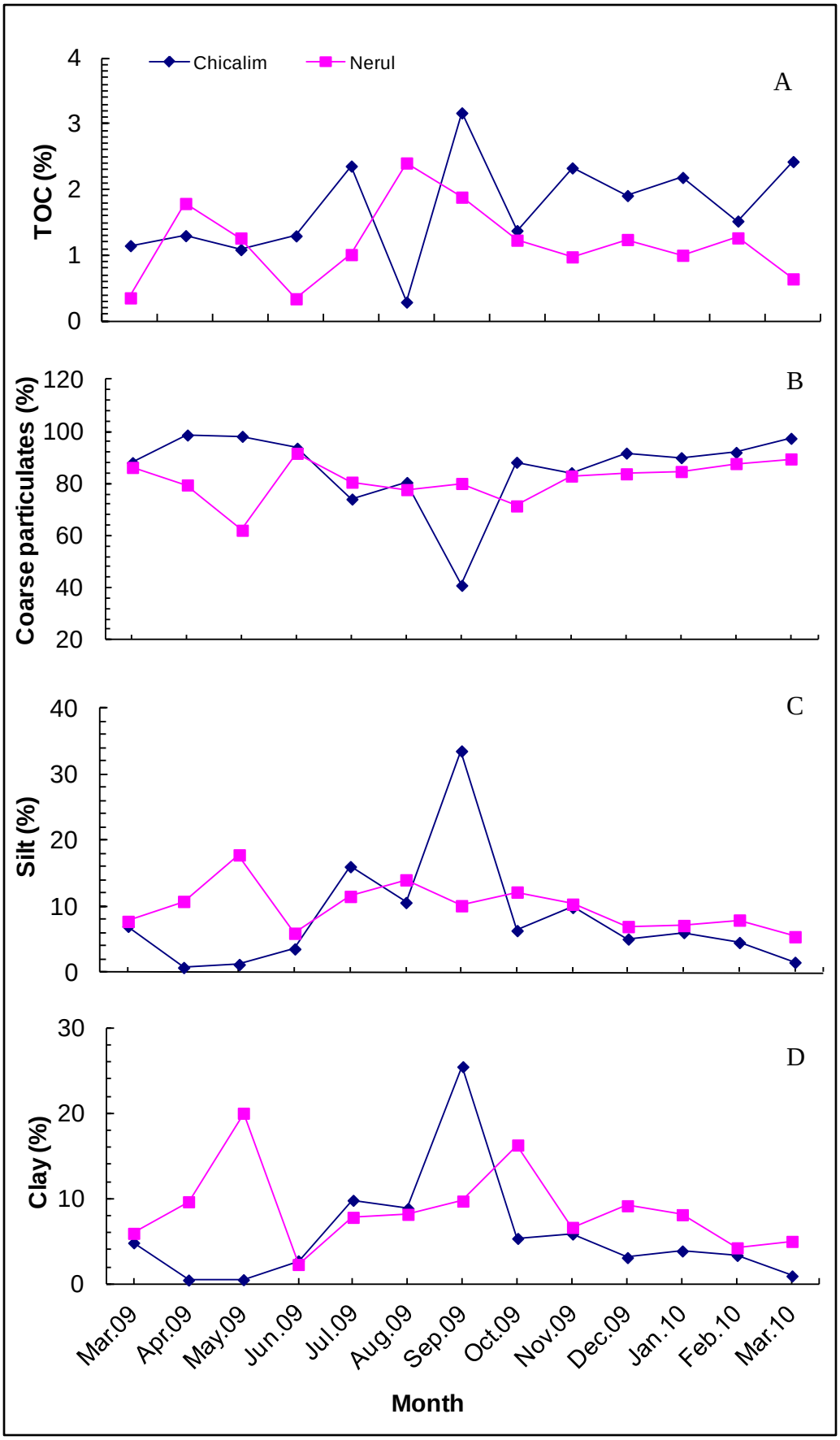


Figure 3.6: Monthly variations in sediment parameters from study area (A) TOC (B) Coarse particulates (C) Silt (D) Clay.

ALLOMETRIC RELATIONSHIPS Chapter-IV

4.1 Introduction

Growth of an individual organism from any habitat is the function of the quality of an ecological and environmental condition in its ambience. In bivalve variety, the size of the organism is directly related to its age. Cumulative increase in biomass with respect to time is referred as "absolute growth", while the percentage increase in biomass per unit time is termed as "relative growth" (Seed, 1976). Bivalve growth is mostly estimated by measuring the shell dimensions, rings or the volume of the animal (Deval, 2001). Allometry involves study of the change in proportion of various parts of an organism as a consequence of growth (Reiss, 1989). Such relationships are observed between shell parameters and body weight that help to estimate the soft body biomass of living bivalves (Dame, 1972). Length-weight relationships help to study life history and morphological comparisons between species or between populations of a species from different habitats and/or regions (Gaspar *et al*., 2001). It also helps in estimation of weight from length for individuals (Anderson and Gutreuter, 1983), by conversion of growth-in-length equations to growth-in-weight, for prediction of weight-at-age and further use in stock assessment models (Pauly, 1993). Shell length and shell height are commonly used for estimation of growth in both fresh and marine water bivalves (Rodhouse *et al*., 1984; Bailey and Green, 1988; Harvey and Vincent, 1990; Smith *et al*., 1992; Franz, 1993). Both the measures provide a nondestructive means of monitoring growth in bivalve population. However, various factors such as reproductive state, population density and habitat have been found to independently influence the rate of increase in tissue weights and in shell dimension i.e. length, height and width, as a result, shell length in bivalves

may not always be an accurate indication of the biomass of an organism (Bayne and Worrall, 1980; Borrero and Hilbish, 1988). Food availability has also been reported to influence tissue growth, storage and utilization, hence can alter the ratio of body mass to shell length (Frechette and Lefauvre, 1990; Nakaoka, 1992; Alunno-Bruscia *et al*., 2001).

Bivalve shell growth and shape are influenced by abiotic (environmental) and biotic (physical) factors (Gaspar *et al*., 2002). Burrowing behavior, ability and efficiency also affect the growth of the bivalve species (Eagar, 1978). Studying allometric relationship and growth are useful information for managing resources and understanding environmental changes. Studies on morphological variations among populations continue to play an important role in stock identification, despite the advent of biochemical and molecular genetics techniques (Swain and Foote, 1999). Different populations of the same species living in different geographical areas are known to differ morphologically (Winans, 1984). A number of environmental factors like latitude, depth, shore level, tidal level, currents, water turbulence, wave exposure, type of bottom and type of sediment (Dame, 1972; Claxton *et al*., 1998; Akester and Martel, 2000) are known to influence shell morphology and relative proportions of any bivalve species. Various allometric relationships in bivalves have been reported (Thippeswamy and Joseph, 1992; Rivonkar *et al*., 1993; Gaspar *et al*., 2001). The literature review revealed lack of an allometric information on *Paphia malabarica*. The present document describes allometry of this particular bivalve from estuarine habitats of Goa.

4.2 Results

Shell length-shell breadth

The allometry data showed that the morphometric relationships between length-breadth and length-depth variables of *P. malabarica* shell were linearly related at both stations (Figure 4.1A,B; Table 4.1,4.2). The relationship between length and breadth of the specimen showed highly significant correlation ($r = 0.9531$, $p < 0.001$ at Chicalim and $r = 0.9192$, $p < 0.001$ at Nerul). It is expressed by the following linear regression equation as

$$B = -0.5797 + 0.7326L \text{ (Chicalim)}$$

$$B = -1.4414 + 0.7594L \text{ (Nerul)}$$

Where, L is length and B is breadth of shell.

Shell length-shell depth

Shell length and depth also expressed highly significant (Figure 4.1A,B) correlation ($r = 0.9220$, $p < 0.001$ at Chicalim and $r = 0.8774$, $p < 0.001$ at Nerul). The linear regression equation obtained was

$$D = -1.5972 + 0.5552L \text{ Chicalim}$$

$$D = -1.9859 + 0.5761L \text{ Nerul}$$

Where, L is length and D is depth of shell.

Shell length-total weight

The relationship between shell length-total weight of an organism, shell length-shell weight, shell length-wet tissue weight, and shell length-dry weight exhibited nonlinear trend at both the stations. The shell length and the total weight (Figure 4.2,4.4; Table 4.1,4.2) also expressed a highly significant correlation ($r = 0.9486$, $p < 0.001$ at Chicalim and $r = 0.9232$, $p < 0.001$ at Nerul). It is expressed by the following logarithmic regression equation as

$$\text{Log } W = -3.7153 + 3.0604 \log L \text{ Chicalim}$$

$$\text{Log } W = -3.7982 + 3.1374 \log L \text{ Nerul}$$

Its corresponding parabolic equations may be represented as,

$$W=0.0002L^{3.0604} \text{ Chicalim}$$

$$W=0.0002L^{3.1374} \text{ Nerul}$$

Where, W is the total weight and L is the shell length of individual organism.

Shell length-shell weight

The two parameters were highly correlated ($r = 0.9343$, $p < 0.001$ at Chicalim and $r = 0.8969$, $p < 0.001$ at Nerul). The logarithmic regression equation which expresses the relationship between the shell length and the shell weight, is as

$$\text{Log } W = -3.9089 + 3.112 \log L \text{ Chicalim}$$

$$\text{Log } W = -3.9658 + 3.1825 \log L \text{ Nerul}$$

Its corresponding parabolic equation may be represented as,

$$W=0.0001L^{3.112}\ \text{Chicalim}$$

$$W=0.001L^{3.1825}\ \text{Nerul}$$

Where, W is weight and L is length of shell.

Shell length-wet tissue weight

Highly significant correlations between shell length and wet tissue weight ($r = 0.9069$, $p < 0.001$ at Chicalim and $r = 0.9123$, $p < 0.001$ at Nerul) were observed, and expressed by logarithmic regression equation as,

$$\text{Log } W = -3.5904 + 2.4426 \log L \text{ Chicalim}$$

$$\text{Log } W = -4.4856 + 3.0584 \log L \text{ Nerul}$$

Its corresponding parabolic equation may be represented as

$$W=0.0003L^{2.4426}\ \text{Chicalim}$$

$$W=3E\text{-}05L^{3.0584}\ \text{Nerul}$$

Where, W is the wet tissue weight and L is the shell length of individual organism.

Shell length-dry tissue weight

The shell length and dry tissue weight too found to be significantly correlated ($r = 0.7534$, $p < 0.001$ at Chicalim and $r = 0.8448$, $p < 0.001$ at Nerul), and expressed by logarithmic regression equation as

$$\text{Log } W = -3.9356 + 2.161 \log L \text{ Chicalim}$$

$$\text{Log } W = -5.4429 + 3.1967 \log L \text{ Nerul}$$

Whereas, its corresponding parabolic equation may be represented as

$$W=0.0001L^{2.161} \text{ Chicalim}$$

$$W=4E\text{-}06L^{3.1967} \text{ Nerul}$$

Where, W is the dry tissue weight and L is the shell length of individual organism.

Shell breadth-total weight relationship

The shell breadth and total weight showed highly significant correlation (Figure 4.3,4.5; Table 4.1,4.2) between them ($r = 0.9472$, $p < 0.001$ at Chicalim and $r = 0.9209$, $p < 0.001$ at Nerul), and expressed as the logarithmic regression equation as

$$\text{Log } W = 2.9917 + 2.8527 \log B \text{ Chicalim}$$

$$\text{Log } W = -2.7807 + 2.7246 \log B \text{ Nerul}$$

Its corresponding parabolic equation may be represented as

$$W=0.0001B^{2.8527} \text{ Chicalim}$$

$$W=0.0717B^{2.7246} \text{ Nerul}$$

Where, W is the total weight and B is the shell breadth of individual organism.

Shell depth-total weight

The two parameters showed a highly significant correlation ($r = 0.9040$, $p < 0.001$ at Chicalim and $r = 0.9633$, $p < 0.001$ at Nerul). The logarithmic regression equation obtained was

$$\text{Log } W = -1.8402 + 2.2346 \log D \text{ Chicalim}$$

$$\text{Log } W = -2.2074 + 2.5758 \log D \text{ Nerul}$$

Its corresponding parabolic equation may be represented as

$$W=0.0144D^{2.2346} \text{ Chicalim}$$

$$W=0.0062D^{2.5758} \text{ Nerul}$$

Where, W is the total weight and D is the shell depth of individual organism.

Shell breadth-wet tissue weight

The correlation between the shell breadth and wet tissue weight was highly significant ($r = 0.8848$, $p < 0.001$ at Chicalim and $r = 0.8788$, $p < 0.001$ at Nerul). The logarithmic regression equation obtained was as

$$\text{Log } W = -2.9454 + 2.2246 \log B \text{ Chicalim}$$

$$\text{Log } W = -3.372 + 2.5647 \log B \text{ Nerul}$$

Its corresponding parabolic equation may be represented as

$$W=0.0011B^{2.2246} \text{ Chicalim}$$

$$W=0.0004B^{2.5647} \text{ Nerul}$$

Where, W is the wet tissue weight and B is the shell breadth of individual organism.

Shell depth–wet tissue weight

Shell depth and wet tissue weight showed a highly significant correlation ($r = 0.8149$, $p < 0.001$ at Chicalim and $r = 0.8435$, $p < 0.001$ at Nerul). The logarithmic regression equation obtained was as

$$\text{Log } W = -1.9784 + 1.6817 \log D \text{ Chicalim}$$

$$\text{Log } W = -2.5954 + 2.2251 \log D \text{ Nerul}$$

Its corresponding parabolic equation may be represented as

$$W=0.0105D^{1.6817} \text{ Chicalim}$$

$$W=0.0025D^{2.2251} \text{ Nerul}$$

Where, W is the wet tissue weight and D is the shell depth of individual organism.

Parabolic and its corresponding linear relationships between various allometric parameters, described above, are depicted in Figures 4.1-4.5 and Table 4.1,4.2.

Population density

Population density varied significantly between stations ($P<0.001$) and months ($P<0.001$). An average density of an organism ranged from 66 nos./m^2 – 500 nos./m^2 with maximum (500 nos./m^2) in the month of December 2009 and the minimum (66 nos./m^2) in September 2009 at Chicalim (Figure 4.6A). However, the population density was observed to be relatively poor (103 nos./m^2 – 252 nos./m^2) from the beds at Nerul with the maximum (252 nos./m^2) in November 2009 and minimum (103 nos./m^2) in March 2009 (Figure 4.6A).

Biomass

The biomass values ranged from 8.68 g/m^2 to 25.21 g/m^2 at Chicalim, while at Nerul it was in the range of 10.50 g/m^2 to 37.40 g/m^2. The biomass of an organism was highest (37.40 g/m^2) in July 2009 and lowest in (10.50 g/m^2) February 2010 at Nerul. While at Chicalim highest (25.21 g/m^2) biomass value of *P. malabarica* was observed

in June 2009 and lowest (8.68 g/m^2) in November 2009 (Figure 4.6B). Biomass too, significantly varied between stations (P<0.001) and months (P<0.001).

Shell length

There was significant variation in allometry of *P. malabarica* among stations (P<0.001) and months (P<0.001). The shell length of specimens ranged between 23.7 ± 2.95 mm to 36.0 ± 3.20 mm at Chicalim. The lowest value (23.7 ± 2.95 mm) was recorded during November 2009, whilst the highest (36.0 ± 3.20 mm) were observed during March 2009. Organism from Nerul beds exhibited the lowest value (26.2 ± 2.53 mm) of shell length in the month of February 2010, whereas highest (36.2 ± 2.74 mm) during July 2009 (Figure 4.6C). The annual mean length of *P. malabarica* from Nerul station (31.2 ± 3.62 mm) was higher than those from the Chicalim beds (30.6 ± 4.86 mm).

Shell breadth

At Chicalim maximum shell breadth recorded was 26.4 ± 2.42 mm in the month of March 2009, while the lowest was 16.4 ± 1.78 mm in November 2009. Highest breadth recorded from Nerul sample was 26.7 ± 2.28 mm in the month of July 2009, while the lowest breadth observed was 17.8 ± 1.70 mm in February 2010 (Figure 4.7A).

Shell depth

The depth of the specimen at Chicalim varied from a minimum of 11.3 ± 1.45 mm in November 2009 to a maximum of 18.7 ± 2.86 mm in June 2009. While at Nerul the depth measurements varied from a minimum of 12.8 ± 1.45 mm in October 2009 to a maximum of 19.4 ± 1.96 mm in July 2009 (Figure 4.7B).

Total weight

Clams collected from Chicalim exhibited the maximum total weight in the month of June 2009 with the weight of 13 ± 4.91 g. The lowest value of 3.1 ± 1.05 g at this station was recorded in November. At Nerul the highest total weight of clam observed was 14.1 ± 3.32 g in July 2009 and the lowest of 4.5 ± 1.30 g in February 2010 (Figure 4.7C).

Wet tissue weight

Wet tissue weight of *P. malabarica* ranged between 0.54 ± 0.17 g to 1.5 ± 0.42 g at Chicalim. The lowest value (0.54 ± 0.17 g) was recorded during November 2009, whilst the highest (1.5 ± 0.42 g) were observed during June 2009. Organism from Nerul beds exhibited the lowest value (0.65 ± 0.19 g) of wet tissue weight in the month of February 2010, whereas highest (2.3 ± 0.53 g) during July 2009 (Figure 4.8A).

Shell weight

The highest shell weight of 9.8 ± 3.98 g was recorded in the month of June 2009 and the lowest of 2.4 ± 0.87 g in November 2009 at Chicalim. While maximum shell weight (11.0 ± 2.75 g) of the specimen was observed in the month of July 2009 at Nerul and minimum recorded was 3.5 ± 1.15 g in October 2009 (Figure 4.8B).

Dry tissue weight

Highest dry tissue weight recorded for Chicalim samples was 0.27 ± 0.08 g in June 2009, while lowest of 0.09 ± 0.03 g was observed in November 2009. At Nerul the

highest dry tissue weight of *P. malabarica* recorded was 0.43 ± 0.11 g in May 2009 which decreases in September 2009 with lowest value of 0.11 ± 0.02 g (Figure 4.8C).

4.3 Discussion

The dimensional relationships are important to understand the various aspects of a particular organism such as growth, ecology and physiology (Gaspar *et al*., 2001). The morphometric relationships between length-breadth, length-depth (Table 4.1,4.2) variables in *P. malabarica* at both stations were linearly related and showed that the short individuals (less height) have low thickness, whereas, long individuals (more height) have greater thickness (Figure 4.1A,B). It indicated that length, breadth and depth are strongly proportional parameters of the individual shells (Ramesha and Thippeswamy, 2009). Some individuals of same length show different breadth and width and such differences results in shape variation (Thippeswamy and Joseph, 1992). Shell dimensional relationship in other edible bivalves like *Perna viridis*, *Donax incarnates, D. faba, D. cuneatus,* and *Meretrix casta* were reported to be linearly related (Durve and Raja, 1965; Nair *et al*., 1978; Thippeswamy and Joseph, 1992; Thippeswamy and Hemachandra, 2008). However, the values of intercept and slope are different, representing variations in morphological traits since these populations inhabit different habitats with varying environmental parameters. Size of clams is more affected than their shape by fluctuation in ambient environment, whereas shape is controlled by its genetics (Thippeswamy and Joseph, 1991). Hence, Shape of bivalves provides significant information on the dimensional relationship of species type (Thippeswamy and Joseph, 1992).

The values of 'b' of morphometric relationships can be used to compare between dimensional growth of related species or same species in different habitats

(Thippeswamy and Hemachandra, 2008). The 'b' values of length-breadth and length-depth equations for *P. malabarica* in the present study were lower (Table 4.3) than that of *M. casta* reported earlier from Athankarai estuary, Tamil Nadu (Durve and Raja, 1965) and *P. viridis*, Karnataka (Thippeswamy and Hemachandra, 2008).

Length-weight relationship analysis shows that dots are skewed on each diagram representing short individuals are light and long individuals are heavy, indicating increase in weight with age in *P. malabarica* (Table 4.1 and 4.2). However some individuals of the equal age show different weight, which may be related to variations in environmental parameters such as salinity, temperature etc and physiological condition of clams (Thippeswamy, 1985; Thippeswamy and Joseph, 1988). The nonlinear relationship observed in *P. malabarica* (Figures 4.2-4.5) were also reported in other bivalves species like *D. incarnates*, *Anomia ephippium, Acanthocardia aculeate, D. trunculus, Mactra glauca, M. stultorum*, *Pandora albida, Venus fasciata*, *P. viridis*, *D. semistriatus, Glycymeris violacescens*, *Solen marginatus, Tellina nitida*, *T. planate, Crassostrea madrasensis, C. gryphoides* (Thippeswamy and Joseph, 1992; Gaspar *et al*., 2001; Thippeswamy and Hemachandra, 2008; Charef *et al*., 2011; Nagi *et al*., 2011) from tropics including India (Table 4.3). Variation in shape of the shell are induced by the differential growth vectors operating at distinct locations around the mantle edge (Seed, 1980), organ that plays a key role in the shell secretion. Various environmental parameters such as tidal height, substrates etc have been reported to be the major factors affecting growth in length and tissue of organism (Bloom *et al*., 1972; Chardy and Clavier, 1988; Baron and Clavier, 1992). Submergence of mussels for many hours increased the weight of tissues and shell deposition than those in high intertidal zone (Fox and Coe, 1943; Wilbur and Jodrey, 1952).

The temporal variation in values of 'b' obtained for shell length-wet tissue weight relationship in *P. malabarica* from Chicalim and Nerul ranged from 1.05-3.05 and 1.37-3.287, respectively (Table 4.1,4.2). Whereas, 'b' for dry tissue weight and shell length ranged from 0.664-3.54 and 1.56-3.52 for Chicalim and Nerul samples, respectively (Figures 4.9,5.0). A Systematic increase in 'b' values in case of *P. malabarica* was observed from July 2009 to March 2010 at Chicalim (Figure 4.9) relative to the 'b' value variation in Nerul (Figure 4.10). Such differences observed may be due to different ecological condition. The b value for the length-weight relationship is within the range for other bivalves (Thippeswamy and Joseph, 1992; Thippeswamy and Hemachandra, 2008; Charef *et al*., 2011). The fully grown shells make the live animals heavier, either because of increased shell mass or a higher capacity to hold water. Currey (1988) have reported that the need for strong shells and capable to live longer in adverse conditions in intertidal areas may direct the part of energy to shell growth instead of the soft tissue. This investment of energy on the shell limits the growth of an individual.

The density and biomass reported for *P. malabarica* in the present study was lower than those reported from Kali estuary, Mandovi estuary and Ashtamudi lake (Harkantra, 1975; Ansari, 1978; Appukuttan *et al*., 2002). The differences noted may be due to extensive exploitation of these clams. It could also be related to the differences in sampling strategies such as variation in depth and season of sampling, variations in sediment properties and other environmental conditions prevailing in the area. Mohite (2010) reported the breeding and spawning in *P. malabarica* during September to January viz after monsoon, along the west coast of India. The rise in population density and the corresponding lowering in biomass (Figures 4.6 A,B) during postmonsoon period may be attributed to the breeding and settlement of young

ones in the previous months as supported by smaller shell length (Figure 4.6 C) during the said period (Modassir, 1990; Sivadas *et al*., 2011). In comparison, the higher biomass values coupled with low population density and larger shell length measured for the premonsoon period imply, when the large-sized individuals as the dominant component of the population at both the stations. Nerul creek is navigated by small fishing boats which releases various waste such as petroleum waste etc. in the area. Relatively low population density in Nerul compared to that of Chicalim (Figure 4.6 A) possibly indicates less conducive environmental condition for larval growth and survival in Nerul during the sampling months. This issue needs to be investigated in future. In addition low population density in Nerul compared to that of Chicalim would create less competition for food in Nerul which may promote higher biomass and size. The local fishermen observe a moratorium of about 5 to 6 months (August to January) on fishing clams at Nerul. During this period the clams get opportunity to reproduce and grow. Such ban on clam fishing is not observed at Chicalim and thereby the crop available is smaller in size due to over exploitation. However, the availability of clams from Chicalim and Nerul throughout the seasons indicates their tolerance to the different types of environmental conditions and adaptability to different season and region. Results of the present study give an overall idea about the relative growth of body parts of *P. malabarica*. However for specific biological evaluation of the growth progression, long-term monitoring is required to understand the dynamics of this species so that the data generated can be used for growth enhancement in commercial cultivation of the species concerned. Further long term ecological studies should also be undertaken to carefully monitor shellfish exploitation.

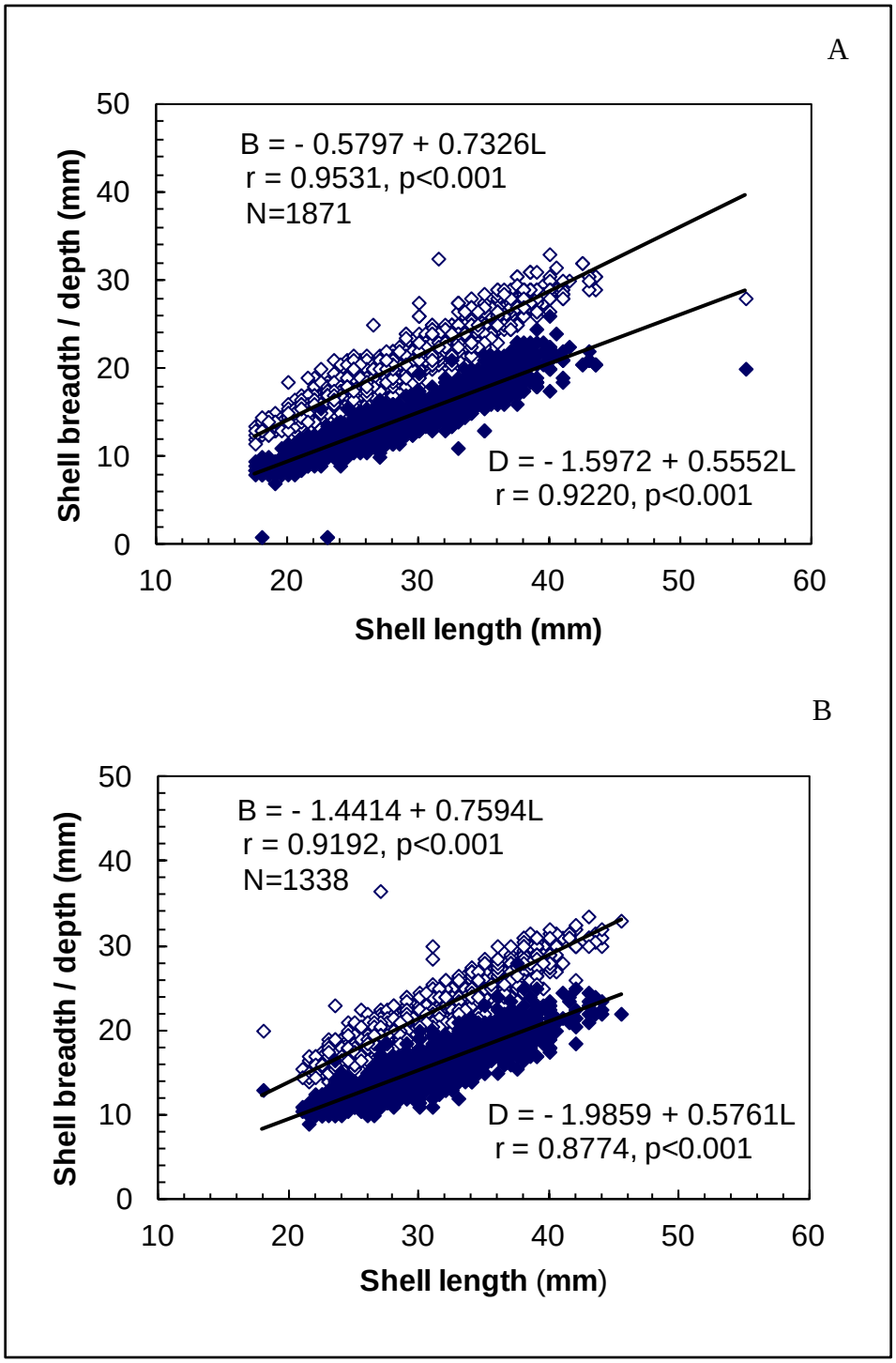


Figure 4.1: Shell length-shell breadth and shell length-shell depth relationship of *P. malabarica* at (A) Chicalim and (B) Nerul.

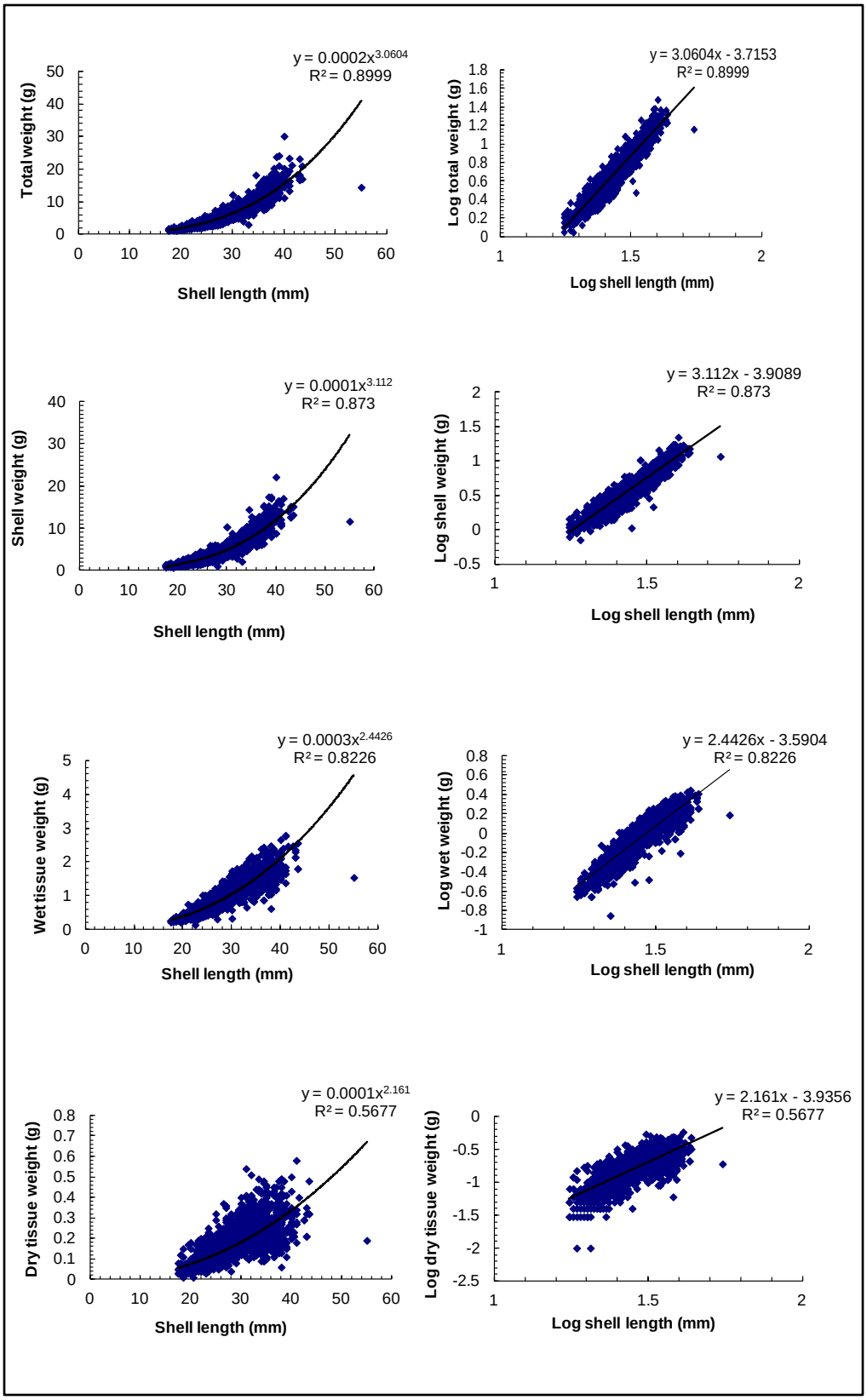


Figure 4.2: Allometric relationship of *P. malabarica* at Chicalim.

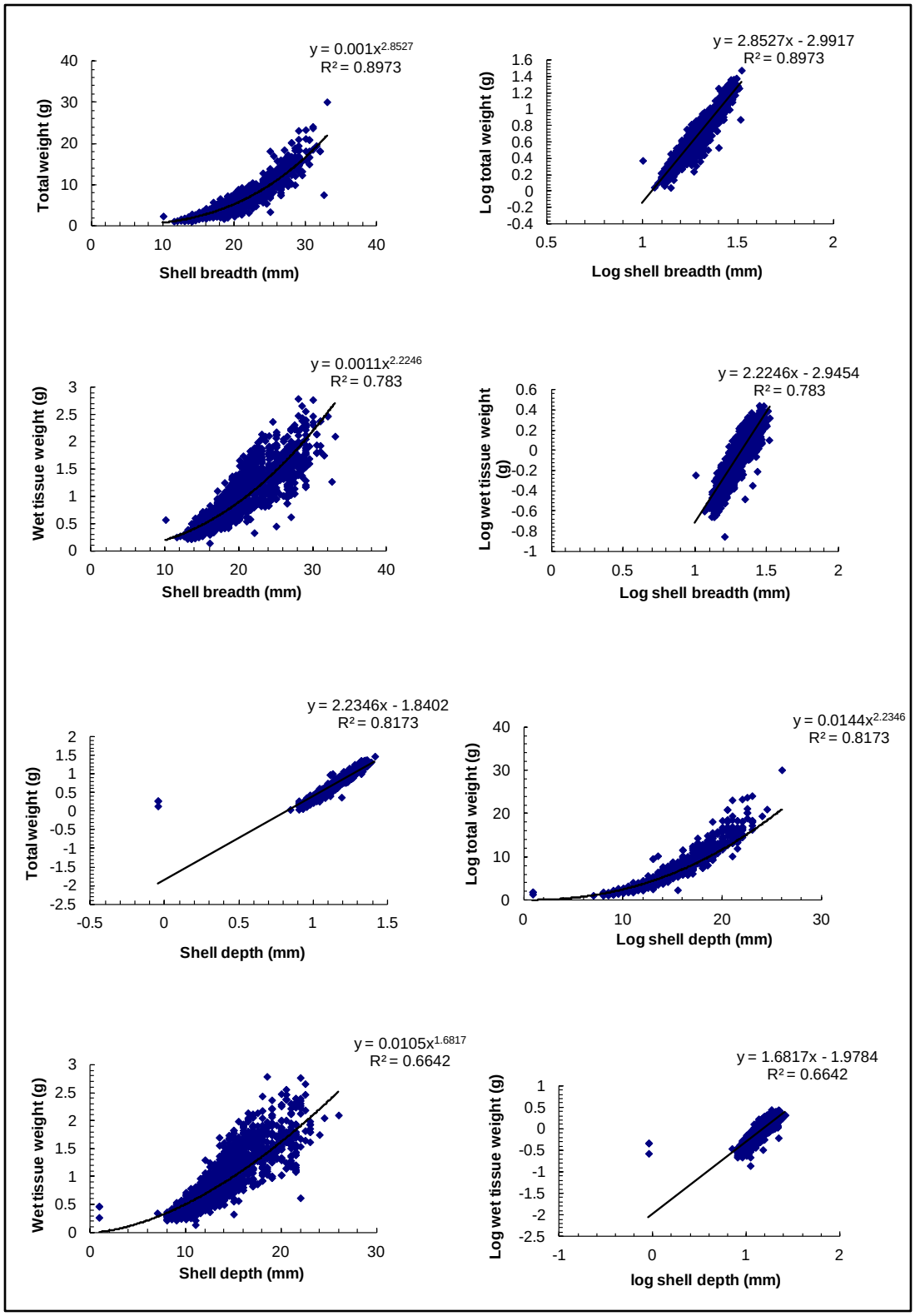


Figure 4.3: Allometric relationship of *P. malabarica* at Chicalim.

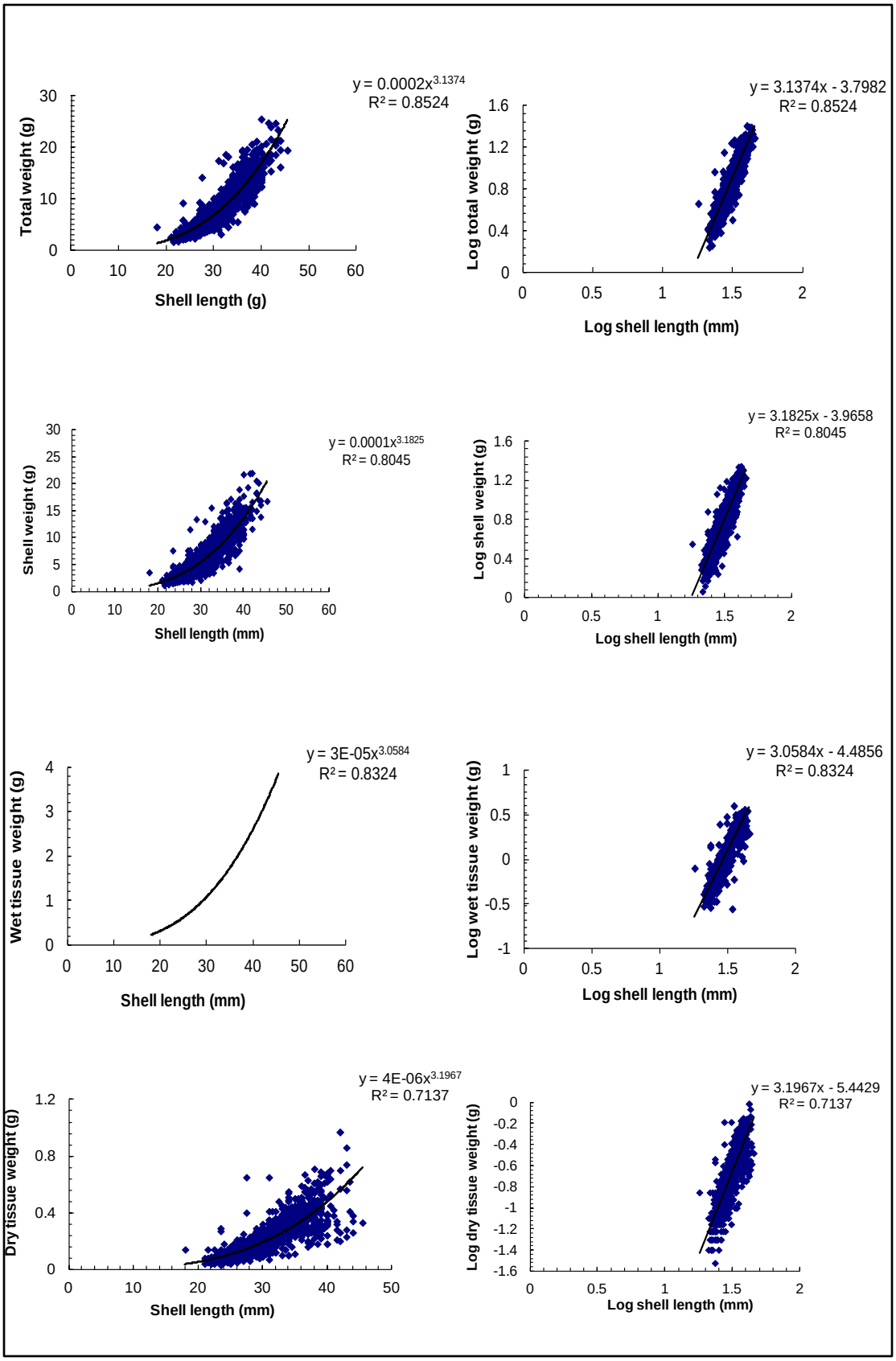


Figure 4.4: Allometric relationships of *P. malabarica* at Nerul.

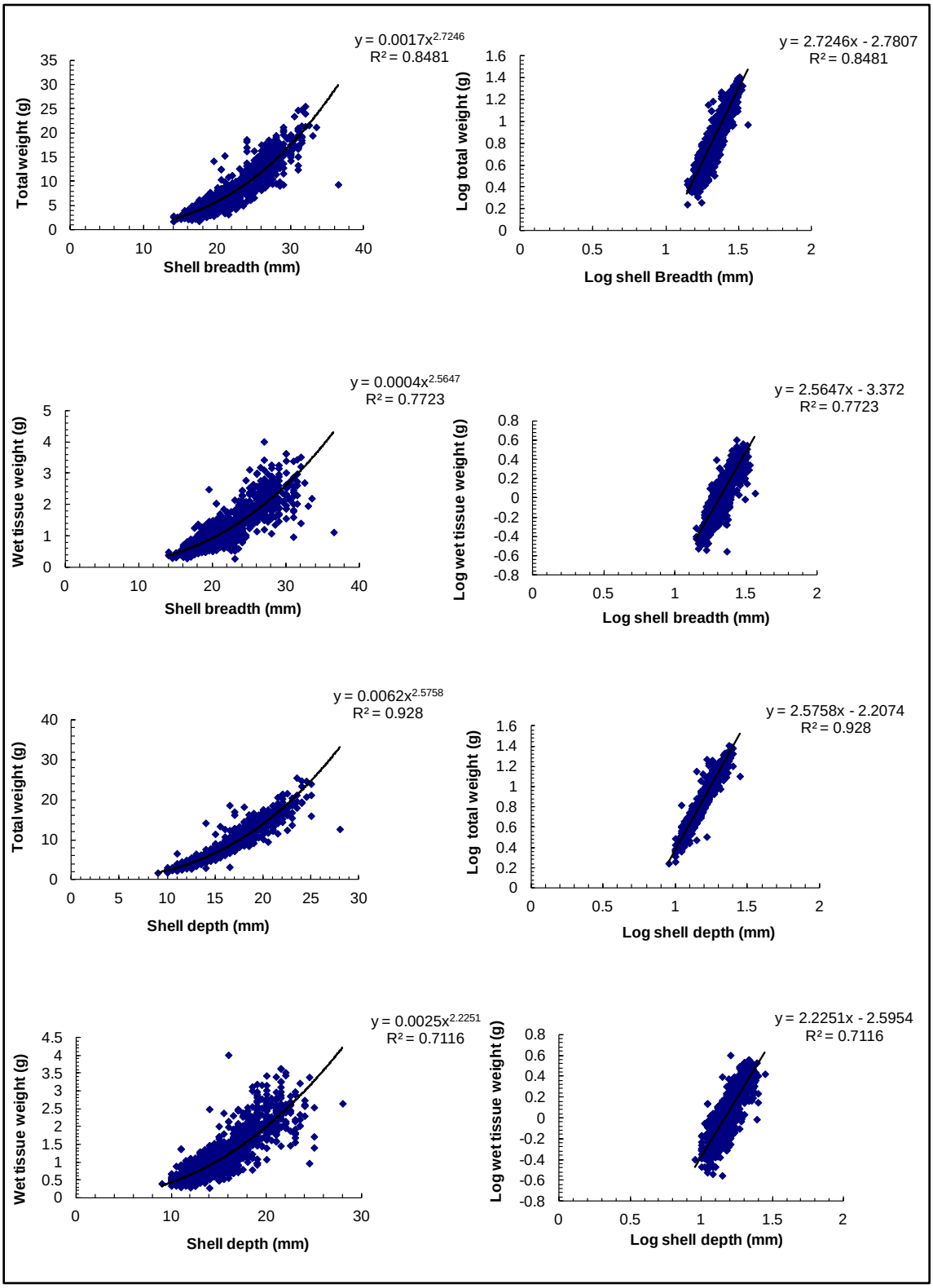


Figure 4.5: Allometric relationship of *P. malabarica* at Nerul.

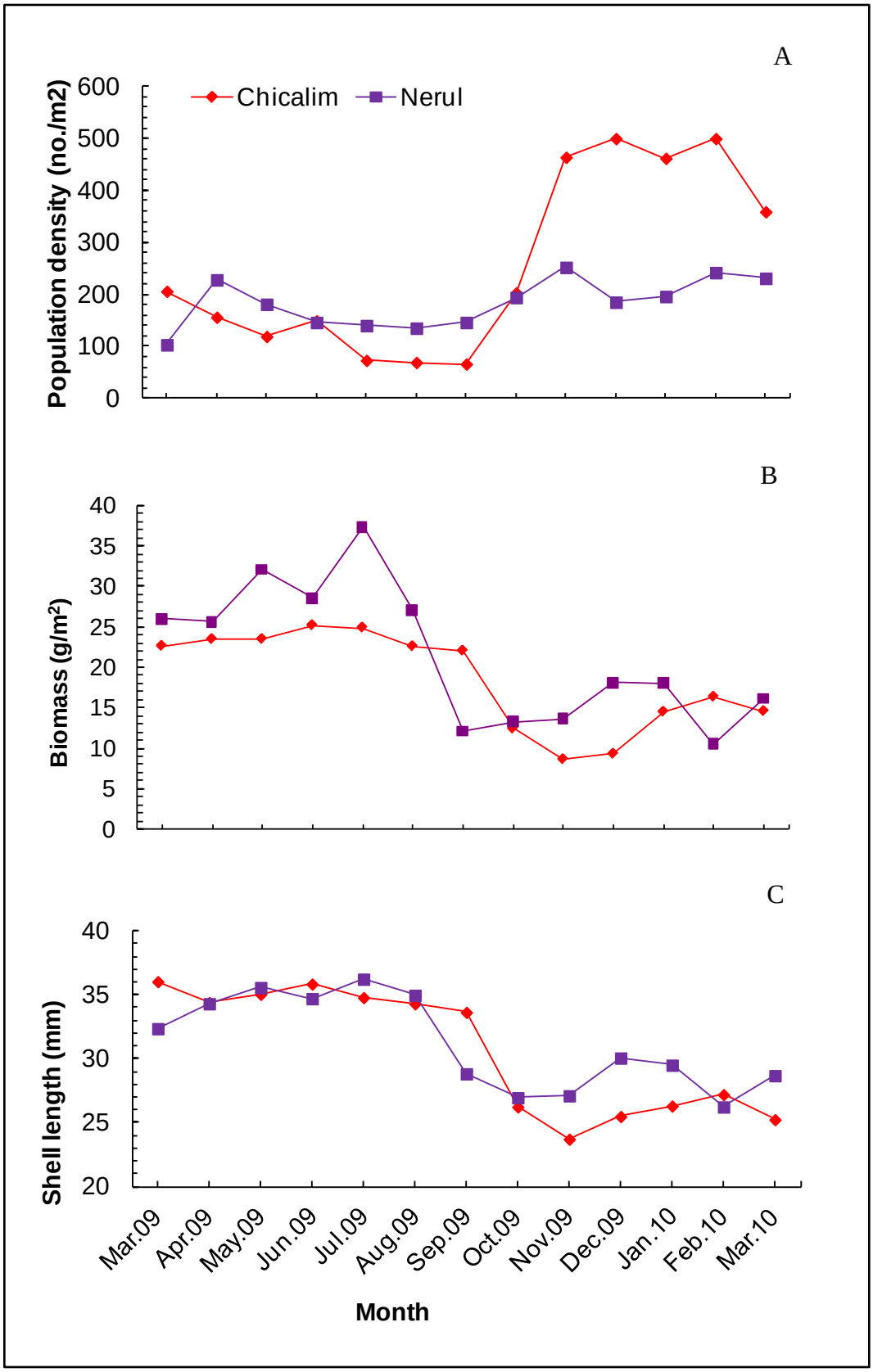


Figure 4.6: Average (A) population density, (B) biomass and (C) shell length of *P. malabarica* during the entire study period.

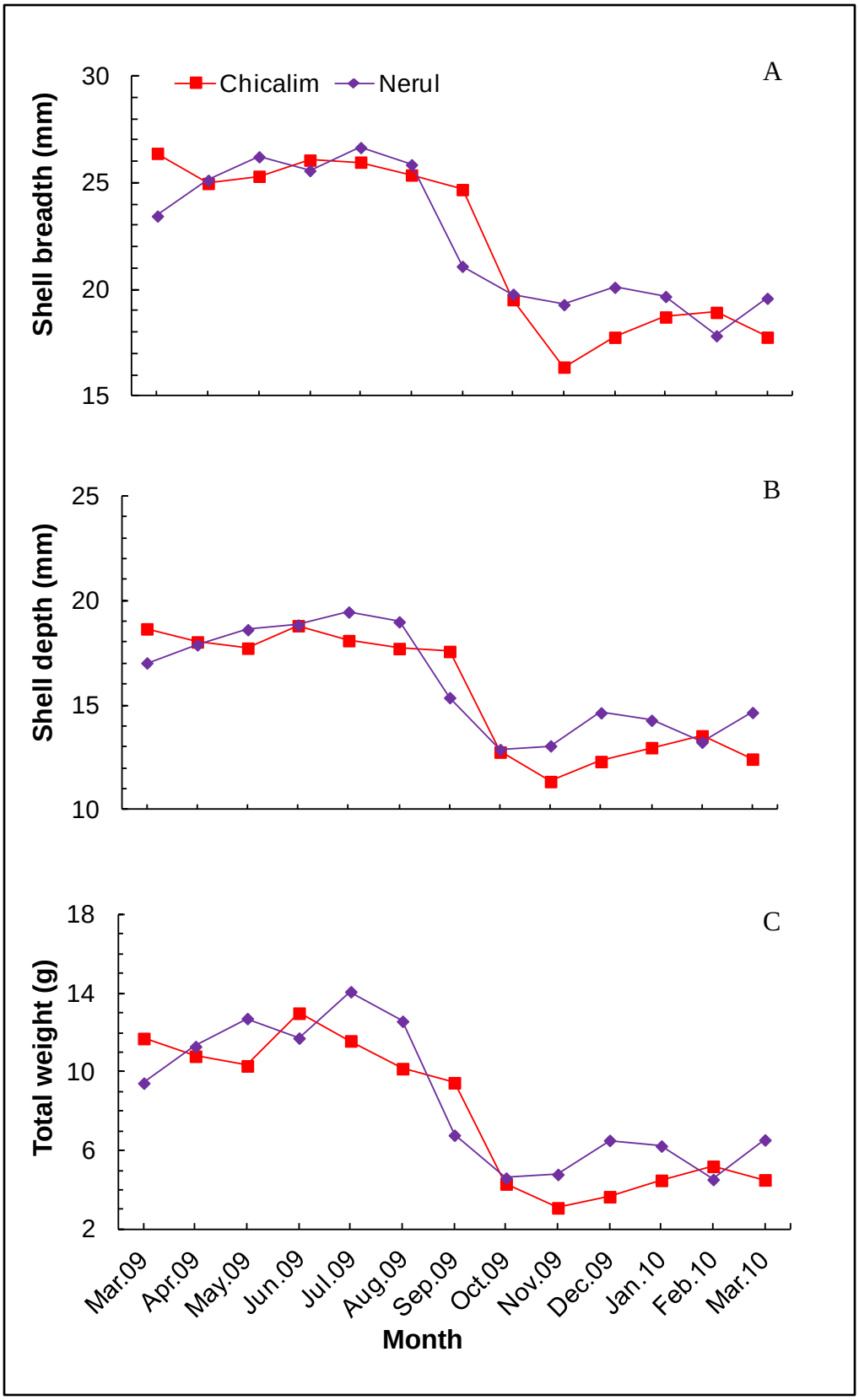


Figure 4.7: Monthly average variation in (A) shell breadth, (B) depth and (C) total weight of *P. malabarica* during the study period.

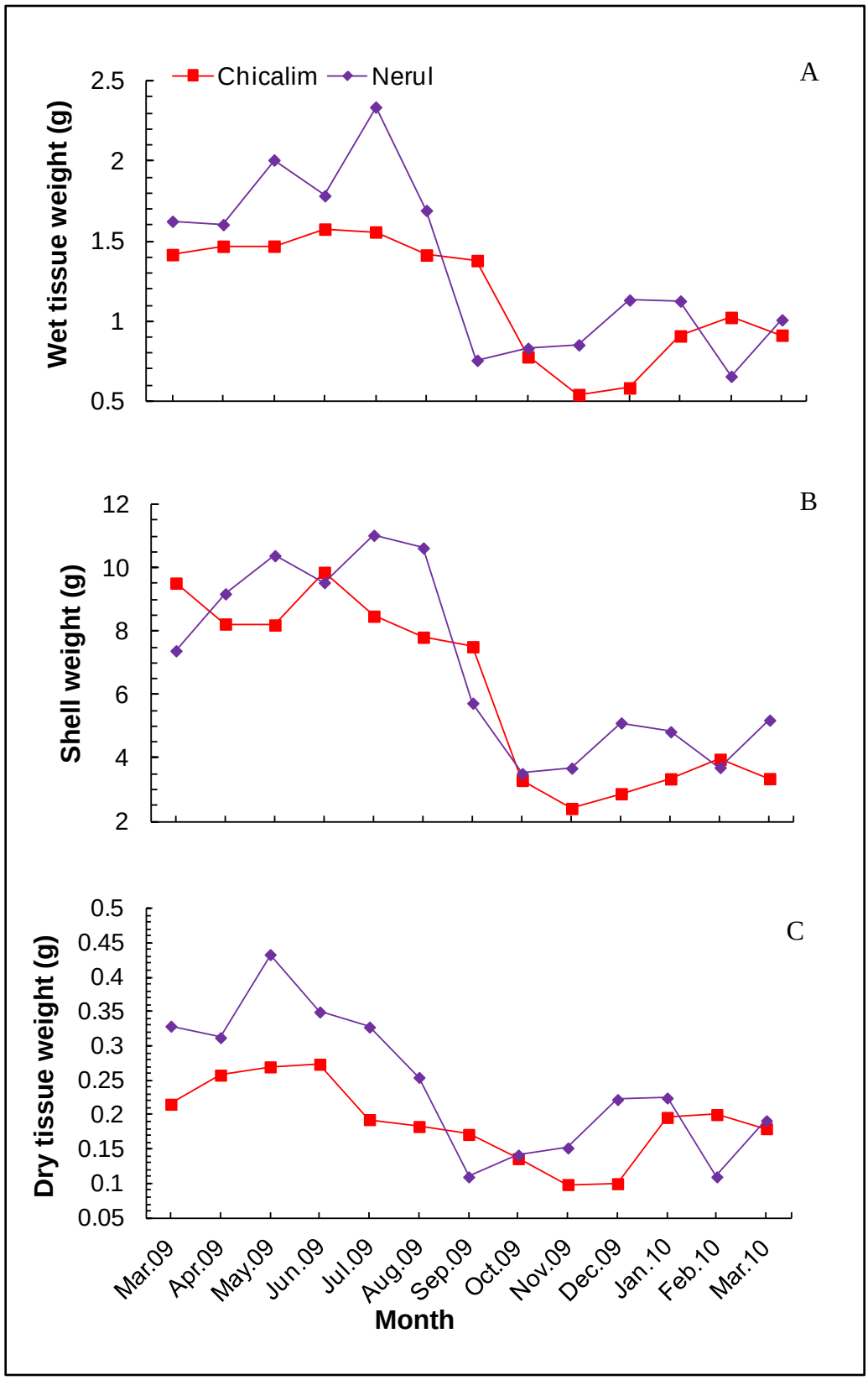


Figure 4.8: Monthly average variation in (A) wet tissue weight, (B) shell weight and (C) dry tissue weight of *P. malabarica* during the study period.

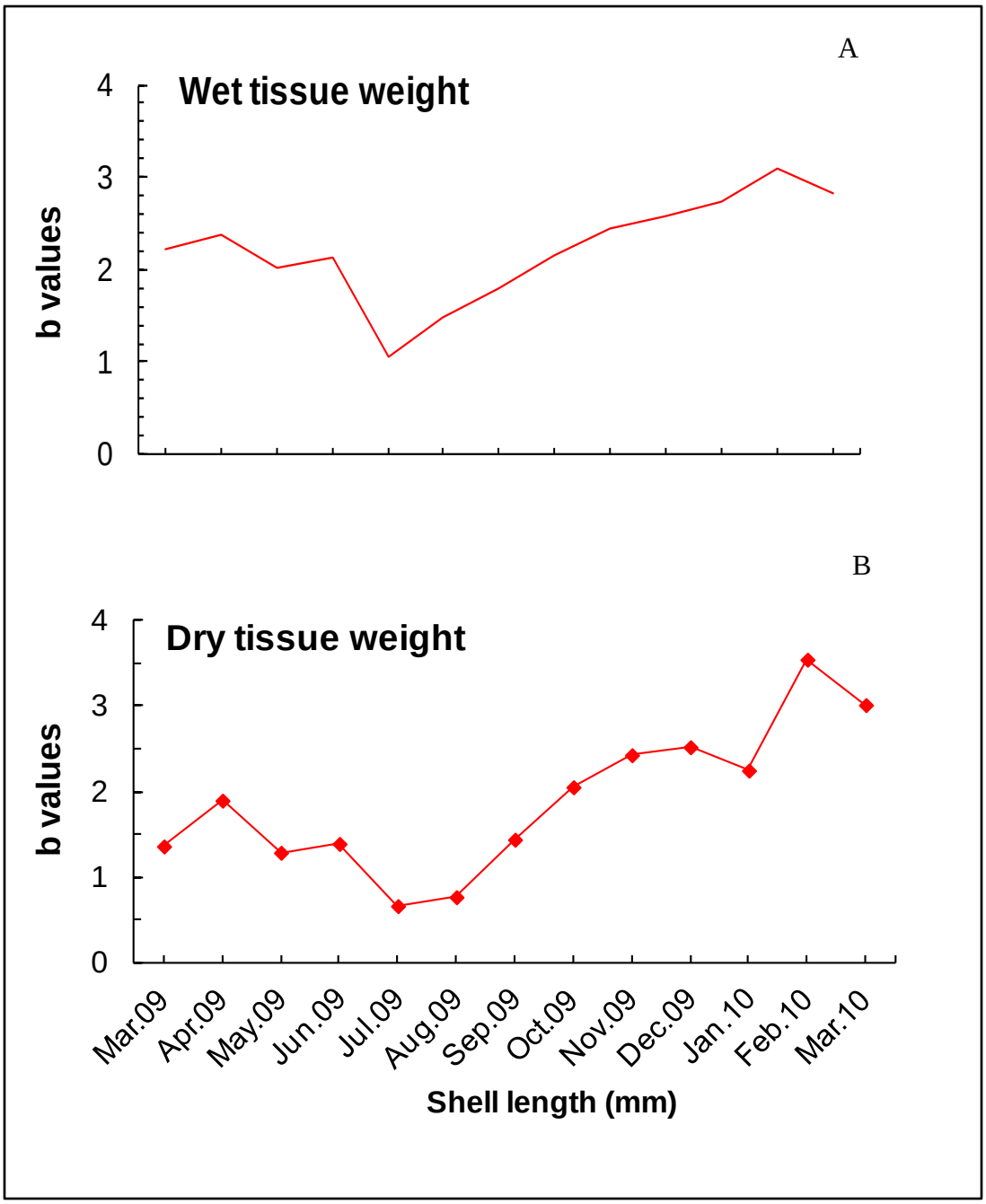


Figure 4.9: Monthly variation in the b values of (A) shell length-wet tissue weight and (B) shell length-dry tissue weight relationships of *P. malabarica* at Chicalim .

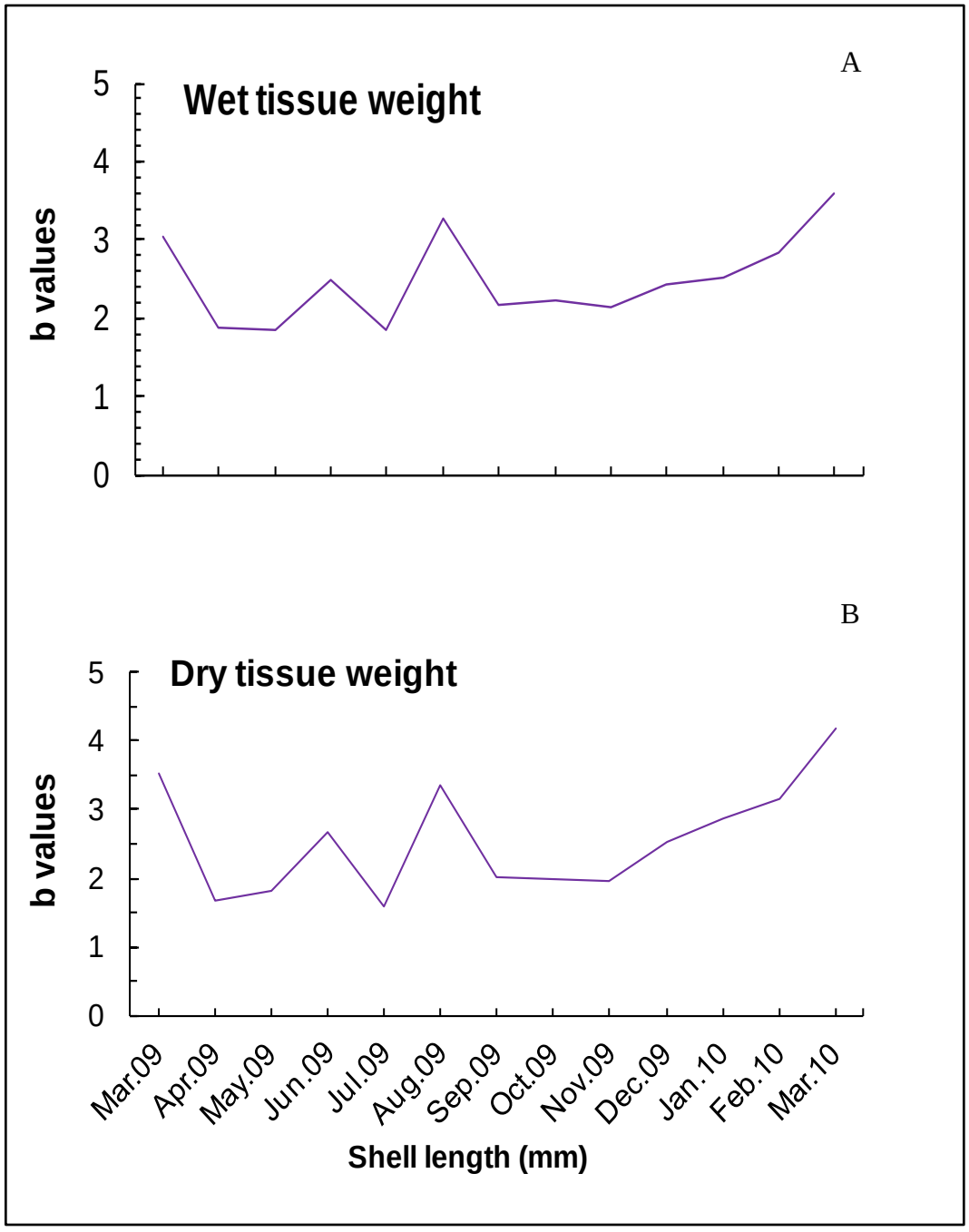


Figure 4.10: Monthly variation in the b values of (A) shell length-wet tissue weight and (B) shell length-dry tissue weight relationships of *P. malabarica* at Nerul.

Table 4.1: Summary of statistical analysis of allometric data on *Paphia malabarica* collected from Chicalim (P<0.0001).

Sl. No.	x	y	a	b	SE of b	Regression equation	Parabolic equation	r	Significance
***Chicalim*, D.F. (Degree of freedom) = 1869**									
1	Shell length	Shell breadth	- 0.5797	0.7326	0.0053	y = -0.5797 + 0.7326 x		0.9531	***
2	Shell length	Shell depth	- 1.5972	0.5552	0.0053	y = -1.5972 + 0.5552 log x		0.9220	***
3	Shell length	Total weight	- 3.7153	3.0604	0.0236	Log y = -3.7153 + 3.0604 log x	$Y = 0.0002\ x^{3.0604}$	0.9486	***
4	Shell length	Shell weight	- 3.9089	3.1120	0.0274	Log y = -3.9089 + 3.1120 log x	$Y = 0.0001\ x^{3.1120}$	0.9343	***
5	Shell length	Wet tissue weight	- 3.5904	2.4426	0.0262	Log y = -3.5904 + 2.4426 log x	$Y = 0.0003\ x^{2.4426}$	0.9069	***
6	Shell length	Dry tissue weight	- 3.9356	2.161	0.0436	Log y = -3.9356 + 2.161 log x	$Y = 0.0001\ x^{2.161}$	0.7534	***
7	Shell breadth	Total weight	- 2.9917	2.8527	0.0223	Log y = -2.9917 + 2.8527 log x	$Y = 0.001\ x^{2.8527}$	0.9472	***
8	Shell breadth	Wet tissue weight	- 2.9454	2.2246	0.0270	Log y = -2.9454 + 2.2246 log x	$Y = 0.0011\ x^{2.2246}$	0.8848	***
9	Shell depth	Total weight	- 1.8402	2.2346	0.0244	Log y = -1.8402 + 2.2346 log x	$Y = 0.0144\ x^{2.2346}$	0.9040	***
10	Shell depth	Wet tissue weight	- 1.9784	1.6817	0.0276	Log y = -1.9784 + 1.6817 log x	$Y = 0.0105\ x^{1.6817}$	0.8149	***

Table 4.2: Summary of statistical analysis of allometric data on *Paphia malabarica* collected from Nerul (P<0.0001).

Sl. No.	x	y	a	b	SE of b	Regression equation	Parabolic equation	r	Significance
***Nerul,* D.F. (Degree of freedom) = 1336**									
1	Shell length	Shell breadth	- 1.4414	0.7594	0.0088	y = -1.4414 + 0.7594 x		0.9192	***
2	Shell length	Shell depth	- 1.9859	0.5761	0.0086	y = -1.9859 + 0.5761 x		0.8774	***
3	Shell length	Total weight	- 3.7982	3.1374	0.0357	Log y = -3.7982 + 3.1374 log x	$Y = 0.0002\ x^{3.1374}$	0.9232	***
4	Shell length	Shell weight	- 3.9658	3.1825	0.0429	Log y = -3.9658 + 3.1825 log x	$Y = 0.0001\ x^{3.1825}$	0.8969	***
5	Shell length	Wet tissue weight	- 4.4856	3.0584	0.0357	Log y = -4.4856 + 3.0584 log x	$Y = 0.0000\ x^{3.0584}$	0.9123	***
6	Shell length	Dry tissue weight	- 5.4429	3.1967	0.0553	Log y = -5.4429 + 3.1967 log x	$Y = 0.0000\ x^{3.1967}$	0.8448	***
7	Shell breadth	Total weight	- 2.7807	2.7246	0.0315	Log y = -2.7807 + 2.7246 log x	$Y = 0.0017\ x^{2.7246}$	0.9209	***
8	Shell breadth	Wet tissue weight	- 3.372	2.5647	0.0381	Log y = -3.372 + 2.5647 log x	$Y = 0.0004\ x^{2.5647}$	0.8788	***
9	Shell depth	Total weight	- 2.2074	2.5758	0.0196	Log y = -2.2074 + 2.5758 log x	$Y = 0.0062\ x^{2.5758}$	0.9633	***
10	Shell depth	Wet tissue weight	- 2.5954	2.2251	0.0387	Log y = -2.5954 + 2.2251 log x	$Y = 0.0025\ x^{2.2251}$	0.8435	***

Table 4.3: Morphometric and length-weight relationships b values of marine bivalves.

Species	L-B	L-D	L-TW	L-WW	L-DW	Location	Source
Donax incarnatus	0.6754	0.3821				Panambur beach, Manglore	Thippeswamy &Joseph, 1992
D.semistriatus			2.442			Gulf of Tunis coasts	Charef et al., 2011
Glycymeris violacescens			2.195			Gulf of Tunis coasts	Charef et al., 2011
Mactra stultorum			2.939			Gulf of Tunis coasts	Charef et al., 2011
Solen marginatus			2.45			Gulf of Tunis coasts	Charef et al., 2011
Tellina nitida			1.48			Gulf of Tunis coasts	Charef et al., 2011
Tellina planata			0.827			Gulf of Tunis coasts	Charef et al., 2011
Perna viridis	0.39	0.32	2.95	2.72	2.93	St. Mary's Islands	Hemachandra & Thippeswamy, 2008
Anomia ephippium			3.097			Algarve coast, Southern Portugal	Gaspar et al., 2001
Acanthocardia aculeate			3.423			Algarve coast, Southern Portugal	Gaspar et al., 2001
D. trunculus			2.572			Algarve coast, Southern Portugal	Gaspar et al., 2001
Mactra glauca			3.450			Algarve coast, Southern Portugal	Gaspar et al., 2001
Pandora albida			2.761			Algarve coast, Southern Portugal	Gaspar et al., 2001
Venus fasciata			2.626			Algarve coast, Southern Portugal	Gaspar et al., 2001
Crassostrea madrasensis			2.0670	2.0757		West coast, Goa	Nagi, 2008
C. gryphoides			1.4655	1.2439		West coast, Goa	Nagi, 2008
P. malabarica						Chicalim, West coast, Goa	Present study
P. malabarica						Nerul, West coast, Goa	Present study

BIOCHEMICAL COMPOSITION **Chapter-V**

5.1 Introduction

Qualities and quantities of biochemical constituents in a particular biota provide information on its nutritional value and health status (Rattan, 1994; Norziah and Ching, 2000; Chakraborty and Santra, 2008; Laxmilatha, 2009; Gopalakrishnan and Vijayavel, 2009; Ersoy and Şereflişan, 2010; Gressler *et al*., 2011). Generally proximate or percentage biochemical composition means quantum of five basic constituents namely protein, carbohydrate, lipid, ash and water. It varies widely within the same individual depending on several factors like species, size, sex, maturity, season and feeding regimes (Xavier, 1996; Ajaya, 2002). Proteins are fundamental macro biomolecules in all aspects of cell structure and function, while carbohydrates are major sources of energy in all living matters. Human diet requires nutritious source rich in protein as well as other constituents. An increasing demand for good quality of animal protein for the exploding human population has led to effective and increasing exploitation of the aquatic resources (Babu *et al*., 2010). For bivalves condition index (CI) is considered as a standard criterion to select the best product and also serves as a useful biomarker reflecting the ability of bivalves to withstand natural and anthropogenic stress (Bressan and Marin, 1985). Biochemical composition, physiological condition and PE (percentage edibility) of an organism generally exhibit seasonal variations and governed by various ecological and environmental elements in ambience (Saraswathy and Nair, 1969; Stephen, 1980b). The seasonal variation in energy metabolism necessary for growth and reproduction shows latitudinal control in addition to water temperature and food availability (Ojea *et al*., 2004).

In a living organism, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major forms of free radicals formed during normal metabolic activities and their overproduction may leads to debilitating oxidative stress and cell death (Halliwell and Gutteridge, 1999). To cope up with such adverse effects living organism utilizes self antioxidant defense systems. However, some of the essential antioxidants are also need to be supplied through external diet. In context to the same, various products used for consumption are being continuously evaluated using in vitro antioxidant determination methods. The most commonly used 2,2-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging has been found to have direct correlation with free radical scavenging, whereas, reducing power and ferric reducing antioxidant power (FRAP) activities potentially equates total antioxidant capacities and presence of related bioactive compounds (Henriquez *et al*., 2011). Moreover, evaluating hydroxyl radical scavenging and inhibition of lipid peroxidation helps to overcome the deteriorative effects on cell membrane damages and food quality (Lee and Hendricks, 1995).

Applications of naturally occurring antioxidants in food or medicinal materials have been increased significantly as compared to that of synthetic antioxidants (Ito *et al*., 1983). The antioxidant properties of food material like spices, herbs, hulls (Kohchi, 1995), wheat, alfalfa (Boveris and Puntarulo, 1998), and meat samples (Nair and Latha, 2005) as well as some of the industrial byproducts like Kraft black liquor (Perez-Perez and Rodriguez-Malaver, 2005) and wine (Campos and Lissi, 1996) have been studied. However, a few nutritional compounds like Glycoprotein (Xiu-Ping *et al*., 2008a) and Glycosaminoglycans (Xiu-Ping *et al*., 2008b) have been isolated from bivalves to study its free radical scavenging activities.

Paphia malabarica, though commonly consumed in coastal region of India (Jones and Alagarswami, 1973), very limited studies have been attempted to evaluate its nutritional potential (Appukuttan and Aravindan, 1995). Since *P. malabarica* clams are staple food for a large section of population along west coast of India and particularly in Goa, efforts were made to understand its nutritive value and antioxidant properties. The results described below would be of great help for sustainable management of such natural resources.

5.2 Results

5.2.1 Biochemical constituents

Water contents

The water content in *P. malabarica* was significantly ($p<0.001$) higher in Chicalim samples (ranging from 77.9 ± 6.19 to 87.5 ± 1.74%) compared to those (Figure 5.1A; Table 5.1) from Nerul (ranging from 78.5 ± 2.97 to 85.8 ± 2.94%). Tukey's post hoc test confirmed significantly high water content (87.5 ± 1.74%) in the month of September 2009 in organisms collected from Chicalim and low (77.9 ± 6.19%) in the month of January 2010. Nerul exhibited the lowest value (78.5 ± 2.97%) of water content in the month of May 2009, whereas highest value (85.8 ± 2.94%) was found during July 2009 (Figure 5.1A).

Protein contents

The protein content varied significantly ($p<0.001$) in the range of 15.6 ± 3.47% to 83.8 ± 9.41% in organism from Chicalim and 15.8 ± 1.69% to 86.8 ± 4.16% in organism from Nerul (Figure 5.1B). Tukey's post hoc test showed significantly low content (15.6 ± 3.47%) of protein during September 2009, while it was highest (83.8

± 9.41%) during March 2010 in organism from Chicalim (Table 5.1). High content (86.8 ± 4.16%) of protein in *P. malabarica* was observed in the month of March, 2010 and low value (15.8 ± 1.69%) in September 2009 at Nerul.

Carbohydrate contents

Total carbohydrates content ranged between 7.4 ± 1.5% to 72.7 ± 0.51% with highest value (72.7 ± 0.51%) recorded during September 2009 and the lowest (7.4 ± 1.5%) during March 2010 (Figure 5.1C; Table 5.1) in organism from Chicalim. A steady increase in carbohydrate was recorded from March till September 2009 and declined thereafter (Figure 5.1C). Highest value (49.4 ± 1.1%) of carbohydrate was recorded in September 2009, whereas, lower values (4.3 ± 0.1%) were recorded in the month of March 2010 in organism from Nerul. Post hoc test showed significantly greater values of carbohydrate for organisms from Chicalim with annual average of 34.3 ± 20.05% while in clams from Nerul have an average of 22.9 ± 10.82% (Table 5.1).

Lipid contents

The lipid contents of *P. malabarica* from Nerul (range 2.6 ± 0.18 to 4.7 ± 0.14%) was found to be significantly ($p<0.001$) greater than in the organism of Chicalim (range 2.3 ± 0.13 to 5.2 ± 0.00%) with annual mean value of 3.3 ± 0.68% and 3.1 ± 0.98%, respectively (Figure 5.2A; Table 5.1). Tukey's post hoc test showed significantly high mean value (5.2 ± 0.00%) of lipids in organisms from Chicalim during May 2009 and low (2.3 ± 0.13%) in January 2010. At Nerul, high lipid content (4.7 ± 0.14%) was observed during May 2009 and low (2.6 ± 0.18%) in the month of September 2009.

Ash content

Ash content in *P. malabarica* from Chicalim was maximum (17.1 ± 0.07%) during November 2009 and minimum (4.7 ± 0.01%) in September 2009. Maximum (15 ± 0.06%) ash content was observed in the month of November, December 2009 and minimum (3.8 ± 0.01%) during September 2009 in organism from Nerul (Figure 5.2B).

Calorific value

Calorific value ranged from 2.9 to 6.5 Kcal/g with significantly ($p<0.001$) highest value in February 2010 and lowest during December 2009 in organisms from Chicalim (Figure 5.2C; Table 5.1). Whereas, Nerul sample showed maximum calorific content (5.8 Kcal/g) in May 2009 and minimum (3.0 Kcal/g) in December 2009 (Figure 5.2C).

Condition index (CI)

The average CI of *P. malabarica* varied from 24.0 ± 10.39 to 62.1 ± 7.82 at Chicalim with maximum value (62.1 ± 7.82) in April 2009 and minimum (24.0 ± 10.39) in December 2009. Whereas, organism collected from Nerul ranged from 20.0 ± 5.37 to 47.9 ± 12.16 with highest value (47.9 ± 12.16) in May 2009 and lowest in November 2009 (Figure 5.3A). Organism from Chicalim showed high CI (annual mean 38.6 ± 12.68) compared to organism from Nerul (annual mean 36.9 ± 8.71). Post hoc test confirmed these values significantly ($p<0.001$) higher in organism from Chicalim during April 2009 whereas, significantly ($p<0.001$) lower in organisms collected from Nerul in November 2009 (Table 5.1).

Percentage edibility (PE)

Percentage edibility ranged from 12.5 ± 2.86 to 21.0 ± 3.27 with significantly ($p<0.001$) greater values (21.0 ± 3.27) in May 2009 and lowest (12.5 ± 2.86) during November 2009 in organisms from Chicalim. While PE in organisms from Nerul varied from 11.5 ± 2.32 to 18.7 ± 3.45 with maximum value (18.7 ± 3.45) during May 2009 and minimum (11.5 ± 2.32) during November 2009 (Figure 5.3B; Table 5.1).

5.2.2 Correlation between biochemical parameters and environmental parameters

Salinity showed significant (p <0.001) negative correlation (r = -0.59 at Chicalim and r = -0.69 at Nerul) with water content of *P. malabarica* whereas it was positively correlated (r = 0.51 at Chicalim and r = 0.46 at Nerul) with ash content and (r = 0.43 at Chicalim and r = 0.44 at Nerul) PE. Chl *a* was positively correlated (r = 0.47) with protein in organisms from Chicalim, whereas, positively correlated (r = 0.51 at Chicalim and r = 0.64 at Nerul) with ash content in organism from both stations (Table 5.2). Significant (p <0.001) negative correlation (r= -0.70 at Chicalim and r = -0.83 at Nerul) was recorded between protein and carbohydrate, while protein was positively correlated with (r = 0.64 at Chicalim and r = 0.54 at Nerul) ash content and (r = 0.81 at Chicalim and r = 0.96 at Nerul) calorific content. Lipid contents too were found to be significantly (p <0.001) correlated with (r = 0.36 at Chicalim and r = 0.52 at Nerul) ash and (r = 0.35 at Chicalim and r = 0.40 at Nerul) calorific content as well as with (r = 0.58 at Chicalim and r = 0.44 at Nerul) CI and (r = 0.75 at Chicalim and r = 0.54 at Nerul) PE values (Table 5.2).

5.2.3 Antioxidant concentrations

Data obtained in the present investigation was compared with standard synthetic antioxidant and presented as relative activity in case of DPPH, in vitro inhibition of lipid peroxidation (LPX) and hydroxyl radical scavenging assays. Whereas, reducing powers and FRAP values are expressed in terms of increasing absorbance and µg/ml of AsA equivalents, respectively.

DPPH scavenging

The DPPH scavenging potential was measured by decrease in its absorbance. A significant dose dependent DPPH scavenging was observed (Figure 5.4A; $P < 0.01$) which was higher for standard as compared with sample extract. The relative percent scavenging activity for initial 0.1 ml was 18.72 ± 3.94% followed by 27.68 ± 2.39% and 36.80± 3.18% for 0.2 and 0.3 ml of sample respectively, as compared to that of standard compound (BHT) used. Similarly the significant correlation ($r = 0.952$; $P < 0.01$) was observed between DPPH radicals and reducing action (Table 5.3) by methanolic extracts of *P. malabarica*.

Reducing power

Dose dependency in absorbance values (Table 5.3) was exhibited by methanolic extracts (0.06 ± 0.01 < 0.13 ± 0.02 < 0.17 ± 0.02) in the present study (Figure 5.4B; $P < 0.01$). Correlation between DPPH scavenging potential and reducing action ($r = 0.952$; $P < 0.01$) and LPX and reducing action ($r = 0.843$; $P < 0.001$) was also observed (Table 5.3).

Lipid peroxidation

The inhibition of LPX levels in terms of relative activities were 30.20 ± 14.11% for 0.1ml, 67.82 ± 5.92 for 0.2 ml, 69.3 ± 16.42% for 0.3 ml of samples, respectively. Figure 5.4C indicates approximately similar inhibitory activities for 0.2 and 0.3 ml of sample concentrations. A positive correlation with DPPH radical scavenging ($r = 0.729$; $P < 0.01$) and reducing power ($r = 0.843$; $P < 0.001$) as well as significant inhibition of LPX was observed (Table 5.3) when compared to control (Figure 5.4C; $P < 0.01$).

Hydroxyl scavenging activity

Methanolic extracts of *P. malabarica* also exhibited prominent hydroxyl radical scavenging activities as compared to commercial antioxidants like BHT (Figure 5.5A; $P < 0.01$). The relative scavenging abilities for 0.1 ml, 0.2 ml and 0.3 ml of samples were 24.30 ± 8.38%, 44.27 ± 8.65%, and 56.96 ± 8.44%, respectively. Significant correlation between LPX and ·OH radical scavenging ($r = 0.774$; $P < 0.01$) were also observed (Table 5.3).

FRAP activity

Antioxidant values of the sample extracts were found to be in the order of 1.54 ± 0.021 < 2.8 ± 0.046 < 3.53 ± 0.039 µg/ml of AsA equivalent for 0.1, 0.2 and 0.3 ml, respectively (Figure 5.5B). Increasing FRAP values shows presence of active antioxidant compounds in sample extracts. Correlation of these values with DPPH scavenging potential ($r = 0.880$; $P <0.01$), reducing power ($r = 0.945$; $P <0.01$), LPX ($r = 0.758$; $P <0.01$) and OH radical scavenging ($r = 0.769$; $P <0.01$) were also observed (Table 5.3).

5.3 Discussion

5.3.1 Biochemical composition

The data reveals significant spatio and temporal variations in biochemical composition, CI and PE of *P. malabarica* which could be attributed to various life stages and influence of ambient environment (Ojea *et al*., 2004; Dridi *et al*., 2007). The variations in percentage of tissue water content could be attributed to salinity concentration around bivalve bed (Nagabhushanam and Bidarkar, 1978). Molluscs may lose salts or gain water in to their tissue during unfavorable conditions of salinity (higher or lower concentrations). The high percentage (Figure 5.1A) of tissue water content during monsoon (September 2009) might be due to reduced salinity (Figure 3.2B) of estuarine water leading to more absorption of water in to the tissues i.e. the replacement for salt in tissue (Appukuttan and Aravindan, 1995; Rivonker and Parulekar, 1995; Nagi, 2008; Mohite, 2010). However, during summer, these values were relatively low in the tissue due to higher salinity in ambient water. Mussels adopt an appropriate compensatory mechanism to counteract the increasing salt content during summer season (Parulekar *et al*., 1982). The significant negative correlation ($r = -0.59$ at Chicalim and $r = -0.69$ at Nerul) between water content in tissue of *P. malabarica* and ambient water salinity (Table 5.2) further strengthen the present findings. Such inverse relation was also reported in *P. viridis* and *C. madrasensis* earlier (Rivonker and Parulekar, 1995; Nagi, 2008).

Maximum protein content in *P. malabarica* (Figure 5.1B) during March 2010 (pre-monsoon) could be a mechanism of storage of reserves during gametogenesis to meet energy requirement during spawning season (Qasim *et al*., 1977; Appukuttan and Aravindan, 1995). Other possibility for elevated proteins content could be the

increased feeding efficiency with increased chl *a* during pre-monsoon, resulting in proper assimilation of food and better metabolic condition (Qasim *et al*., 1977). The significant positive correlation (r = 0.47) between protein and chl *a* (Table 5.2) justifies the above views. The low proteins in an organism during September 2009 may be due to the spawning activity and decreased chl *a* value as reported by Wafar *et al*. (1976); Rivonker and Parulekar (1995) and Mohite and Mohite (2009b). Reduced protein contents (Figure 5.1B) during post-spawning period, suggest that much of the energy spent during active spawning period was contributed by protein (Rivonker and Parulekar, 1995). The spawning period of *P. malabarica* was reported to be from September to January (Mohite and Mohite, 2009b), hence the lower values of protein could be attributed to spawning activity.

Lipid contents in an organism are more influenced by the clam's annual reproductive cycle because of their relationship with gonad maturation (Rivonker and Parulekar, 1995; Dridi *et al*., 2007). Lipids also serve as an energy reserve in conditions of either imposed or natural nutritional stress. Higher values of lipid during pre-monsoon could be correlated with high storage of fat before spawning (Venkataraman and Chari, 1951). However, the lower amounts of lipid reported during monsoon may be due to utilization of accumulated lipid for building up of tissue materials (Rivonkar, 1991). It could also be due to initiation of gametogenesis and utilization of energy reserve for development of gametes (Qasim *et al*., 1977; Zandee *et al*., 1980). Lower values of fat content could also be correlated to the low content of chl *a* as seen by inverse relationship (r = -0.03) in the present study.

Carbohydrate concentration profiles show a reverse trend with respect to protein and lipid contents. The same relationship has been observed by Lakshmanan and

Nambisan (1980) in *V. cyprinoids* and *M. casta*. Carbohydrate contents reached minima during March 2010 (pre-monsoon), when protein and lipid contents were higher (Figure 5.1C). The decrease in carbohydrate content during pre-monsoon period could be due to unfavourable conditions causing stress to an organism. Bayne (1973) reported that, stress in bivalves results in utilization of carbohydrate reserves and a decline in the rate of excretion of ammonia-N. Carbohydrate serves as an index of high glycogen metabolism during the period of high environmental stress (Parulekar *et al*., 1982). High values of carbohydrates and low values of protein and lipids during monsoon indicate accumulation of carbohydrates in the tissue and greater reliance of organism on proteins and lipids. It has been reported that, in mussel *M. edulis*, the food shortage between January and April caused a significant loss of protein (75%) and some of lipid reserves (15%), while carbohydrate contributed 10% of the total energy loss (Dare and Edwards, 1975). During spawning season, energy requirements have been reported (Rivonkar, 1991; Rodriguez-Astudillo *et al*., 2005) to be met by proteins and lipid to a greater extent as compared to carbohydrates. This is because gonads consist mainly of protein and fat in bivalves (Pieters *et al*., 1980). Marked changes observed in carbohydrate content of *P. malabarica* during present investigations may be the result of the processes mentioned above.

The increased ash content during pre and post monsoon period (Figure 5.2B) coincided with increase in protein content. It was inferred that the increase ash content might possibly be due to an increased inorganic content in the body constituents (Mohite, 2010; Singh *et al*., 2012), which could be confirmed by highly significant correlation of ash content with the protein (r = 0.64 at Chicalim and r = 0.54 at Nerul) and (r = 0.36 at Chicalim and r = 0.52 at Nerul) lipid contents (Table 5.2). Further, the lower value of ash content during monsoon period was correlated (r = 0.51 at

Chicalim and r = 0.64 at Nerul) with low content of chl *a* and (r = 0.51 at Chicalim and r = 0.46 at Nerul) lower salinity. The calorific value of *P. malabarica* is comparable with other edible bivalves (Rivonker and Parulekar, 1995; Appukuttan and Aravindan, 1995; Mohite, 2010). In general, energy stored prior to gametogenesis is utilised in the production of gametes, when metabolic demand remains high (Appukuttan and Aravindan, 1995). *Paphia malabarica* too showed the high energy storage during pre-monsoon (Figure 5.2C) as noticed by high protein and lipid values. Higher and lower calorific values were related to proteins and lipids content (Table 5.2), which directly influenced the energy content (Rivonker and Parulekar, 1995).

The environmental conditions appear to influence certain biological processes within a population so that the reproductive cycle usually becomes tuned to local seasonal conditions (Walter, 1982). Values of CI and PE during pre-monsoon commenced with the maturation stages (Mohite, 2010) while low values during post-monsoon (Figure 5.3 A,B) could be attributed to spawning season (Appukuttan and Aravindan, 1995). These values of clam are considered as very important criteria for bivalve producers, and the CI in particular, gives an indication of the general physiological status of the animals (Marin *et al*., 2003). Condition index values observed were >14, indicative of high meat quality of *P. malabarica*. Similar pattern in CI and PE values of *P. malabarica* from Ashtamudi estuary was reported by Appukuttan and Aravindan (1995). However, these values were slightly higher than those reported in *P. malabarica* from Kajali and Kalbadevi estuary (Ratnagiri) and Mulki estuary (Mohite *et al*., 2009; Rao, 1988). The correlations of CI and PE with lipid contents (Table 5.2) suggest that the energetic variations in CI and PE in *P. malabarica* may be related to variations in lipid content produced by gamete emission and gamete maturation (Morriconi *et al*., 2002). In other words, rise in protein and lipid concentrations results

in an increase in CI and PE. The present findings match well with studies carried out on other edible bivalves (Rivonker and Parulekar, 1995; Nagi, 2008; Appukuttan and Aravindan, 1995 and Mohite *et al*., 2009).

5.3.2 Antioxidant properties

The exponential decrease (Figure 5.4A) in the absorbance of DPPH solution caused by test samples may be due to its reducing capability. Some of the fat-soluble antioxidant compounds from animal tissue have been reported for their efficient solubility in methanol (Ekanayake *et al*., 2004). Lipophilic (fatty acid) antioxidants from muscle tissue of *Tapes decussatus* showed prominent antioxidant properties (Passi *et al*., 2002). Recent findings on Indian bivalves such as *Perna viridis, Crassostrea spp., Placuna placenta* and *Polymesoda erosa* revealed their potency towards radical scavenging activities (Shenai-Tirodkar *et al*., 2012). Moreover, antioxidant peptides from *M. casta* (Nazeer et al., DOI 10.1007/s13197-011-0395-z), Glycoprotein (Xiu-Ping *et al*., 2008a) and Glycosaminoglycans (Xiu-Ping *et al*., 2008b) from *Paphia undulate* were shown to have radical scavenging activity. Radical scavenging effects observed in methanolic extract of *P. malabarica* might be due to the presence of similar types of antioxidant pepetides.

Compounds, exhibiting an increase in absorbance, can be considered as iron reductants and such reducing action serves as a significant indicator of potential antioxidant activity (Meir *et al*., 1995; Pouvreau *et al*., 2008). The exponential increase in reducing potential observed in *P. malabarica* could be due to the donation of electrons by active compounds of sample extracts (Figure 5.4B). Methanolic extracts of other bivalve species from India have shown similar type of trend in their reducing abilities (Jena *et al*., 2010; Shenai-Tirodkar *et al*., 2012). Moreover, different types of reactions such as binding of transition metal ion catalysts, decomposition of

peroxides and prevention of continued hydrogen abstraction are also known to take part in reducing actions (Yildirim *et al*., 2001). Thus, the overall reducing activitiy (Figure 5.4B) might be due to the quenching effect of reductants of *P. malabarica* which might have possibly extracted in methanolic extracts.

In vitro studies on guinea pig aorta cell cultures showed that α-tocopherol has inhibitory activities on LPX reactions and supportive role in cell proliferation (Victor *et al*., 1981). Protein, carbohydrate and vitamin contents of the bivalves, *P. viridis, Donax cuneatus*, *Meretrix meretrix* (Gopalakrishnan and Vijayavel, 2009) and *P. malabarica* (Raghavan *et al*., 2009) have been attributed to various nutritional and bioactive properties. Such biomolecules may take part in the inhibiting reactions of lipid peroxidation. A positive correlation (r = 0.95) with DPPH radical scavenging and reducing power as well as significant inhibition of LPX (Figure 5.4C) emphasize role of *P. malabarica* extracts in inhibiting chain reactions of lipid peroxidation. In addition, xenobiotics produced by carbohydrate detoxification reactions, organochlorinated compounds and trace metals are able to generate ROS and free radicals in vivo which consequently cause induction of lipid peroxidation and oxidative stress (Doyotte *et al*., 1997).

Hydroxyl radical is one of the most commonly involved ROS in biological damages of lipids, proteins and DNA (Spencer *et al*., 1994). In vivo, it may be produced from irradiation (X-ray) or from H_2O_2 in metal catalyzed reactions. Further due to its high reactivity, it acts on surrounding target molecules which leads to chain reactions e.g. initiation of lipid peroxidation due to abstraction of hydrogen atoms from (PUFA) polyunsaturated fatty acids (Cheeseman and Slater, 1993; Aruoma, 1998). Increasingly scavenging effects on hydroxyl radicals in methanolic extract of *P. malabarica* indicates potential biomolecule in *P. malabarica* like in other bivalves

(Xiu-Ping *et al*., 2008a,b) which could prevent degradation of PUFAs. It is well known that food rich in fat content is protected by natural antioxidants. Conjugated linoleic acid and phospholipids possess antioxidant potential (Saito and Ishihara, 1997); however, similar kind of fatty compound in *P. malabarica* may be one of the important factors involved in antioxidant potential. However, significant correlation (r = 0.77) between LPX and ·OH radical scavenging in extracts of *P. malabarica* supports inhibitory activities on LPX induced due to reactive oxygen species (ROS). Prominent hydroxyl scavenging in various types of bivalve species (Jena *et al*., 2010; Shenai-Tirodkar *et al*., 2012) further confirmed potential role of *P. malabarica* extract in deterring ROS induced free radical reactions.

The increasingly exponential values of FRAP measurement represents higher content of antioxidants in the sample extract (Smet *et al*., 2006). Carotenoproteins expressed by cloned bivalve species possess significant FRAP or total antioxidant capacity values which also help for in vivo stabilization of free radicals (Zheng *et al*., 2012). Amino acids, oligosaccharides and unsaturated fatty acids of aqueous and alcoholic extracts from clam *Mactra veneriformis*, (widely consumed as a delicious food in China and also used in traditional Chinese medicine) have contributed significant antioxidant activities (Luan *et al*., 2011). Synergistic effects of carotenoids, vitamins and minerals are also found to impart antioxidant properties to biological substances (Ratnam *et al*., 2006). One or more of these types of compounds might have contributed for antioxidant properties in *P. malabarica*.

In addition to the efficient in vitro radical scavenging activities, bivalve species are also found to exhibit in vivo antioxidant responses when exposed to natural or artificial stressors (Conners, 2004). These determinations heavily rely (Conners, 2004) on surrogate measurements of elevated antioxidant defenses (e.g. enzymes) and

indicators of oxidative damage (e.g. DNA strand breaks). Studies on bivalves like *Mytilus galloprovincialis* (Cavaletto *et al*., 2002), *Laternula elliptica* (Park *et al*., 2008) and *Scrobicularia plana* (Ahmad *et al*., 2011) challenged with either chemicals, temperature or metals shown to express elevated levels of antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione (GSH). The enzymatic reactions between free radicals and antioxidative enzymes results in conversion of ROS to the less harmful or reactive forms and helps to prevent cellular damages. Despite the involvement of various cellular (in vivo) defense pathways against ROS, the biochemical data (in vitro) obtained, further strengthens protective role of *P. malabarica* against free radicals. It demonstrates the importance of seafood clam, in terms of radical scavenging, reducing capabilities and inhibition of lipid peroxidation. These findings suggest its role in balancing normal metabolic functions of living organism when supplied through diet. This forms a first comprehensive report on the nutraceutical property of a seafood *P. malabarica* as a natural source of antioxidants.

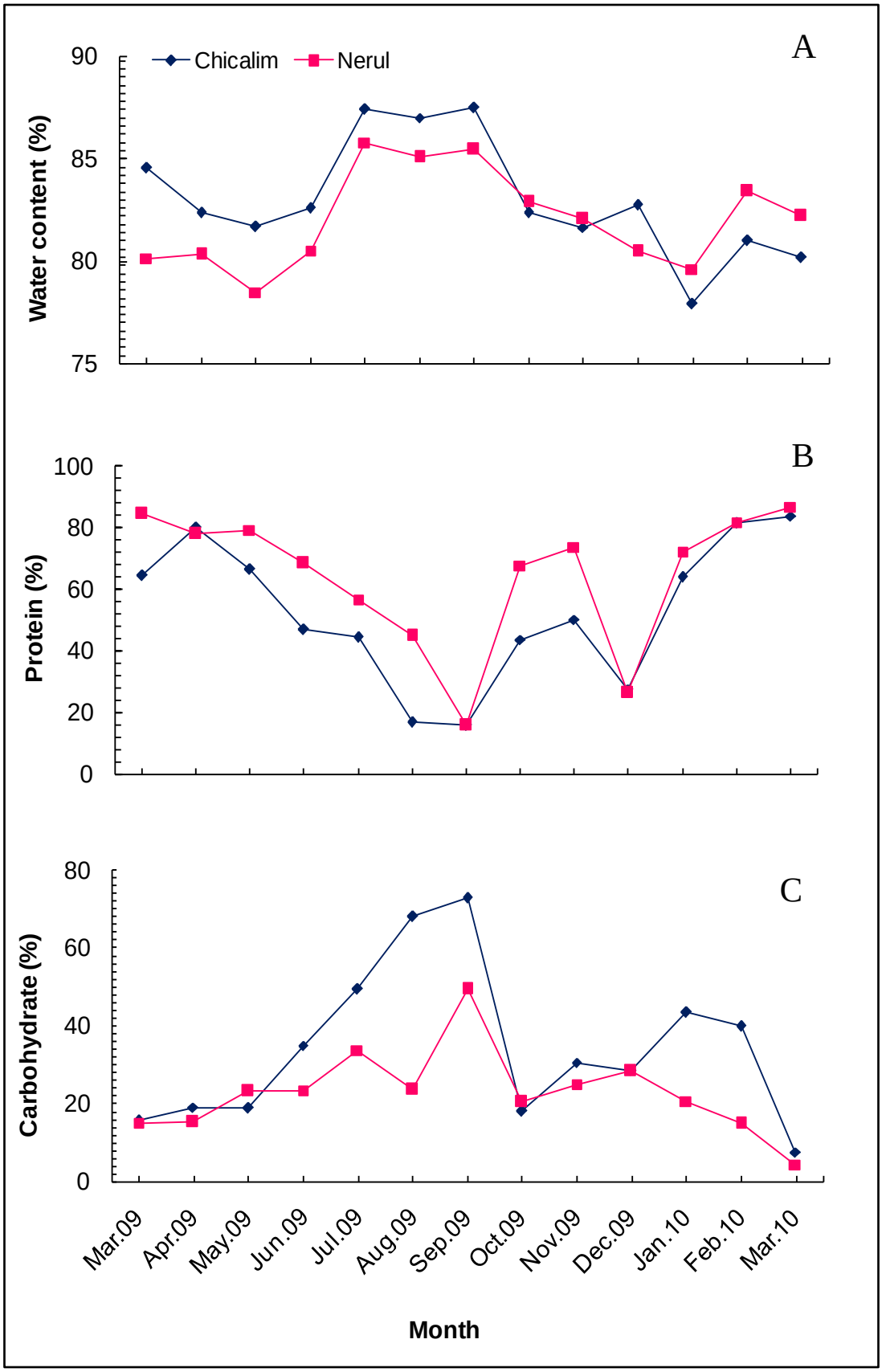


Figure 5.1: Monthly variations in (A) water content (B) Protein (C) Carbohydrate (mean±standard deviation) of *P. malabarica*.

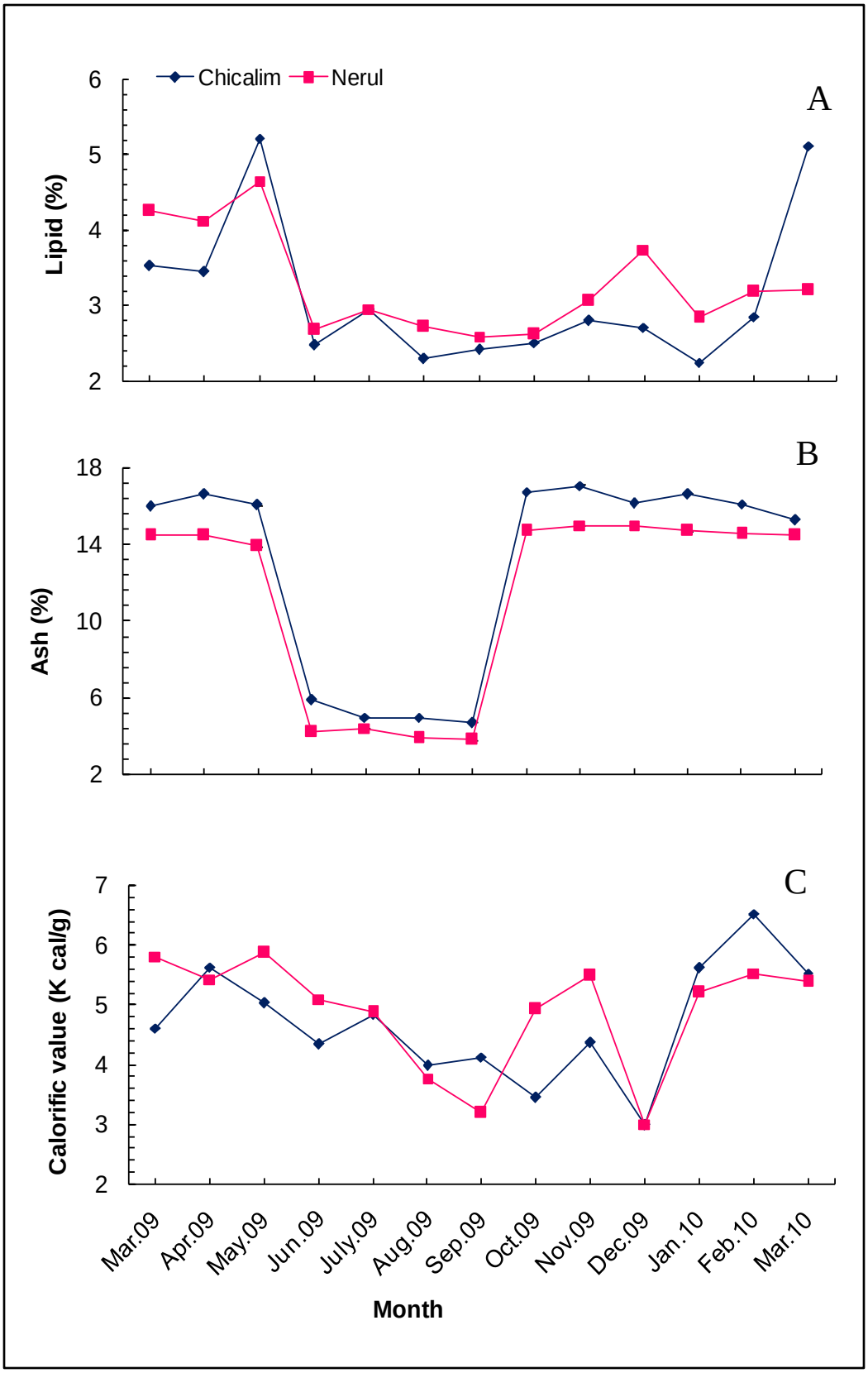


Figure 5.2: Monthly variation in (A) Lipid (B) Ash (C) Calorific value (mean±standard deviation) of *P. malabarica*.

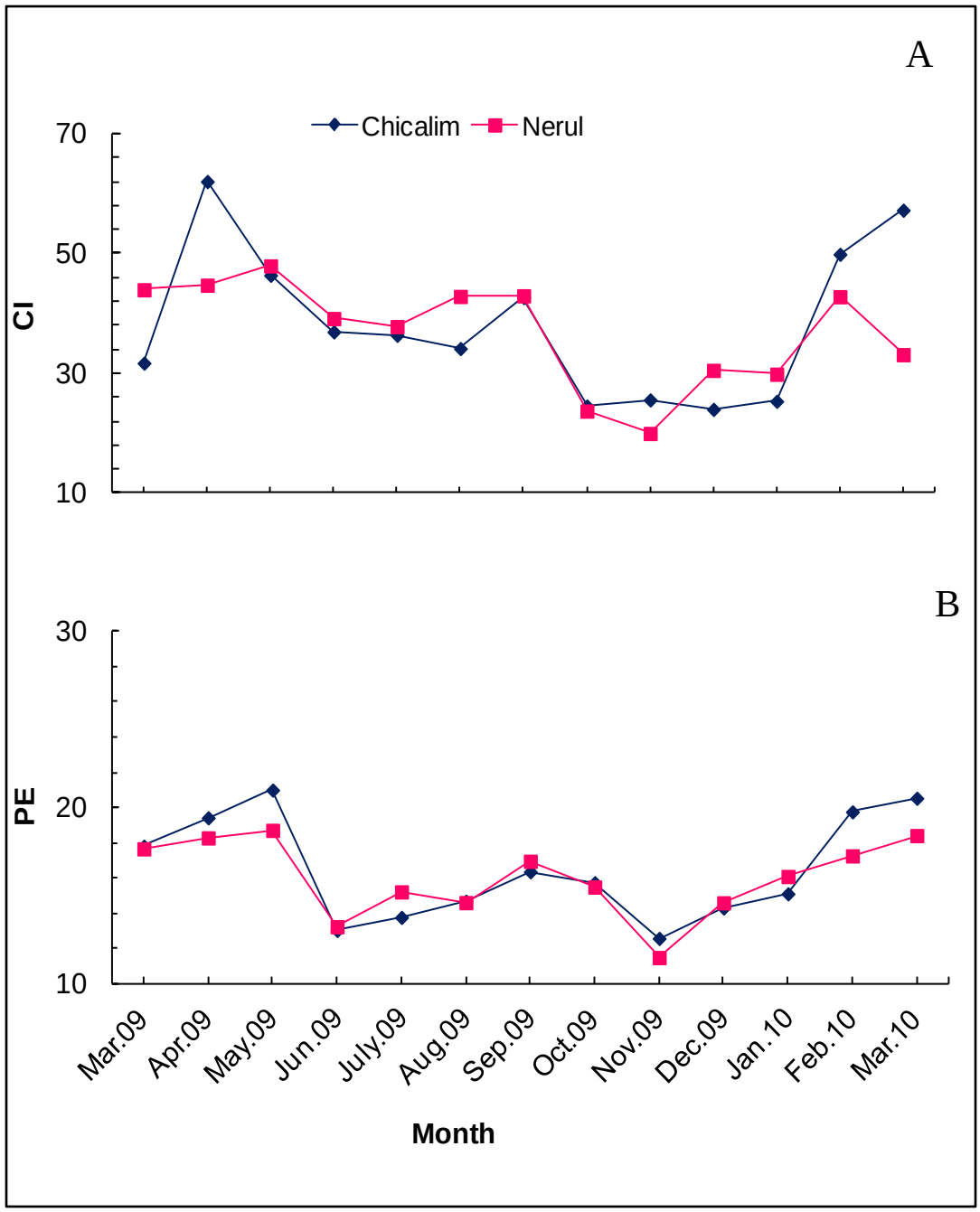


Figure 5.3: Monthly variations in (A) CI and (B) PE values (mean±standard deviation) of *P. malabarica*.

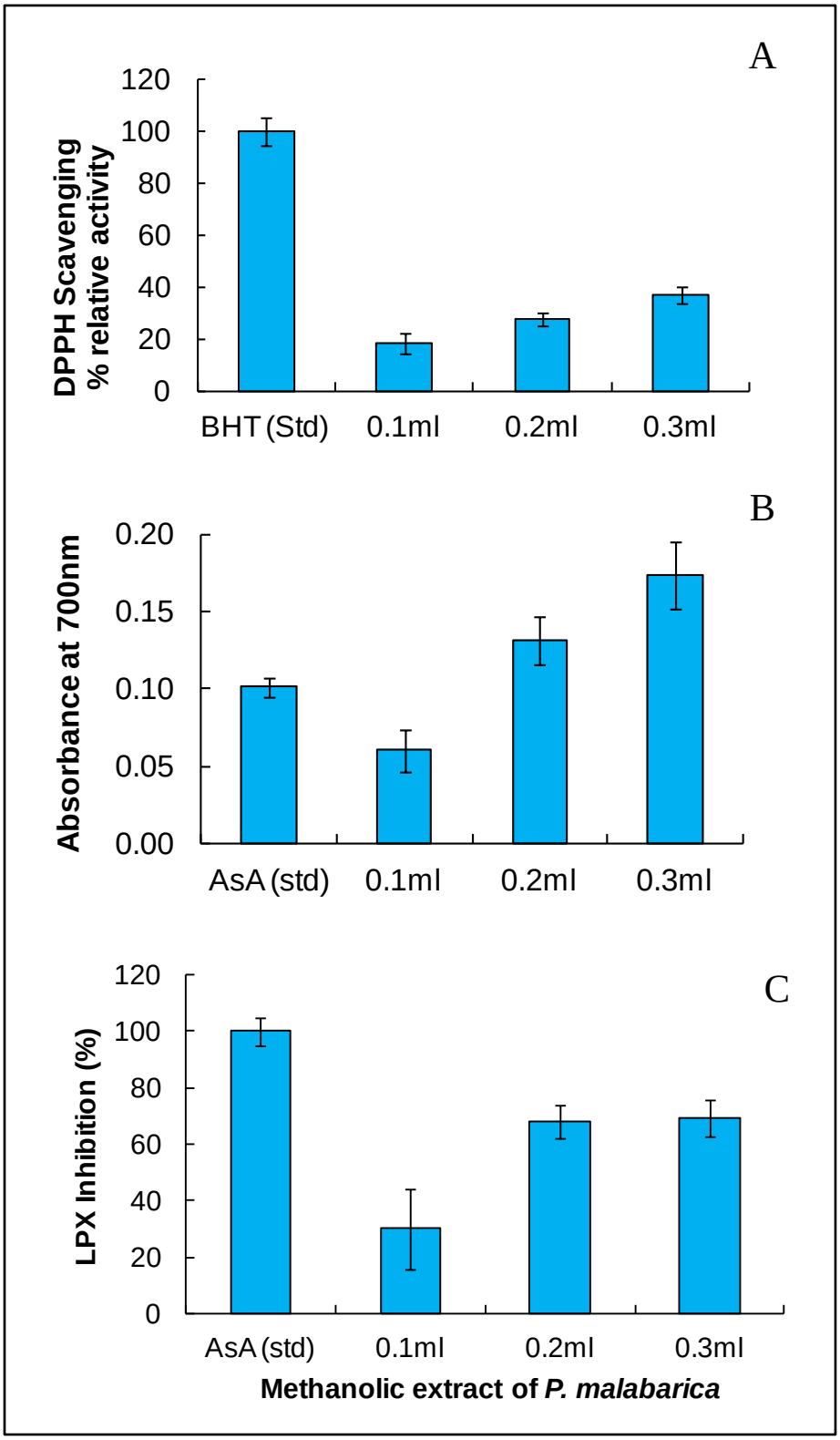


Figure 5.4: (A) DPPH scavenging activity, (B) reducing power and (C) in vitro inhibition of lipid peroxidation of the extract of *P. malabarica*. Values are the means of triplicate determination ± SD (n= 3).

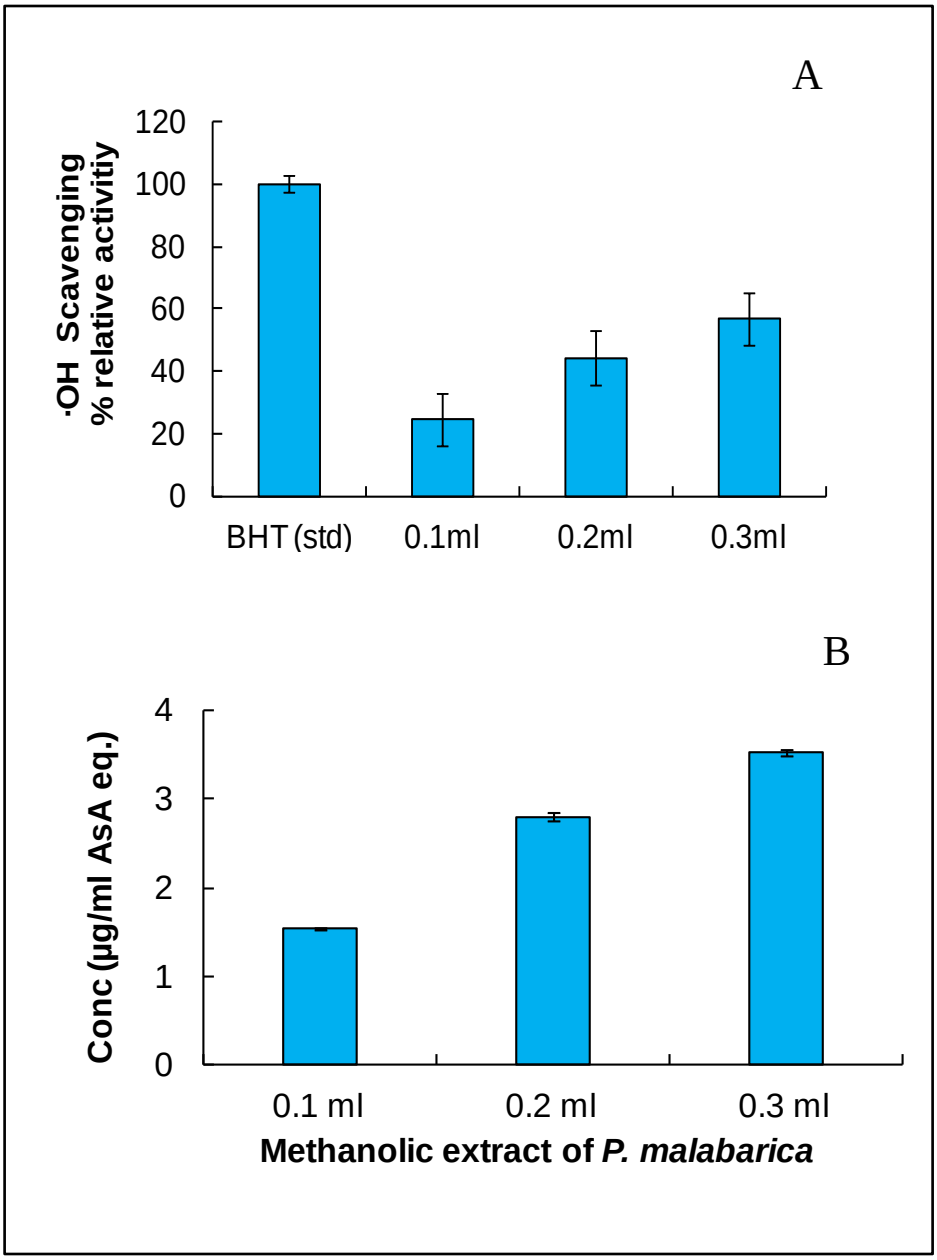


Figure 5.5: (A) Hydroxyl scavenging activity and (B) FRAP activity of the extract of *P. malabarica*. Values are the means of triplicate determination ± SD (n= 3).

Table 5.1: Results of ANOVA per parameters studied in *P. malabarica* from study area.

Parameters	Factor	*df*	MS	F value	*P* value	Post hoc test
Water (%)	Station	1	544.89	33.45	$<10^{-6}$	
	Month	12	86473	53.08	$<10^{-6}$	
	Station-Month	12	269.68	16.55	$<10^{-6}$	Chicalim-Sep
Protein (%)	Station	1	2639.46	30.81	$<10^{-6}$	
	Month	12	2954.82	34.56	$<10^{-6}$	
	Station-Month	12	179.93	2.10	0.032	Nerul-Mar
Carbohydrate (%)	Station	1	2572.63	1859.02	$<10^{-6}$	
	Month	12	1221.92	882.98	$<10^{-6}$	
	Station-Month	12	335.59	242.50	$<10^{-6}$	Chicalim-Sep
Lipid (%)	Station	1	0.47	29.29	$<10^{-7}$	
	Month	12	3.54	218.13	$<10^{-7}$	
	Station-Month	12	0.81	49.84	$<10^{-7}$	Chicalim-May
Calorific (Kcal/g)	Station	1	0.74	2.68	0.10	
	Month	12	5.48	19.80	$<10^{-7}$	
	Station-Month	12	1.25	4.52	5.9×10^{-5}	Chicalim-Feb
Condition index	Station	1	1059.04	5.82	0.01	
	Month	12	17072.53	93.96	$<10^{-7}$	
	Station-Month	12	6358.61	34.99	$<10^{-7}$	Chicalim-Apr
Percentage edibility	Station	1	171.68	17.44	3.04×10^{-5}	
	Month	12	1348.75	137.01	$<10^{-7}$	
	Station-Month	12	82.79	8.41	6.85×10^{-16}	Chicalim-May

df: degree of freedom, MS: mean squares.

Table 5.2: Correlation coefficients (r values) between environmental parameters and biochemical composition, CI, PE of *P. malabarica.*

	Temp	**Salinity**	**Chl. *a***	**Protein**	**Carbohydrate**	**Lipid**	**Ash**	**Calorific value**	**PE**	**CI**	**Water content**
Chicalim											
Protein	0.05	0.40*	0.47*	1							
Carbohydrate	0.00	-0.45**	-0.08	-0.70***	1						
Lipid	-0.24	0.25	-0.03	0.60***	-0.64***	1					
Ash	0.21	0.51**	0.51*	0.64***	-0.76***	0.36*	1				
Calorific value	0.04	0.18	0.56***	0.81***	-0.14	0.35*	0.24	1			
PE	-0.02	0.43*	0.29	0.67***	-0.44	0.75***	0.42*	0.59***	1		
CI	-0.14	0.25	-0.07	0.56***	-0.19	0.58***	0	0.65***	0.76***	1	
Water content	-0.09	-0.59***	-0.65***	-0.68***	0.59*	-0.26	-0.76***	-0.43*	-0.28	-0.05	1
Nerul											
Protein	0.31	0.40*	-0.08	1							
Carbohydrate	-0.09	-0.46**	0.00	-0.83***	1						
Lipid	0.27	0.37*	0.25	0.36*	-0.33	1					
Ash	0.21	0.46**	0.64***	0.54**	-0.62***	0.52**	1				
Calorific value	0.39*	0.34	-0.09	0.96***	-0.65***	0.40*	0.46*	1			
PE	-0.01	0.44*	0.19	0.29	-0.31	0.54**	0.27	0.27	1		
CI	-0.29	0.26	-0.38	0.00	0.06	0.44*	-0.35*	0.06	0.62***	1	
Water content	-0.33	-0.69***	-0.45**	-0.48**	0.46**	-0.66***	-0.63***	-0.46**	-0.21	-0.01	1

*Significance (P<0.05) **Significance (P<0.01) ***Significance (P<0.001).

Table 5.3: Correlation between variables with respect to the antioxidant assays.

Variables	DPPH savenging	Reducing Power	LPX inhibition	Hydroxyl scavenging	FRAP activity
DPPH Savenging	-	-	-	-	-
Reducing Power	$y = 0.0075x - 0.0433$ $R^2 = 0.9069$	-	-	-	-
LPX inhibition	$y = 0.893x + 9.1288$ $R^2 = 0.5317$	$y = 0.0082x + 0.0049$ $R^2 = 0.7108$	-	-	-
Hydroxyl scavenging	$y = 1.243x + 2.0802$ $R^2 = 0.5273$	$y = 167.53x + 8.881$ $R^2 = 0.6011$	$y = 1.6232x + 6.0932$ $R^2 = 0.5995$	-	-
FRAP activity	$y = 0.046x - 0.082$ $R^2 = 0.880$	$y = 6.020x + 0.195$ $R^2 = 0.945$	$y = 0.052x + 0.178$ $R^2 = 0.758$	$y = 0.025x + 0.189$ $R^2 = 0.769$	-

Values are given with linear regression equation and R^2 values respectively, while interpreted with correlation value (r) in the text.

METAL CONCENTRATION Chapter-VI

6.1 Introduction

Various fresh and marine water bodies are of great ecological and socioeconomic values (Jones, 1999; Carballo *et al*., 2008; Kulkarni *et al*., 2011). Being located in the low lying regions serves as sink for organic and inorganic substances originated from hinterlands of natural and anthropogenic origin as well as from the marine seas and oceans. Trace metal concentrations in any aquatic habitat are of critical concern, owing to their toxicity and accumulation in its various components of abiotic and biotic constituents (Islam and Tanaka, 2004; Barua *et al*., 2011). Marine ecosystems receive metal contaminants through atmospheric and anthropogenic pathways (Sen Gupta and Qasim, 2001). Discharges carrying different trace metals from industries and municipalities find their way into various aquatic systems before eventually reaching the marine environment. Freshwater aquatic bodies transport trace metals to the oceans in dissolved, colloidal and particulate forms (George, 1989). Coastal habitats including estuaries, mangroves, saltmarshes and seagrass beds serve as natural reservoirs for various terrigenous materials arising from natural and anthropogenic processes. Subsequently, these regions remain constantly under risk from various types of contaminations. Number of aquatic organisms, particularly edible ones, contains elevated concentrations of trace metals due to the contamination of their ambient environment. Bivalves, among molluscs are considered to be the sentinel organisms for studying and monitoring metal pollution in marine environments (Phillips, 1977a; Popham, 1980). Due to their bio-accumulative properties, they play a key role as indicator organisms in pollution monitoring programs (Phillips, 1976a; 1976b; Talbot, 1987; Lannig *et al.*, 2006).

Marine sediments serve as a source of toxic contaminants in marine food chains. However, the bioavailability of various metals from sediments to marine invertebrates is largely unknown. Information on assimilation of metal from ingested contaminated sediment is essential to assess the potential importance of sediment as a source of metal for marine organisms. The presence of different types of binding sites on sediment particle makes it difficult to predict the bioavailability of particle bound metal (Luoma, 1989). Also, the gut conditions of organisms such as Eh, pH, enzyme types and surfactant activity in the digestive processes are known to affect the release of ingested metals in the gut and subsequent accumulation in the tissue (Griscom *et al*., 2002).

Paphia malabarica may be used to monitor the environmental changes and hence forms a very good tool in studying environmental health and pollution (Wilfred and Abdul, 1994; Modassir and Ansari, 2000; kumari *et al.*, 2006). The hinterlands of Goa have rich deposits of iron ore, which have been intensively mined in the past five decades. Mining (Iron ore, manganese, bauxite, high magnesia, limestone and clay) forms the major activity in the economic history of modern Goa that brings significant foreign revenue for the state. The mining belt of Goa covers approximately 700 sq. km and is mostly concentrated in four talukas namely, Bicholim of North Goa district and Salcete, Sanguem and Quepem of South Goa district. The maximum area under mining is in Sanguem taluka followed by Bicholim, Sattari and Quepem. The iron ore industry forms the main industry of mining with annual production of around 15 million tones and are mainly exported to Japan, European countries, China and South Korea through the Mormugao harbor. However, in the process removal of these iron ore about 40 to 50 million of mining waste is generated. Such a huge quantity of mining waste creates a problem for its storage thereby causing severe environmental

pollution. Mining and associated activities have greatly affected the natural landscape in an around areas, which is characterized by the presence of pits and waste rejects. The damage occurs mostly during monsoon where the rain water carries the washed out material from the waste dumps to the adjoining low-lying agricultural field and water streams. The major estuarine complex (Mandovi-Cumbarjua canal-Zuari) is intensively used for transporting iron ore to the loading centers or ships anchored at harbour (Marmagao) and offshore. As a result the estuarine habitats of Goa and its surrounding regions remain under great threat from mining activities. It is therefore, necessary to evaluate the health status of edible organisms to understand their suitability for consumption. It is therefore, attempts have been made to evaluate concentrations of selected metals (Fe, Mn, Cd, Pb and Zn) in tissues of *Paphia malabarica*, and the bioavailability of sediment bound metal in gut of the organisms. *Paphia malabarica* are filter feeders. They feed by straining suspended matter and food particles from water, typically by passing the water over a specialized filtering structure. Particulate ingested by the clams are digested at a gut pH of ~ 5 to 6 (Owen, 1966) indicating acidic nature of gut could be of help in processing the food particles at the initial digestive process. In order to understand the extent of metal leaching (extraction of minerals) from the ingested sediment particulates in the gut, leaching experiments with sediment aliquots were carried out at pH of 5. The leaching was carried out using 10^{-5} N HNO_3. Since enzymatic leaching using bivalve gut enzyme (eg. amino acid) was beyond the scope of the study. The piece of work forms the baseline for the sustainable management of commercial bivalves in Goa. Further the study may be applicable to other such coastal habitats around the tropics.

6.2 Results

6.2.1 Metal concentrations in Tissues

Iron (Fe)

Fe concentrations from Chicalim ranged from 242.7 ± 1.4 to 1316 ± 6.2 ppm (avg: 570.2 ± 3.6 ppm). Whereas Fe concentration ranged from 14.6 ± 1.2 to 1547 ± 1.4 ppm (avg: 495 ± 5.2 ppm) in organism from Nerul. The peak Fe concentrations at both stations were observed during the period of July 2009 to December 2009 (Figure 6.1A). Peak concentrations at Chicalim were measured in August and November 2009, whereas, at Nerul, the highest Fe concentration recorded in September 2009. In contrast, the Fe concentrations in organisms from both followed similar trend during March 2009 to June 2009 and December 2009 to March 2010 (Figure 6.1A).

Manganese (Mn)

Manganese concentrations in organism from Chicalim varied from 28.8 ± 0.04 to 189.3 ± 0.16 ppm (avg: 84.2 ± 0.56 ppm) while concentration in organism from Nerul ranged from 9.1 ± 0.01 to 197.9 ± 3.2 ppm (avg: 69.8 ± 0.82 ppm). Peak Mn concentrations were recorded from July 2009 to December 2009 in tissue of *P. malabarica* from the both stations (Figure 6.1B). Chicalim showed peak concentration of Mn in August and November 2009, whereas, maximum concentration was observed in September 2009 in organisms from Nerul (Figure 6.1B). The Minimum concentration of Mn was recorded in July 2009 (28.7 ± 0.04 ppm at Chicalim and 9.1 ± 0.01 ppm at Nerul) in organism from the both stations.

Cadmium (Cd)

Cadmium concentrations in organism from Chicalim ranged from 0.82 ± 0.03 to 4.2 ± 0.16 ppm (avg: 1.8 ± 0.08 ppm) and showed a maxima in March and August 2009 (Figure 6.1C). At Nerul, Cd concentration in *P. malabarica* ranged from 0.84 ± 0.04 to 7.1 ± 0.01 ppm (avg: 1.9 ± 1.63 ppm). The maxima (7.1 ± 0.01 ppm) in Cd concentration were noted in September 2009 and minima (0.84 ± 0.04 ppm) in March, 2009 in organism from Nerul. Relative to Nerul, organism from Chicalim showed Cd enrichment from March to August 2009 and depletion from September 2009 to March 2010 (Figure 6.1C).

Lead (Pb)

Lead concentration from Chicalim ranged from 3.1 ± 0.02 to 18.9 ± 0.01 ppm (avg: 7.5 ± 0.07 ppm), whereas concentration in organism from Nerul varied from 2.8 ± 0.03 to 64.4 ± 0.45 ppm (avg: 12.1 ± 0.11 ppm). Chicalim showed peak Pb concentration in *P. malabarica* during March, May and August 2009 while minimum (3.1 ± 0.02 ppm) in January, 2010 (Figure 6.2A). At Nerul, high concentration of Pb was observed in tissue of organism from May 2009 to January 2010 with highest peak in September 2009 (64.4 ± 0.45 ppm) and lowest (2.8 ± 0.03 ppm) in January 2010 (Figure 6.2A).

Zinc (Zn)

Zinc concentration ranged from 21.7 ± 0.04 to 300.2 ± 3.2 ppm (avg: 98.3 ± 0.88 ppm) and 19.6 ± 1 to 106.7 ± 0.55 (avg: 48.04 ± 0.79 ppm) in organism from Chicalim and Nerul respectively (Figure 6.2B). At Chicalim, a broad Zn concentration peak was recorded between June to November 2009 other than the minor fluctuations

recorded prior to June 2009. Whereas, at Nerul high concentrations of Zn were observed in tissue of *P. malabarica* from June 2009 to January 2010 with maximum concentration in November 2009 (106.7 ± 0.55 ppm) and minimum (19.6 ± 1 ppm) in January 2010 (Figure 6.2B).

6.2.2 Metal concentrations in Sediment leachate (0.00001N HNO3; pH =5)

Iron (Fe)

Iron concentration in the sediment leachate (SL) varied from 0.63 ± 0 to 39.1 ± 0.2 ppm (avg: 6.7 ± 1.9 ppm) at Chicalim where as at Nerul concentration ranged from 0.54 ± 0 to 12.1 ± 0.43 ppm (avg: 5.5 ± 0.10 ppm). At Chicalim, marked fluctuation was recorded throughout the sampling period with maxima (39.1 ± 25 ppm) in June 2009 and minima (0.63 ± 0 ppm) in July 2009 (Figure 6.3A). In contrast, the maxima (12.1 ± 0.43 ppm) in leachate Fe concentration at Nerul were considerably lower than that recorded at Chicalim. From November 2009 to March 2010, Fe concentrations at Nerul remain steady (Figure 6.3A).

Manganese (Mn)

Chicalim showed Mn concentration in the range from 0.03 ± 0 to 1.75 ± 0.49 ppm while Nerul showed concentration ranging from 0.02 ± 0 to 0.18 ± 0.01 ppm (Figure 6.3B). Highest Mn concentrations were observed in June 2009 and February 2010 at Chicalim. Comparatively, no noteworthy Mn concentration maxima were recorded at Nerul (Figure 6.3B). The lowest concentration of Mn was recorded (0.03 ± 0 ppm) in September 2009 at Chicalim and (0.02 ± 0 ppm) October 2009 at Nerul.

Cadmium (Cd)

Cadmium concentration ranged from 0 to 4.3 ± 0.12 ppb at Chicalim, whereas, it varied from 0.3 ± 0.10 to 0.98 ± 0.23 ppb at Nerul (Figure 6.3C). Chicalim showed

peak Cd concentration between March-June 2009 and in January 2010. Comparatively steady concentration profile was recorded from Chicalim (Figure 6.3C). The minimum concentration was observed in June 2009 at Chicalim and in October 2009 (0.3 ± 0.10 ppm) at Nerul.

Lead (Pb)

Pb concentration at Chicalim varied from 0.02 ± 0 to 14.3 ± 0.70 ppb, while at Nerul it ranged from minimum of 0.28 ± 0.07 ppb (August 2009) and maximum (November 2009) of 8.03 ± 0.60 ppb (Figure 6.4A). Highest (14.3 ± 0.70 ppb) concentration of Pb in sediment was observed from Chicalim during January 2010 and lowest (0.02 ± 0 ppm) in June 2009 (Figure 6.4A).

Zinc (Zn)

Zn concentration ranges from 0.53 ± 0.38 to 78.1 ± 0.38 ppb at Chicalim. Highest (78.1 ± 0.38 ppb) concentration was observed in February 2010 while lowest (0.53 ± 0.38 ppb) was in the month of June 2009 (Figure 6.4B). At Nerul, Zn concentration ranged from 10.6 ± 0.7 to 55.2 ± 1.3 ppb. Maximum concentration (55.2 ± 1.3 ppb) was in February 2010 and minimum (10.7 ± 0.7 ppb) in October 2009 (Figure 6.4B).

6.3 Discussion

6.3.1 Metal concentration in Tissues

The extent of metal accumulation in filter feeding bivalve tissues depends on several factors such as the metal concentration in food (Bryan, 1973), ingested sedimentary particulates, physico-chemical factors, body mass, physiological conditions (Rajendran *et al.*, 1987; Kanakaraju *et al*., 2008) and the efficiency with which the animal assimilates the ingested metal (Gagnon and Fisher, 1997). Substantial

variation occur in concentration of trace metals amongst the individual of the same species from the same area or habitat (Segar *et al*., 1971; Popham *et al*., 1980) suggesting marked variation in metabolism.

Average metal concentration in tissue of *P. malabarica* at Chicalim and Nerul follow the order Fe>Zn>Mn>Pb>Cd. Fe, Mn and Zn are essential elements required by animals for metabolic processes and show greater accumulation in the tissues relative to the non-essential elements like Cd and Pb (Duruibe *et al*., 2007).

Fe and Mn concentration observed in *P. malabarica* from Chicalim and Nerul, could be attributed to the inflow of mine drainage (Zingde *et al.*, 1976), as well as an increasing influx of pollutants from shipyard activities, mining transport (Rivonkar and Parulekar, 1998) which discharges various trace metal into the environment thereby posing threat to the beds of *P. malabarica* inhabiting this region.

Attri and Kerkar (2011) reported that mining activities adjoining the Mandovi river estuary forms the main source and cause of heavy metal pollution in the area. Mining waste has created a degraded environment and such damages occur more during monsoon where the rain water carries the washed out material from the waste dumps to the adjoining low-lying agricultural field and water streams. Hence high concentration of Fe and Mn in tissues of *P. malabarica* during monsoon to post monsoon period (Figure 6.1A,B) could be due to high discharge of Fe-Mn particulates in suspension. Kumari *et al*. (2006) observed high Fe concentrations in tissues of *P. malabarica* during monsoon period and attributed it to high suspended matter having enriched Fe content. High iron (~2-8%) and Mn (0.05 to 0.3 %) contents in the sediments of Zuari river estuary during post-monsoon and monsoon was recorded by Singh *et al*. (2009). They linked the observed Fe and Mn concentrations in estuarine

sediments during monsoon and post-monsoon to several factors such as particle size (Clay and clayey silt) and organic matter content (Aston and Chester, 1976; DevaVarma *et al*., 1993). Higher concentrations during monsoon may be due to the higher inputs from land runoff and influx of metal rich fresh water. Fe and Mn migrate as suspended matter, insoluble hydrated iron compounds, complexed to inorganic and organic ligands. In contrast, Alagarsamy (2006) observed higher metal contents specially Fe (10-16%) and Mn (0.2-0.25%) in sediments of Mandovi estuary during the pre-monsoon period. This observation has been attributed primarily to anthropogenic input namely iron ore processing and spillage during transportation. Walting and Walting (1976) stated that, Fe accumulation in marine organisms is regulated by additional factors such as sex, salinity, season, turbidity and depth. Zingde *et al*. (1976) and Sankaranarayanan *et al*. (1978) attributed low levels of Mn concentration in *C. madrasensis* as an indicator of its poor accumulating ability, while high concentration of Mn in the oyster tissues were attributed to a greater rate of pollution from automobiles, small crafts and substrate composition (Rivonkar and Parulekar, 1998). Shells of fresh water molluscs such as *Anodontal sp*. were reported to have higher contents of Mn compared to those recorded from marine molluscs (Segar *et al*., 1971).

Lead (Pb), Zn and Cd showed enrichment in the tissues during the monsoon period (Figure 6.1; Figure 6.2A,B). However, non consistency was observed between Chicalim and Nerul. Nerul showed peak in Pb and Cd, whereas Chicalim shows peak Zn concentration during the monsoon season. Association of these trace metals with the Fe-oxide (Fe_2O_3, Fe_3O_4) and oxy-hydroxide (Fe-OOH) fluxes associated with high discharge during monsoon could be the reason for high concentrations. Lead is a toxic element, which enters coastal waters from atmospheric and various anthropogenic

sources such as terrestrial runoff, industrial waste waters and antifouling paints (Nolting *et al*., 1999; Mitra *et al*., 2011). In present study, Pb concentrations in *P. malabarica* were observed to be higher than permissible limits (Table 6.1). Antifouling paints contain Pb as an important component and are used to prevent growth of marine organisms that may attach to bottom of boats, which ultimately is transported to the sediment and aquatic compartments (Fowler and Oregioni, 1976; Phillips, 1976b; Mitra *et al*., 2011). Organisms accumulate metals from aquatic environment either passively from water or by facilitated uptake. The input of metals to the environment from anthropogenic activities is difficult to distinguish since there are large natural inputs such as erosion, windblown dust, volcanic activity, industrial dumping and sewage sludge etc. Zinc is an essential element required for normal growth and metabolism of living organism (Mitra and Banerjee, 2011). They are naturally present or are introduced into the ecosystem by anthropogenic processes such as runoff containing motor oil, liver and brake things, galvanized metal roofs (Hannikeri, 2005). High concentration of Zn was observed during monsoon and were found to be beyond permissible limit (Table 6.1). Cd is one of the toxic heavy metal and is known to accumulate in marine food chains (Romeo *et al*., 1995). It may occur naturally or as a contaminant from sewage sludge, fertilizers, polluted groundwater and mining effluents. Cd concentrations recorded in the tissues were also above the permissible limits (Table 6.1). kumari *et al*. (2006) reported Cd values of 3.8 ppm in *P. malabarica* from Verem, Mandovi estuary. Various factors such as food availability, changes in the run-off of particulate material into the environment, variations in reproductive cycle, etc. are some of the reasons for seasonal variations in metal content (Fowler and Oregioni, 1976; Latouche and Mix, 1981). Salinity and temperature are also known to play an important role in the speciation and

bioavailability of metals. High concentration of Pb, Zn and Cd during monsoon (Figure 6.1C; Figure 6.2A,B) in tissue of *P. malabarica* could be attributed to the speciation and bioavailability of metals due to low pH, salinity and increase precipitated form of metals coupled with a higher rate of filtration and excess of contaminants entering the environment due to land run-off (Kumari *et al*., 2006).

6.3.2 Metal accumulation in tissues and biochemical/ physiological pathways

Filter feeding clams tend to accumulate metal through the food chain (Bryan, 1973). In Lamellibranchs, the inhalant water passes into the inhalent part of the mantle-cavity and later through the gills to the exhalent chamber. Food and suspended materials are accumulated on the inhalent faces of the gill. They are then accumulated in the food grooves with some mucus. Later, by the action of cilia, the food material passes along the gills to the labial palps. Fine material is carried by cilia into the mouth and then into the oesophagus and stomach. Coarser particles accumulated at the edges of the palps, are rejected periodically onto the mantle wall (Russel-Hunter, 1968). The process during food intake accompanies the intake of fine sediment particles. Suspension feeding bivalve are known to accumulate metals by assimilating sediment bound metals those ingested by them (Griscom and Fisher, 2004). It appears that bioavailability of sedimentary metals to organism is a function of dissolved gut amino acids (AA), gut retention time, sedimentary metal loading and species of organisms, and hence is a product of sediment-organism interactions. Study by Chen and Mayer (1999) showed that metals bound to sediments are subjected to significant solubilization during passage of the sediments through the digestive systems of an organism. In clam tissue, metal accumulation pathways is complex process and is controlled by range of biochemical and physiological process, which in turn are

influenced by ambient environmental conditions like salinity, temperature, pH etc. In order to understand the extent of metal leaching from ingested sediment particulates within the clam gut, the experiments were carried out to simulate the approximate gut pH (~5: Owen, 1966) and food passage time (~4-5 hrs: Griscom *et al*., 2002). The observation and some known biochemical pathways which influence metal accumulation in the tissues have been discussed. However, rigorous experiments need to be carried out in future to understand these biochemical pathways.

The leached metal concentrations (Figure 6.3A-C; Figure 6.4A,B) were found to be significantly lower compared to the level in the tissues of *P. malabarica* (Figure 6.3A-C; Figure 6.4A,B) and the reported concentrations in the sediments (Alagarsamy, 2006; Singh *et al*., 2009). Leaching of metals from sediments depends on speciation, grain size and solvent (HNO_3 in the present study). Silicate and oxide bound metals are minimally leached relative to the oxy-hydroxide and carbonate species. On the other hand, the rate of leaching (reaction kinetics) is strongly dependent on grain size. Smaller the grain size greater the rate of reaction. The complexation of leached metal cations is possibly influenced by the solvent used. Although temporal variations were observed in the metal concentrations in the leachates, no synchronous relation was obvious with the concentrations in the tissues. One reason for such lack of coherence could be the inherent assumption that the sedimentary particulates in the gut and the ambient sediments are of similar composition. However, the filtration mechanism of *P. malabarica* may have significant influence on grain size of ingested particulates relative to the suspended load. The grain size fractionation may have strong influence on particulate chemistry and solubility.

Digestive processes of bivalves are also known to influence assimilation and possess two phases, i.e. extracellular and intracellular digestion (Purchon, 1971; Owen, 1974; Bayne and Newell, 1983). Extracellular digestion occurs in the stomach once the food materials are ingested while intracellular digestion occurs primarily in the digestive diverticula. Such partitioning of food material, rate and pattern of particle movement within bivalve's gut affects the extent of digestion and assimilation (Van weel, 1961; Widdows *et al*., 1979; Bayne and Newell, 1983; Decho and Luoma, 1991; 1994; 1996). Intracellular digestions are slower than extracellular digestion which results in the longer retention of particles in the glandular path (Van Weel, 1961). Hence gut passage time are known to affect metal assimilation at different food concentrations in bivalves. Longer the retention of ingested food within digestive tract result efficient digestion and absorption (Willows, 1992). Earlier studies have reported higher assimilation efficiency of ingested Cd, Zn and Ag by clam's processed by glandular digestion (Harvey and Luoma, 1985; Decho and Luoma, 1991; 1994). The redox conditions and digestive enzymes within a bivalve's gut may also affect the fate of some ingested metals (Gagnon and Fisher, 1997). Filter feeding bivalves shows a good correlation of gut passage time (GPT) and (AE) assimilation efficiency (Griscom and Fisher, 2004).

6.3.3 Effect on human

Various trace metals are required to perform physiological processes that are essential to life in living organism. However, at higher concentration (higher than permissible limit), these micronutrients tend to become toxic and disturb various physiological processes causing various health hazards in consumers of contaminated bivalves (Mehra and Juneja, 2003). Iron is an important constituent of hemoglobin, myoblobin

and also enzymes. However, excessive Iron concentration causes various problems such as breathing trouble, acidosis fatal shock discoloration of the skin (Subramanian, 1987; De, 1994). It also leads to diabetes, cancer, kidney problem, and hypertension, nervous system diseases such as Parkinson's disease, Alzheimer's disease and behavioral abnormalities. Sukumar and Subramanian (1992) observed chronic headache and dizziness with higher level of Mn in male worker, working in fire work factory. Higher concentration of Mn also results in mental stress, breathing trouble and disorder in central nervous system. Excess uptake of Cd tends to accumulate in the liver and kidneys of the human body (Abbe and Riedel, 2000), resulting physiological and metabolic changes in an organism by alterating enzyme activity and membrane transport mechanism (Viarengo, 1989). High concentration of Cd also result in hypertension, renal dysfunction and decreased heamoglobin levels (Subramanian, 1987; De, 1994). It also leads to obstructive lung disease, cadmium pneumonitis, resulting from inhaled dusts and fumes. Bone defects, i.e. osteomalacia, osteoporosis and spontaneous fractures, increased blood pressure and myocardic dysfunctions have also been observed with high concentration of Cd. However, severe exposure may cause pulmonary odema and death. (McCluggage, 1991; INECAR, 2000; European Union, 2002; Young, 2005). Accumulation of Pb > 2.5mg kg^{-1} (13.75 ppm dry weight) in animal tissues leads to health hazards if humans consume such contaminated products (Talbot, 1987). Excess Pb concentration causes neurosis, mental retardation in children, gastrointestinal and respiratory cancer (Subramanian, 1987; De, 1994). It is a potential neurotoxin causing memory loss, insomnia, fatigue and drowsiness. Lead has been also reported to cause heart ailments by raising the blood pressure through its attack on the specific sites and cellular elements of the nervous system (Seth, 1998). Inhibition of the synthesis of haemoglobin, dysfunctions

in the kidneys, joints and reproductive systems, acute and chronic damage to the central nervous system and peripheral nervous system have also been reported with excess Pb (Ogwuebgu and Muhanga, 2005). Other effects include damage to the gastrointestinal tract and urinary tract resulting in bloody urine, neurological disorder and can cause severe and permanent brain damage (McCluggage, 1991; INECAR, 2000; Ferner, 2001; Lenntech, 2004). Lead is known to affects children causing poor development of the grey matter of the brain, thereby resulting in poor intelligence quotient (Udedi, 2003). Zinc has been reported to cause similar signs of illness as lead (McCluggage, 1991). It is considered to be relatively non-toxic, if taken orally. However, excess amount can cause system dysfunctions that result in impairment of growth and reproduction (INECAR, 2000; Nolan, 2003). It causes vomiting, diarrhea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure and anemia (Fosmire, 1990).

Fe and Mn concentrations in the tissues of *P. malabarica* were found to be higher at both study stations. Since the stations are in close proximity to mining sites, it is speculated that mining related activities played important role in metal supply to the *P. malabarica* habitat. Cd, Pb and Zn were found to be beyond permissible levels during the study period. The consumption of clams with more than permissible metal concentration might influence human health. The present findings provide baseline information on the contamination levels of toxic metals in edible bivalve species. However, further studies are required to conclusively determine the sources of the metal contaminants. Hence, regular monitoring of fisheries products from estuarine regions need to be carried out on a regular basis to assess their health status. Further observations need to be carried out by increasing the frequency of sampling over a larger area.

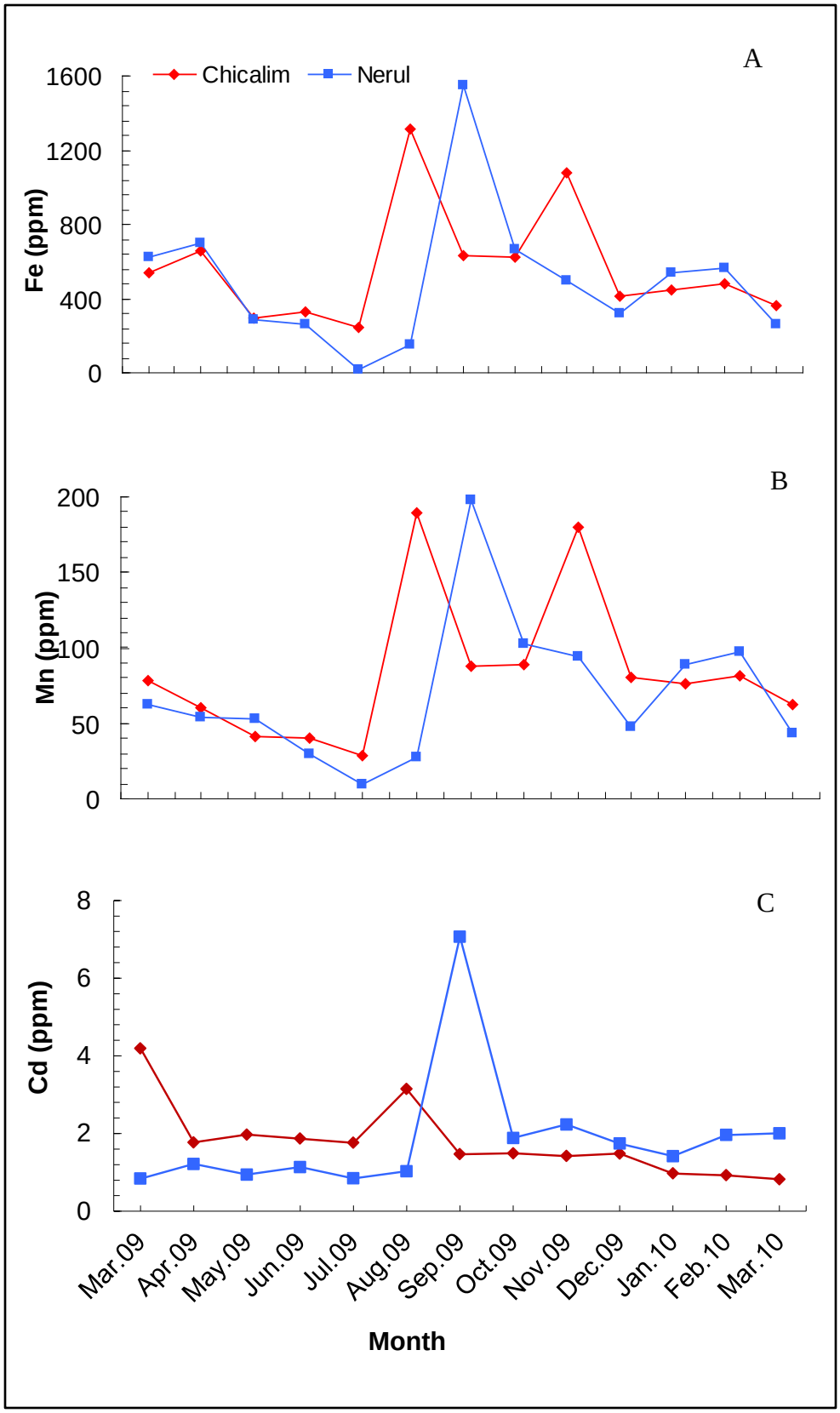


Figure 6.1: Monthly variations in (A) Fe, (B) Mn and (C) Cd concentrations in tissue of *P. malabarica*.

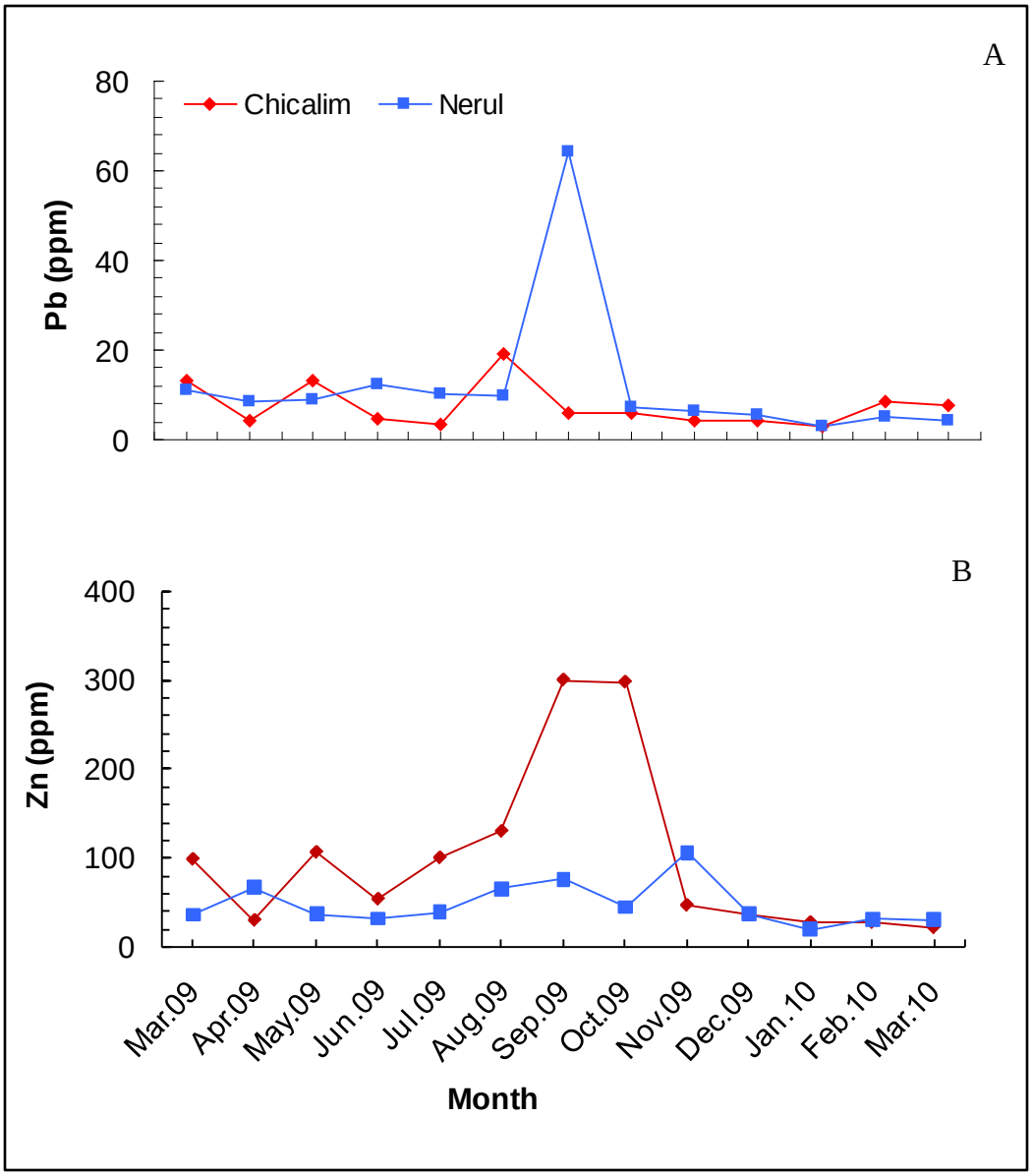


Figure 6.2: Monthly variations in (A) Pb and (B) Zn concentrations in tissue of *P. malabarica*.

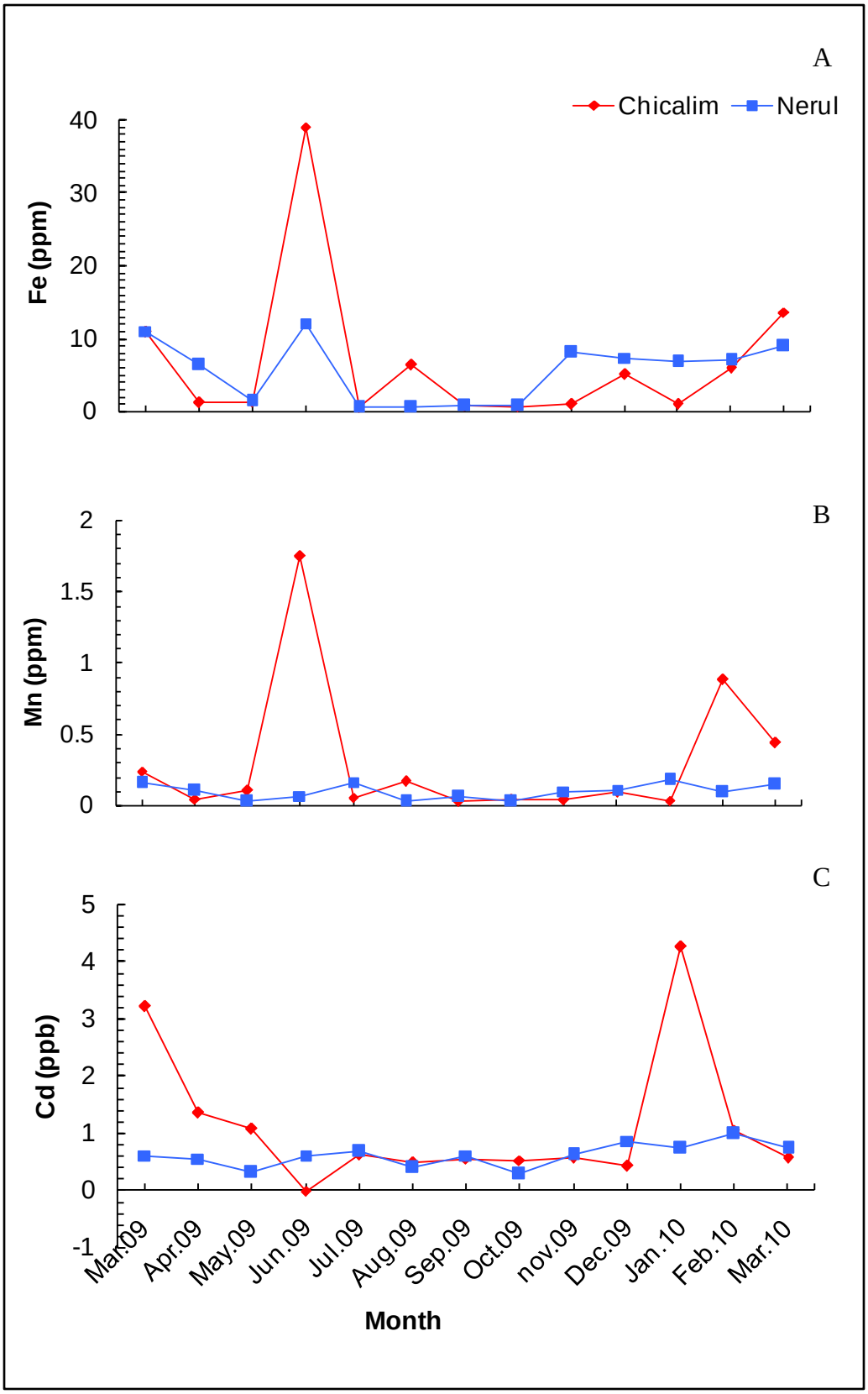


Figure 6.3: Monthly variations in (A) Fe, (B) Mn and (C) Cd concentrations of sediment samples.

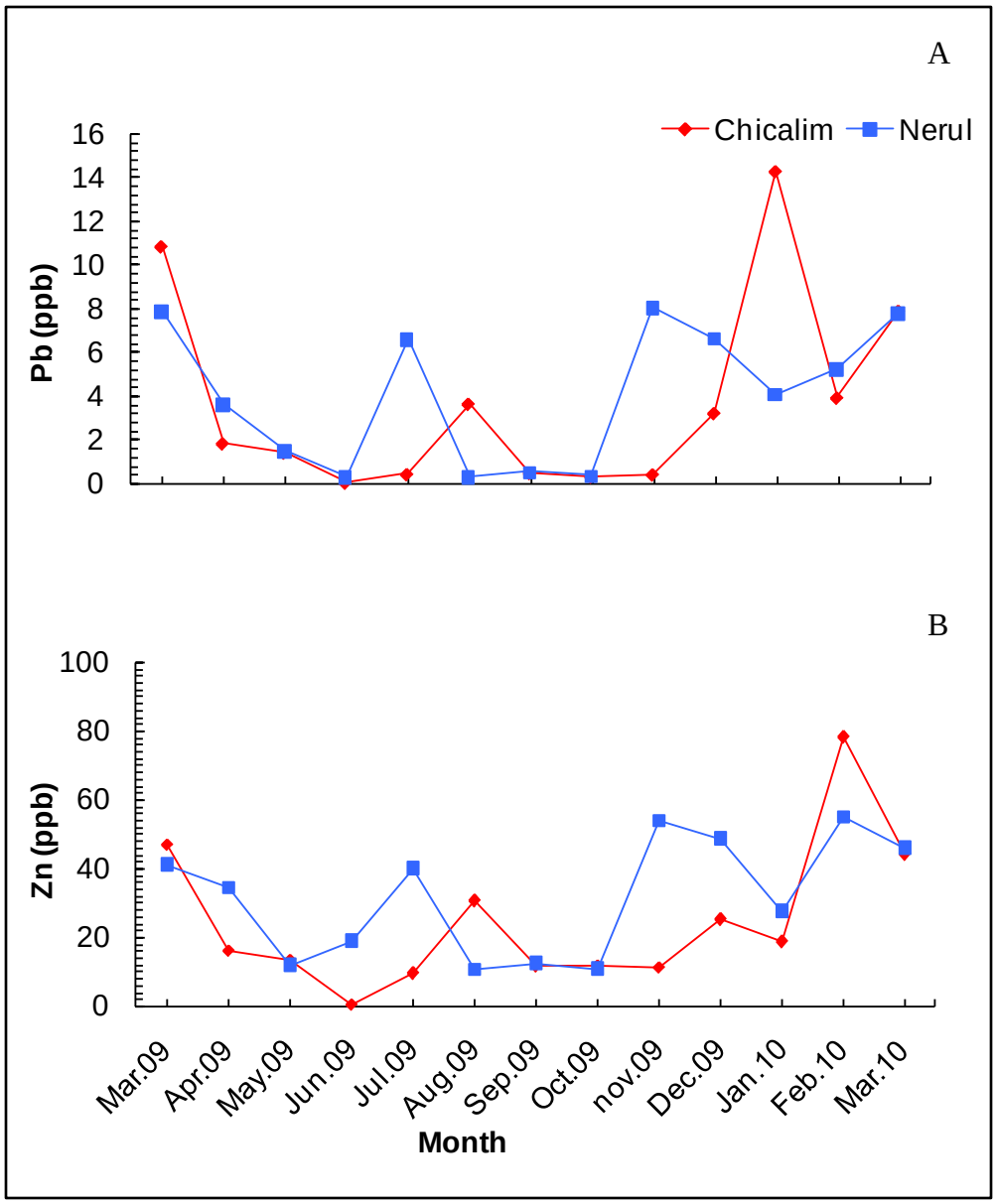


Figure 6.4: Monthly variations in (A) Pb and (B) Zn concentrations of sediment samples.

Table 6.1: Safe level of selected metals in marine organisms.

Metal	Metal Concentration (ppm) in *Paphia malabarica*		Relevant Permissible limit (ppm)	References
	Chicalim	Nerul		
Cd	0.83 – 4.19	0.84 – 7.06	0.5	WHO, 1993
			1	Gregori et al., 1996
			5	CEFAS, 1997
			10	FAO & WHO, 1982 NHMRC, 1987
			0.0018	UNESCO/WHO/UNEP, (1992)
Pb	3.11 – 18.97	2.79 – 64.36	2	WHO, 1993
			25	BOE, 1991
			0.8	FDA, 1993
			50	Great Britain Parlament,1979
Zn	21.72 – 300.2	19.63 – 106.7	1000	Anonymous, 1981
			50	Gregori et al., 1996
			0.03	UNESCO/WHO/UNEP, (1992)
Fe	242.7 – 1316	14.6 – 1547	5	WHO, 1993
			0.3	UNESCO/WHO/UNEP, (1992)
Mn	28.77 – 189.3	9.12 – 197.9	100	WHO, 1993

CONSERVATION AND MANAGEMENT Chapter-VII

7.1 Introduction

Demands for seafood are constantly increasing with the continuous rise in the population and hence required to increase the food production and utilise the same in a sustainable manner. Fish forms an important source of low-priced protein to the people. However, lately exploitation of fisheries resources, particularly in the Indian Ocean has exceeded the natural rate of renewal, resulting in over fishing and dwindling fishery resources (Ansari *et al*., 2006). It is therefore, the conservation and management of fishery has became the need of hour in India. Fishery management is the mechanism required to stimulate, control and regulate developmental processes in fisheries, so as to ensure proper and balanced utilization of various fisheries resources and to provide the maximum benefit without causing damage to the resources and the environment (Sivasubramanian, 1999).

7.2 Molluscan fishery

Molluscs form a valuable resource along the East-West coast of India and Adaman-Nicobar islands. It constitutes important food source production of lime for construction, pearl and decorative shells for shell handicraft trade (Pillai and Preetha, 2011). Due to increasing demand for molluscan meat, both in local and regional markets and for export, there is considerable pressure in fishing efforts leading to increased molluscan landings forming 4-5% of the total fish landings (Appukutan, 1996). India has extensive molluscan resources along the coasts. The annual landing of mollusc reported was 1,15,914 tonnes during the year 1998-2008. Cephalopods

constitute the bulk landing (96.8%) followed by bivalves (2%) and (1.2%) gastropods (Pillai and Preetha, 2011).

Bivalve fishery

A variety of clams, mussels, edible oysters, pearl oysters are distributed along the Indian coast where they are commonly exploited by small fishermen communities either for their meat or shell. Clams and cockles form 73.8%, followed by oysters (12.5%), mussels (7.5%) and windowpane oysters 6.2 % (Moharned, 2012). Along the West Coast, Kerala accounts for a majority of total landings of clam and cockles with 73.8%. The average annual estimated landing of bivalve during 1998-2008 was 2319.18 tonnes (Pillai and Preetha, 2011).

The important species of clams commercially exploited are *Meretrix casta, M. meretrix, Paphia malabarica, Katelysia opima, Villorita cyprinoides, Mactra spp. Sunetta scripta* and *Mercia opima* (Pillai and Preetha, 2011). Other important clams exploited are *Gafrarium tumidum, Mesodesma glabratum, Tellina sp, Anadara rhombea, Donax faba, D. cuneata, D.incarnattis, Mactra violacea, Tridacana maxima, T. crocea and T. squamosa*. In Goa commonly exploited clam are *P. malabrica*, *M. casta*, *V.cyprinoides* and *Polymedosa erosa*.

7.3 Management practises

Inspite of commercial importance, bivalves are least managed resources along the Indian coast. Restriction on the pearl oyster fishery by the Government of Tamil Nadu and management measures on the short neck clam fishery of Ashtamudi Lake in Kerala are some of the important steps taken towards conservation of the these

marine resources. Following management practices are being implemented all over the world to conserve various fishery resources.

Stock assessment

An assessment of the stock is important before any management measures are thought of and implemented. In this process, data such as, total catch statistics along with measures of fishing effort are collected. Catch per unit effort (CPUE) data are used to provide a measure of abundance over time and area. Such data are often collected for commercial fishing operations. This is inexpensive and comprehensive way of collecting data, provided that samples are reasonably large and representative of the population (Grosling, 2003). However, fishermen often concentrate their efforts on areas of high bivalve density as a result CPUE remains high over time, even when the total stock size is reduced substantially, and vice versa.

Shellfish farming

Shellfish farming are practiced by the coastal municipalities for restoration and restocking as well as by private individuals for economic gain. Shellfish Aquaculture Best Management Practices (BMPs) are a set of voluntary procedures that have been developed by the Massachusetts shellfish aquaculture industry in collaboration with the South Eastern Massachusetts Aquaculture Center (SEMAC) to address areas where attention should be focused to improve production while preserving the environment (Leavitt, 2004). Shellfish production in farms means using good management so that the crop is properly managed to maintain it healthy. However, the best management practice depends on site-specific circumstances, economic opportunities and environmental considerations (Leavitt, 2004).

Propagation Hatchery

Hatcheries may be used to increase shellfish populations for harvest, but they can also be used to support restoration. Successful shellfish hatcheries have been established in Maryland and Virginia to support Chesapeake Bay restoration sites (CES, 2010). Hundreds of millions of spat-on-shell are produced at Horn Point Laboratory's Shellfish Cultivation Facility and later transplanted to restoration sites (CES, 2010). Several small commercial hatcheries also exist along the Atlantic Coast and the University of Delaware maintains a small research hatchery in Lewes (PDE, 2011).

Spat Collection and Relaying

Relaying is the process of transplanting live bivalves to a new location. Oyster relaying has been used as a management technique for centuries with spat and adult relaying occurring in the Delaware Bay on the upper seed beds (Kreeger *et al*., 2011).

Culture possibilities

An aquatic animal possesses a greater productive potential than terrestrial animals for many reasons such as body temperature, which remains very close to the ambient environment and body density too remains similar to habitat (Parker, 2002). Another important feature of aquatic animal is that they inhabit multidimensional environment, where different species inhabit different space and position within the aquatic body. Such properties of an aquatic organisms help in their culture practices. Aquaculture has been the world's fastest growing food production system. Bivalves have advantage as culture candidates as they are efficient converters of primary production into animal protein of high value. The clam culture is eco-friendly and involves a low cost culture technology. It also helps in employment generation and socio-economic

uplifting of coastal rural communities. Clam cultures are being practised in Malaysia's since 1948 and farming of blood clam, *Anadara granosa* is the most organised culture system in Malaysia's. In India, attempts were made to transplant indigenous clam species for fattening (Ranade, 1964). In 1972, CMFRI, Cochin, India, started a programme on clam and mussel culture by transplanting clam seed into manageable areas in the estuaries and bays. National Institute of Oceanography (CSIR-NIO), Goa and college of Fisheries, Ratnagiri also took such similar programmes on culture of mussels. Culture of various clam species such as Manila clam (*Ruditeps philippinarum*), the colourful clam (*R. Variegate*), the meretrix clam (*Meretrix meretrix*), the mud cockle (*Tegillarca granosa*) and the razor clam (*Sinovnovacula constricta*) have been carried out in China.

7.4 The Coastal Regulation Zone (CRZ)

Estuary and coastal areas are the part of human life since prehistoric time. Man has always used the estuary as a resource: initially for food, then for transport and communication and recently as a site for industry and development. However, these areas are mainly affected by industrial and sewage discharges, atmospheric deposition and terrestrial drainage. Rapid developments in the coastal regions and ever increasing demands for land and natural resources, have been constant threat for the existing sensitive coastal marine habitats. In India, these regions have been protected by the coastal Regulation Zone (CRZ) notification of 1991 (MoEF, 1991). The legal system of coastal zone management in India came into force in 1991 (Anonymous, 1999). The Coastal Regulation Zone (CRZ) notification, under the environment Act, is one of the major norms limiting the activities in the coastal zone. It includes various

laws for regulation of anthropogenic interferences by permitting environmental friendly developments.

The new CRZ notification 2011 (MoEF, 2011) includes the land area between high tide mark to 500 m on the landward side along the sea front. The CRZ notification classifies the zones into four different categories as follow:

CRZ-I

It includes area that are ecologically sensitive such as mangrove wetlands, national parks, sanctuaries, wild life habitats, places of outstanding natural beauty or historical heritage. Areas close to breeding and spawning grounds of fish, those likely to be inundated due to sea level rise (consequent upon global warming), and the area between LTL and HTL are covered under this category. New construction are not permitted within 500 meters of the HTL of CRZ-I. However treated effluent or waste water discharge, for cooling process with sea water or construction activities for laying of oil or gas pipelines are permissible activities with LTL and HTL regions.

CRZ-II

CRZ-II indicates sufficiently developed urban area with facilities of drainage, approach roads, water supply and sewerage mains. The new constructions are not permitted seaward side or along the existing roads or existing buildings. However, new constructions are permitted landwards, subject to regulations and existing norms of Floor Space Index/Floor Area Ratio (FSI/FAR) by the Town and Country Planning Department. Reconstruction of authorized buildings is also governed by these norms. The design and construction of the new buildings have to be maintained consistent with the surrounding landscape and local architectural style.

CRZ-III

The relatively undisturbed areas are included under this category. It includes coastal zones in developed or undeveloped and areas within municipal limits or in other legally designated urban areas, inadequately developed. Area within 200 m from the HTL is declared as a No Development Zone (NDZ), where no constructions other than repairs of existing authorized structures are permitted. The designated coastal zone authority may allow limited construction for water supply, drainage, and sewerage facilities for local inhabitants. Agriculture, horticulture, gardens, pastures, parks, play fields, forestry and salt manufacture from seawaters are also permissible. Traditional rights and customary uses of existing fishing villages are recognized but permission to construct or reconstruct village units have to be strictly governed in accordance with certain set norms. Construction is allowed for permissible activities under the Notification.

CRZ-IV

The aquatic area from low tide line upto territorial limits is classified as CRZ-IV including the area of the tidal influenced water body. In CRZ-IV areas, there is no restriction on the traditional fishing and allied activities undertaken by local communities. However, no untreated sewage, effluents or solid waste shall be let off or dumped in these areas. A comprehensive plan for treatment of sewage generating from the city must be formulated within a period of one year from the date of issue of this Notification and be implemented within two years thereafter.

Provisions in the 2011 Notification to benefit the fishing community

Fishing communities have been given primary importance in the CRZ Notification 2011, since they live in the coastal areas and depend upon marine living resources. One of the important objectives of the notification is to ensure livelihood security to the fishing communities and other local communities, living in the coastal areas and to promote development through sustainable manner based on scientific principles taking into account the dangers of natural hazards in the coastal areas and sea level rise due to global warming.

The following are the provisions in the 2011 Notification that address the issues relating to fishermen community:-

(i) Water area up to 12 nautical miles and the tidal influenced water bodies have been included under the Coastal Regulation Zone areas in order to:

• Control the discharge of untreated sewage, effluents and the disposal of solid wastes as such activities endanger the fish and their ecosystem;

• Conserve and protect habitats in the marine area such as corals and coral reefs and associated biodiversity, marine sanctuaries and biosphere reserves, seagrass beds etc., which act as spawning, nursery and rearing grounds for commercially and ecological important fauna;

• Regulate activities in the marine and coastal waters such as dredging, sand mining, discharge of waste from ships, construction like groynes, breakwaters, etc. including reclamation which have serious impacts on fishing and allied activities;

• Enable studies of the coastal and marine waters with regard to the impact of climate change and the occurrence of disasters which have serious impacts on the livelihood and property of the fisher-folk communities. No restrictions are being imposed on

any fishing activities and allied activities of the traditional fishing communities in this area.

(ii) At several coastal stretches of the country the fishermen and their dwelling units are in danger due to erosion which is occurring primarily due to manmade activities. The development of such manmade foreshore activities shall be regulated after identifying and demarcating the coast as falling in the high eroding category, the medium eroding category or the stable sites category.

(iii) While preparing the Coastal Zone Management Plans the infrastructures essential for fishing communities must be clearly demarcated and fishing Zones in the water bodies and the fish breeding areas shall also be clearly marked.

(iv) The 2011 Notification requires the Coastal Zone Management Authorities to invite comments on the draft Coastal Zone Management Plan from stakeholders. This will ensure that for the first time, local communities including fishermen communities will have a say in the preparation of the CZMPs.

(v) The Notification allows infrastructural facilities for the local fishing communities to be constructed in the CRZ-III area.

(vi) Reconstruction, repair works of dwelling units of local communities including fisheries in accordance with local Town and Country Planning Regulations has been made permissible.

(vii) In CRZ-III areas where 0-200 m is a NDZ, to meet the demands of dwelling units of traditional coastal communities including fisher-folk, the NDZ has been reduced to 100 m. Hence, dwelling units of such communities can be constructed within 100-200 metres from HTL along the seafront with the approval of the State Government and the MoEF.

Special provisions have also been incorporated for the fishermen communities living along the coastal areas in Maharashtra, Goa, Kerala, Sunderban and other ecologically sensitive areas.

• **Greater Mumbai:** For the traditional fishing communities (namely, the Koliwadas) living in Greater Mumbai a provision has been provided, wherein, the area concerned shall be mapped and declared as CRZ-III and development including construction and reconstruction can be taken up as per local Town and Country Planning Regulations.

• **Goa:** The Government of Goa shall survey and map the fishing villages all along the Goa coast and all facilities required for fishing and allied activities shall be provided. As per the CRZ Notification, 1991, expansion/reconstruction/repair of dwelling units of local communities in CRZ areas were viewed as violations of the Notification if the requisite permission had not been taken from the authorities. Such units (approximately 5,000) were ordered to be demolished by the Hon'ble High Court of Bombay. However, the 2011 Notification provides that reconstruction and repair of the structures of local communities shall also be permissible in CRZ areas.

• **Kerala:** The CRZ area in Kerala is reduced to 50 m from HTL on the landward side. This area is a 'NDZ' where no new constructions can be carried out. However, dwelling units of local communities within this area may be repaired and reconstructed. Necessary foreshore facilities such as fishing jetty, fish drying and net mending yard, fishing processing by traditional methods, boat building yards, ice plant, boat repairs etc. can also be constructed within the 0-50 metres area. Beyond 50 metres from HTL on the landward side, dwelling units of local communities may be constructed with the permission of the local panchayat.

• **Sunderban:** In order to regulate development in Sunderban and other ecologically sensitive areas, and to take up conservation and protection of nine these areas for the

benefit of local communities an integrated management plan (IMP) is required to be prepared in consultation with the local communities. The housing needs of such communities including fisherfolk living in such ecologically sensitive areas shall be also addressed in the IMP (MoEF, 2011).

The special dispensations given to Goa

Specific provisions have been provided for the State of Goa with a stringent regulatory mechanism for sustainable development and ecological protection of coastal areas. Other provisions include the following:

• Since the traditional occupation of the population living along the coast is mainly the fishing and allied activities and fishing communities require basic infrastructure facilities for their livelihood, such facilities shall be provided by the Government of Goa after conducting a comprehensive survey.

• Reconstruction, repair of the structures of local communities shall be permissible in the CRZ areas.

• The eco sensitive low lying areas influenced by tidal action known as khazan lands shall be mapped. All mangroves along such land shall be protected and a management plan shall be prepared. No developmental activities shall be permitted in the khazan land.

• Sand dunes, beach stretches along the bays and creeks shall be surveyed and mapped. No activity shall be permitted on such sand dune areas.

• Beaches such as Mandrem, Morjim, Galgibag and Agonda have been designated as turtle nesting sites and protected under the Wildlife Protection Act, 1972. These areas

shall be surveyed and management plan prepared for protecting these sites. No developmental activities shall be permitted in these areas (MoEF, 2011).

However, the increasing human pressure continues to put constant threat for coastal habitats. Therefore, strict enforcement of CRZ act needs to be implemented for effective conservation and protection of these ecologically sensitive ecosystems.

7.5 Conservation and management of *Paphia malabarica*

The coastal and inshore waters of Goa are known to be very rich in fishery resources (Ansari, 1978; John, 2006). All important marine fish landing centres are situated on the coast which are free from rocks and which have sandy sea-bottom. Although Goa is the smallest maritime state of India, it contributes ~ 3% of the total marine fish catch in the country (John, 2006). The annual marine fish catch in Goa was estimated to be 72, 307 tonnes during 2012 (Rao, 2013).

Paphia malabarica forms an important fishery in Goa. The species is locally referred to as "tisreo". A large proportion of this clam is fished for local consumption (Plate 7.1). Harvesting is done by hand, feet, hand-operated scoop net or with the aid of a small hand-held digging stick (Plate 7.2 and 7.3). The collectors work for three to four hours a day at ebb period. Clams are collected in cone shaped nets, baskets, plastic boxes, cement bags, etc. Small non mechanized crafts are normally used for collection from deeper waters and for transport of bivalves from the collection site to the villages.

Management of clam beds

Management of natural clam beds is an important practice for a long term sustainable yield. Annual survey of clam beds gives information on the abundance of clam in each bed. It also helps in providing continuous monitoring of beds to investigate the temporal changes of the population. Hence regular monitoring of *P. malabarica* beds has to be carried out. Drastic change in factors like temperature, salinity, food, diseases and pollution may adversely influence the health of clams and results in mass mortality. Hence, these factors should be regularly monitored. A better understanding of the habitat of clam that serve as nurseries for various marine species and the factor that affect the quality will help in improving conservation and management of such clam beds. The major estuarine complex (Mandovi-Cumbarjua canal-Zuari) of Goa is intensively used for transporting bauxite/ iron ore to loading centres or ships anchored at harbour (Marmagao) and offshore. As a result the estuarine habitats of Goa and its surrounding regions remain under great threat from mining activities. Hence, water quality of any ecosystem should be protected and conserved. Regular monitoring of water quality for metal contamination should be carried out to avoid health hazards. Proper database on the resource availability of *P. malabarica* and their utilization pattern should be carried out.

Overfishing

Overexploitation is another major problem in fishery throughout the world. In many cases, the rate of harvesting has exceeded the natural rate of renewal, resulting in biological overfishing. Such overexploitation may leads to depletion of clams (Ansari *et al*., 2006). In an overexploited stock, the number of adults may be reduced to a level where reproduction is unable to replace the numbers lost (recruitment

overfishing) or large numbers of individuals may be caught at too small size (growth overfishing) to maximize yield (Sinclair *et al*., 1985). Hence overexploitation of undersized/smaller *P. malabarica* should be avoided by making use of appropriate mesh size. Earlier chapter (Chap-V) of present document reported that biochemical composition of *P. malabarica* was found to be higher during pre-monsoon, hence harvesting should be done during such periods. Total ban on fishing activity during breeding season (September to January, as reported by Mohite, 2010) of *P. malabarica* will allow the young organism to grow to a marketable size. In terms of a management strategy, it is desirable that a proportion of reproductively mature individuals be left in the population to allow breeding to take place. A useful size limit to catches would be 40-50 mm in length and clams below this size should be left undisturbed in order to guarantee recruitment in the population. Necessary information/training should be given to fishermen communities about the time they should halt collecting clams, which will allow small organism to grow to the recommended marketable sizes for better benefits.

Culture

Owing to the natural abundance of the seeds of *P. malabarica* and its demand in the local market, culture of this species should be attempted on a large scale in estuaries. This will increase the yield and generate employment opportunities for the fishermen communities. On-bottom culture method (Modassir and Ansari, 2006) have been attempted to culture *P. malabarica*. Study indicated a high yield can be obtained using this method of culture. Clam farming by semi-culture (transplanting the seed clams from dense beds to other suitable places in the estuary) should be carried out to increase production. Planting of clean shells revitalizes the natural clam beds, expands

and improves habitat for dependant marine life and provides critical habitat for juvenile clam (spat) recruitment, ultimately increasing clams abundance. Cultures (transplantation) of *P. malabarica* have been carried out at Kalbadevi estuary, Ratnagiri (Maharashtra), India and it reported increase in production (Mohite, 2010). Such transplantation technique should also be carried out in estuaries of Goa to increase the productivity of *P. malabarica*.

Establishment of marine parks, total ban on exploitation of breeding stock, mesh-size regulation for commercial exploitation of clams and practice of farming techniques can be some of the recommendations for conservations of *P. malabarica* resources from Goa.

Plate 7.1: Harvesting of clam from estuarine regions of Goa at ebb.

Plate 7.2: Various tools for collection of *P. malabarica*.

Plate 7.3: Marketing of *P. malabarica*.

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