STUDIES ON THE ROLE OF BACTERIA IN THE ASSIMILATION OF ORGANIC INPUTS IN COASTAL WATERS

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

in

MARINE SCIENCES

to the

GOA UNIVERSITY

by

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OCTOBER 2002

STATEMENT

As required under the University ordinance 0.19.8(iv), I state that the present thesis entitled "**STUDIES ON THE ROLE OF BACTERIA IN THE ASSIMILATION OF ORGANIC INPUTS IN COASTAL WATERS**" is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the first comprehensive work of its kind from the area mentioned.

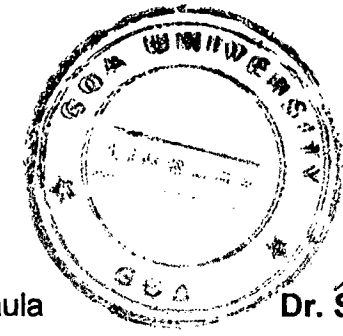
The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

A.S Pradeep Ram

(A.S. Pradeep Ram)

CERTIFICATE

This is to certify that the thesis entitled, "**STUDIES ON THE ROLE OF BACTERIA IN THE ASSIMILATION OF ORGANIC INPUTS IN COASTAL WATERS**", submitted by Mr. **A.S. PRADEEP RAM** for the award of the degree of Doctor of Philosophy in Marine Science is based on his original studies carried out by him under my supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma in any universities or institutes.



Place: NIO, Dona Paula
Dated: 3rd Oct 2002

Dr. SHANTA ACHUTHANKUTTY
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TO MY BELOVED PARENTS

"Microbial ecology has sometimes appeared to be the art of talking about what nobody really knows about in a language that everyone pretends to understand. The challenge in microbial ecology is to seek out the factors determining the growth of micro-organisms in their natural habitats and to talk about these in a language that everybody can understand"

- *Clark 1968*

CONTENTS

ACKNOWLEDGEMENTS

I have no words to express my deep sense of gratitude to my Research Guide ***Dr. Shanta Achuthankutty****, Scientist, Microbiology Department, NIO. I thank her for the scientific freedom, infallible guidance, encouragement and untiring efforts during the course of my study. I take this opportunity to convey my indebtedness and respect to her.*

Dr. D. Chandramohan*, Head, Biological Oceanography, NIO, for his moral support and inspiration that has made me to carry out this work. Thanks are also due to his valuable and critical assessment of the thesis in amazingly short time.*

I am indebted to ***Dr. P. A. Loka Bharathi****, Scientist, Microbiology Department, NIO for her sustained interest and valuable discussions.*

Dr. Jonathan J. Cole*, Institute of Ecosystem Studies, New York and* ***Dr. David L. Kirchman****, University of Delaware, USA for providing me with their valuable suggestions and constructive discussion.*

My deep sense of gratitude to ***Dr. Ehlrich Desa****, Director, NIO, Goa for providing me with the necessary infrastructure and excellent supporting facilities during the course of my Ph.D work.*

I thank ***Dr. G.N. Nayak****, Head, Department of Marine Science,* ***Prof. U.M.X Sangodkar****, Head, Marine Biotechnology Department, Goa University, for their valuable support.*

My sincere thanks to:

***Dr. B.R. Subramanian**, Advisor, Department of Ocean Development, New Delhi for his constant support, concern and encouragement.*

*Senior staff and colleagues of Microbiology department, NIO during the course of my research. **Drs. Krishna Kumari, Wafar, Dalal** (BOD), and **Dileep Kumar** (COD) for their constructive discussion pertaining to my work.*

***Dr. Sugandhini Sardessai** (COD), **Dr. S.G. Prabhu Matondkar** and **Mr. V Subramanian** (BOD), for their valuable assistance in the analyses*
The personnel of drawing section, workshop and photography section for their assistance and cooperation.

Office of Naval Research (ONR, USA) for their support to do my research.
***Dr. Bernard Zahuranec** for coordinating the project.*

Council of Scientific and Industrial Research (CSIR), New Delhi for providing me with a Senior Research Fellowship grant to complete my Ph.D.

Last but not the least, I find no appropriate words to express my feelings and gratitude to my parents for their unending love, unrelented support and guidance. Their great selfless sacrifice, advice and deep affection gave me the determination to fulfill my research study.

A.S. Pradeep Ram

Chapter 1

GENERAL INTRODUCTION

The coastal ocean, represents an area of transition where land, air and sea interact to form a wide variety of diverse habitats and ecosystems viz. estuaries, coral reefs, sea grass beds, mangrove swamps, creeks, lagoons and bays. In the coastal ocean, estuaries are regarded as complex ecosystems, involving interactions of physical, and bio-geochemical processes both spatially and temporally. They are the most productive ecosystems in the world and act as conduits for dissolved and particulate effluents discharged from centers of population, industries and from land drainage to the adjacent coastal environment. While some components are consumed or retained within the estuarine environment, rest of it are transported into the adjoining coastal waters. The coastal ecosystems are also places of hectic human activity, resulting in interference due to rapid development. Thus the concept of "sustainable management" of the coastal and estuarine ecosystems for the future is a "common currency".

In today's context, water quality standards are an outdated and irrelevant approach to control marine pollution. Rather than viewing man as the "Lord of all creation", man should be seen as an integral part of the environmental management. There is a changing view on the environment i.e. in the application of ecosystem concepts like assimilative or environmental capacity, precautionary principle in the management of aquatic systems to combat pollution. Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP 1986) defined assimilative capacity as the "ability to accommodate a particular activity or rate of activity (e.g. volume of discharge per unit time, quantity of dredging dumped per

unit time, quantity of minerals extracted per unit time) without unacceptable impact". Most of the studies carried out so far have attributed the assimilative capacity of aquatic ecosystems to physical forcing (Zdanowski and Figueiras 1999, Walsh et al. 1999). Although physical and chemical processes like circulation, sedimentation, photo-degradation are involved to study the assimilative capacity, the decisive role must be attributed to the biological compartment, particularly the standing stock of natural bacterioplankton, which largely determines the carrying capacity of the system. Recent concern regarding coastal contamination, ecosystem health and long-term changes in food web structures, underscores the relevance of understanding the ecology of coastal assemblages of prokaryotes. Studies pertaining to biology, especially on microbiological processes on the above aspect are relatively less known, particularly with respect to tropical ecosystems. In ecosystems with weak environmental regulations, the main control process is generally attributed to the activity of heterotrophs especially bacteria.

Study on heterotrophic bacteria in coastal and estuarine ecosystems is a central paradigm of any ecological study (Pomeroy et al. 1991, Ducklow and Carlson 1992). They are the most abundant component responsible for transformation and mineralisation of organic matter i.e. in the recovery of organic matter from detritus to living biomass or in its remineralisation back to inorganic compounds (Ducklow and Carlson 1992, Shiah and Ducklow 1994). These bacterioplankton often dominate the biomass of planktonic food webs making their role in nutrient and energy fluxes crucial for the organization of

marine ecosystems (Fuhrman et al. 1989). They are the major consumers of organic matter (DOM) thereby transferring their energy to at least the next trophic level (Sanders et al. 1992), hence forming the link between the dissolved and particulate organic carbon. Over the last 30 years, microbial ecologists have been examining the microbes and processes and this has been summarized in Fig. 1. Therefore, studying the dynamics of heterotrophic bacteria in the coastal ocean is of vital importance in understanding their role in these ecologically sensitive regions. Biologically dominated ecosystems generally show high biomass of heterotrophic bacteria because these organisms are responsible for the bulk of organic carbon utilization and respiration in the sea. The efficiency and versatility with which bacteria use organic substrates for the synthesis of new cell material and for meeting their energy requirements makes them unique (Biddanda et al. 1994).

Bacteria respond quickly to biotic and abiotic changes in the marine environment and probably they may be the first component to reflect environmental perturbations. To link bacteriological variables with ecosystem processes, it is necessary to understand the regulatory mechanisms that controls the distribution of bacterial biomass and activity. The distribution and activity of bacteria in aquatic ecosystems are regulated by a number of factors such as temperature (Shiah and Ducklow 1994), predation (McManus and Fuhrman 1988), substrate supply (organic and inorganic nutrients) (Cole et al. 1988, Rivkin and Andersen 1997) and even viral infections (Proctor and Fuhrman 1992). These factors could greatly vary and may be system specific. The complex

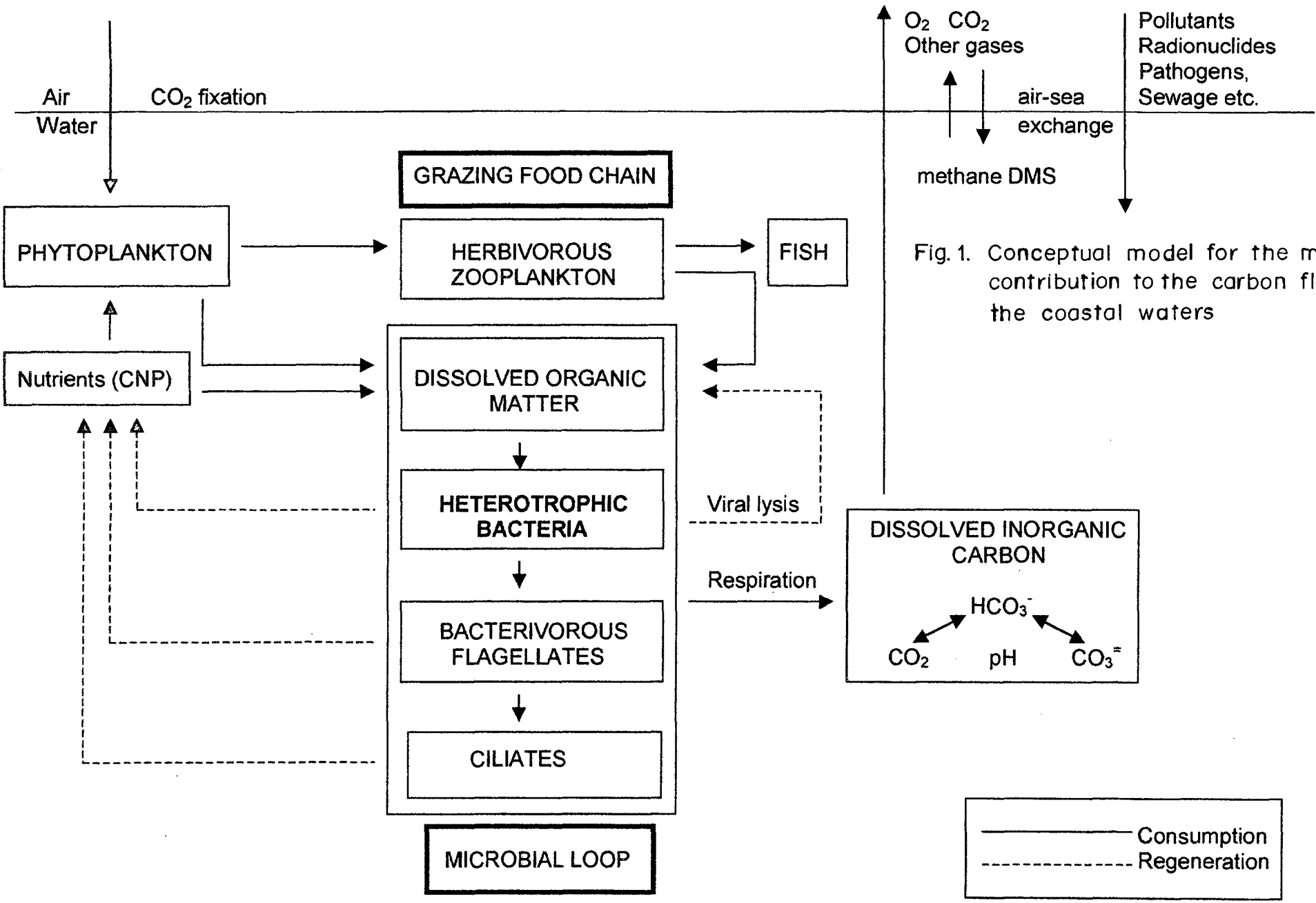

Fig. 1. Conceptual model for the microbial contribution to the carbon flow in the coastal waters

interactions between these environmental parameters make it difficult to determine which factor or set of factors is most important in the control of bacterial dynamics. Hence, it is important to study the influence of the above-mentioned environmental factors on the microbial population, their relationships and mechanisms. Few studies have been carried out on the above aspect worldwide, with studies largely being restricted to temperate and sub tropic regions. In the present scenario, self-purification of coastal waters assumes special relevance in maintaining the cleanliness of the sea. For this reason, a more accurate knowledge on the microbial ecology and processes of coastal waters particularly with reference to estuaries are urgently needed.

India has a long coastline of 7516 km and many rivers through their discharges carry significant quantity of waste laden freshwater into the coastal ocean. The amount of pollutants (industrial, sewage and domestic discharge) entering the sea around India is 24×10^9 m^3 (as of 2001). However this value is expected to increase in the coming years largely due to rapid urbanization and industrialization. The pressures on the coastal ocean are multifaceted and not only they interact among themselves to a large extent but also at many levels. Microbiological pollution of seas has received considerable attention in the past several years through the Coastal Ocean Monitoring and Prediction System (COMAPS) project funded by Department of Ocean Development, Government of India, New Delhi. With the increase in human activities much work is needed on the above lines for effective management of the coastal seas around us, instead of relying more on the presence of indicator species in marine systems.

Studies related to scientific issues associated with the management of coastal water systems are limited and the information available in the literature is scanty. Further, issues mentioned above have not been studied so far in Indian estuaries. Hence, in this study an attempt was made to collect extensive microbiological data together with other physico-chemical and biological parameters in Mandovi-Zuari estuaries and adjoining coastal waters with a view to understand their dynamics. Mandovi-Zuari estuarine system, located in Goa on the southwest coast of India is formed by the perennial connection of two rivers namely, the Mandovi and Zuari, which open into the adjacent coastal waters (Arabian Sea). The coastal zone is influenced by the semi-diurnal tides with maximum range of 2.3 metres. Tidal influence is almost the same in the entire water column, which means good flushing of water takes place twice a day and any pollutant in the river will be diluted more than six times by sea water before it finally reaches the open sea (Qasim and Sengupta 1981). In Mandovi-Zuari estuarine system seasonal variation in primary productivity have been carried out in relation to water chemistry, distribution and abundance (Devassy and Goes 1989). The physico-chemical characteristics of Mandovi-Zuari estuarine system have been reviewed by Qasim and Sengupta (1981). Although earlier studies have indicated this region to be a moderately productive zone, studies in tandem with bacteriological parameters in relation to food chain and trophic status are limited. In this tropical estuarine system, most of the studies have concentrated more on diversity than on microbial processes.

The present study revolves around the heterotrophic bacteria, which have

been used as a tool to study their interrelationships with other physico-chemical and biological parameters, thereby determining their role in mediating carbon flux and coastal metabolism. For the above studies, baseline information regarding the abundance and activity of bacterioplankton are mandatory. Seasonal studies would determine how bacterial properties are regulated over time and allow insight into how the changing environmental conditions affect the bacterial distribution and their activity. Studies pertaining to the above would significantly contribute to the ecological and biogeochemical understanding of this tropical estuaries, about which little is known.

In addition, we propose a simple empirical model, which would give an overall picture predicting bacterial activity and availability of quality substrates for bacterioplankton assimilation in understanding their role for a better management of tropical ecosystems. The above study is the first authentive work which would provide basis for addressing questions regarding the role of bacteria in biotechnology, risk assessment, waste assimilative capacity and global climate.

The objectives of the present study were:

1. to examine the spatial and temporal variations in the bacterial parameters and to bring out their interrelationship with other biotic and abiotic parameters in this tropical system
2. to determine the assimilative capacity of bacteria and the factors regulating it.
3. to determine the trophic status of the ecosystem and
4. to propose certain recommendations which could be applied in future for the management of these coastal waters.

Chapter 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

The coastal zone should always be viewed from a holistic approach, as an integrated system involving the input, output and circulation of mass, energy and information. This zone represents an area of transition, - it embraces a wide variety of diverse habitats and ecosystems (e.g. estuaries, coral reefs, sea-grass beds, mangrove swamps, creeks, lagoons, bays) and provides an opportunity for multidisciplinary activities like recreation, settlement, waste disposal, power generation, aquaculture, fishing, shipping, defense, as heritage areas and natural reserves. At any one locality, the coastal zone may be characterized according to physical, biological or cultural criteria. The coastal resources have a definite role in maintaining the ecological balance and economic vitality of the coastal regions. The measurement of energy and material flows between various ecosystem components and the efficiency with which energy is assimilated, transferred and dissipated provides significant insight into the fundamental structure and function of the system (Ulanowicz and Platt 1985). Understanding the dynamics of coastal waters is very much necessary to study its assimilative capacity for management practices. Assessing the assimilative capacity of an aquatic ecosystem is a broad spectrum of study, involving the physical, chemical and biological processes.

Of the coastal systems, estuaries are considered to be the richest, as they offer a physical and chemical environment which is different from freshwater (e.g. higher ionic strength) and may influence the bio-availability of dissolved terrigenous compounds The autochthonous bacterial communities of aquatic systems provide a means of detecting environmental perturbations. Evidences of

terrestrial organic matter containing substantial amounts of humic substances supplying energy and materials to aquatic food chains in both limnic and coastal marine systems have been reported (Moran and Hodson 1992). The functioning of the tidal estuaries involves the complex interactions of physical, biogeochemical and biological processes.

Among the various compartments in a marine ecosystem, bacteria are considered to be important as they would influence the chemistry and biology of the coastal ecosystem. They are the most abundant and most important biological component involved in the transformation and mineralization of organic matter in the biosphere (Cho and Azam 1988, Pomeroy et al. 1991). The importance of planktonic bacteria as an energy source for the microbial food web and as mineralizers of carbon and nutrients have been major research areas during the last decades (Ducklow and Kirchman 1983, Sherr and Sherr 1996). Their distribution and activity in aquatic systems are thought to be controlled by several abiotic and biotic factors as they are ecologically and biogeochemically (Toggweiler 1989) important to the cycling of energy in marine systems. Tranvik (1988) suggested that the bacterial degradation of terrigenous compounds might therefore have pronounced effects on the bacterial production and carbon balance in lakes and estuaries. Assessing assimilative capacity of bacteria to organic inputs mainly involves determining its metabolic activity and relating it with other biological and chemical parameters to understand its role in the ecosystem. Based on the above aspects, models have been formulated and studied (Connolly et al. 1992, Medrano and Maske 1999, Touratier et al. 1999).

2.2 INTERNATIONAL SCENARIO

Organic matter flux into bacteria has been identified to be one of the major pathways of material and energy flow in pelagic food webs (Azam et al. 1983, Cole et al. 1988, Ducklow and Carlson 1992). Measurement of bacterial growth efficiency (BGE) in natural assemblages is an integrated measure of efficiency of utilization of a large number of organic compounds (del Giorgio and Cole 1998). The efficiency with which they utilize the organic matter largely depends upon the metabolic capability of the residing bacterioplankton community. This efficiency is a fundamental attribute of microbial metabolism, which adequately determines the ecological and biogeochemical roles of bacteria played in the microbial food webs and in aquatic ecosystems (Sherr and Sherr, 1996, del Giorgio and Cole 1998), which ultimately decide the carrying capacity of an ecosystem. The calculation of BGE (using bacterial productivity and respiration) using short-term incubation method has been carried out by many researchers (del Giorgio et al. 1997, Amon and Benner 1998, Roland and Cole 1999). Several microbial ecologists have discussed the importance of using radiolabeled compounds (thymidine and leucine) in bacterial productivity studies (Fuhrman and Azam 1982, Kirchman et al. 1985, del Giorgio and Cole 2000). Generally the reported growth efficiencies vary widely in aquatic ecosystems ranging from<10 to >60%, with most of the values occurring in the range of 5-30%. From the previous studies, the median BGE for open ocean was calculated to be 9% (mean 15 ±12%), 25% (mean 27 ±18%) for coastal areas and 34% (mean 37 ±25%) for estuaries (del Giorgio and Cole 2000). Griffith et al. (1990) found BGE to

decrease with increase in distance from shore (11% in estuarine, 6% in near shore water, 2% in shelf waters). Similarly, Coffin et al. (1993) also reported temporal variations in BGE values, with higher values in the Florida Bays (43%) when compared to the adjacent coastal waters in Gulf of Mexico. Studies on the seasonal fluctuations and diel variations in the growth efficiencies have been carried out in the temperate waters (Chin-Leo and Benner 1992, Roland and Cole 1999). del Giorgio et al. (1997) attributed the higher BGE in estuarine than coastal waters to the DOM arriving from external sources, which fuels bacterial production.

DOM becomes available for bacterial utilization through phytoplankton exudation and lysis, grazing and release by zooplankton and also by physical disaggregation of detrital particles (Conan et al. 1999). DOC generated from primary production by a variety of means is taken up by bacteria and used for their growth and metabolism (Azam et al. 1993, Ducklow and Carlson 1992). The proportion of primary production in marine environments is reported to vary from 10 to over 100% with a mean of 30 to 40% (Cole et al. 1988, Ducklow and Carlson 1992). In such studies understanding the behaviour of the indigenous bacterial population to natural DOM in the ecosystem is mandatory. Both the quality and quantity of DOM have been identified as the primary factors affecting bacterial remineralization and trophic assimilation (Cole et al. 1988, Kirchman 1990, Keil and Kirchman 1994). The C: N ratio of the substrate assumes greater importance when evaluating the efficiency in utilizing the bulk of the DOM by the natural bacterioplankton (Amon and Benner 1998, Biddanda et al. 1994).

Previous studies have indicated that the bacteria in general have a C: N ratio of 4.5:1(Goldman et al. 1987, Kroer 1993, Fagerbakke et al. 1996) and have been shown to be inversely related to BGE (Kroer 1993, Biddanda et al. 2001). Modeling of bacterial growth efficiency influenced by substrate C: N ratio and concentration was attempted by Touratier et al. (1999), who emphasize on the quality of assimilated substrates, which may be a critical factor to understand the role of bacteria in a pelagic ecosystem.

In recent years, the importance of exo-enzymes in the assimilation of high molecular weight organic compounds as the rate-limiting step in microbial growth has been well documented (Chrost et al. 1989). Extracellular enzymatic hydrolyses are direct measure of microbial metabolism which have been used to describe apparent differences in detrital quality among habitats (Sinsabaugh and Findlay 1995) and broad scale pattern of substrate utilization (Christian and Karl 1995). Extracellular hydrolysis is a necessary pre-requisite to assimilation of carbon from complex macromolecules and these macromolecules (molecular weight >10,000 daltons) constitute the bulk of the DOC pool in almost all the aquatic ecosystems (Chrost 1990). Studies on the utilization of wide variety of organic substrates together with exo-enzyme kinetics and growth of the organisms have been conducted (Sinsabaugh et al. 1997). Foreman et al. (1998) studied the extracellular enzyme activity of heterotrophic bacteria in assimilating natural DOM.

Coupling of BGE to system productivity i.e. primary productivity (Kirchman and Rich 1997, del Giorgio and Cole 1998), substrate quality (Coffin et al. 1993,

Biddanda et al. 1994) elemental stoichiometry or nutrient limitation (C: N ratio) (Kroer 1993, Vallino et al. 1996), shift in the bacterial community or species composition and activity (Kroer 1993, Biddanda et al. 1994), temperature (White et al. 1991) salinity (Turley and Lochte 1990) and physiological conditions of cells (Morita 1997) have been studied in the past.

Connolly et al. (1992) studied the growth yield coefficients (=BGE) on various classes of substrates such as amino acids, alcohols, alkanes, sugars esters and organic acids. BGE on different detrital organic matter like phytoplankton (Biddanda 1988), marcoalgae (Robinson et al. 1982) and bivalve faeces (Tupas and Koike 1990) have also been investigated. Cherrier et al. (1996) demonstrated a substantial increase in the bacterial growth efficiency when the water samples were amended with plankton extract-DOM than those amended with model substrates. In laboratory experiments, Foreman et al. (1998) observed the fall in growth rate and bacterial growth efficiency due to addition of phenolic compounds.

The dependence of BGE on the relative availability of mineral nutrients and organic carbon was originally proposed by Fenchel and Blackburn (1979) and later expanded by others (Anderson 1992, Vallino et al. 1996). Depending on the trophic state and seasonal situation of the given environment, studies have indicated that organic carbon (Ducklow and Carlson 1992, Kirchman and Rich 1997); nitrogen (Goldman and Denett 1991, Kroer 1993) or inorganic phosphate (Chin-Leo and Benner 1992, Cotner 1997) or a combination of them could most often limit bacterioplankton growth. Nutrient enrichment studies involving

amendments of C, N and P as model substrates (in various combinations) for determination of nutrient limitation have been carried out to investigate the factor, responsible for limiting bacterial growth in the ecosystem (Chin-Leo and Benner 1993, Carlson and Ducklow 1996, Cherrier et al. 1996). Pomeroy et al. (1995) suggested that the accumulation of dissolved organic carbon (DOC) in oligotrophic waters could suggest phosphate limitation. Research with cultured bacteria (Russel and Cook 1995) as well as models of bacterial energetics and growth in aquatic ecosystems (Anderson 1992, Connolly et al. 1992, Vallino et al. 1996) suggested that the substrate supply and complexity and mineral nutrient availability are the most important variables controlling BGE. However, recent studies have shown that the inorganic nutrients and not the carbon, which has been traditionally assumed, controlled BGE in many of the estuarine environments (Zweifel et al. 1993, Caron et al. 2000).

The production of heterotrophic bacteria in aquatic systems is related to phytoplankton, primary productivity and, dissolved and particulate organic matter (Cole et al. 1988, Biddanda et al. 1994). Biddanda et al. (1994) indicated that carbon flow through bacterioplankton could account for 25% of PP at the highly productive shelf region and 69% of PP at the less productive slope region. Simon and Tilzer (1987) calculated 40% of the PP to pass through bacterioplankton in Lake Constance (Germany). In the western and eastern parts of Mediterranean Sea 110% and 170% of primary production respectively is reported to support the bacterial carbon demand (Turley et al. 2000). Primary production flow into the microbial food web is on a greater proportion in the eastern in

Mediterranean Sea than in the western. Cole et al. (1988) suggested that 60% of PP would be fluxed through bacterioplankton when reviewing data from a large number of marine and freshwater systems. However, recent studies indicate that bacteria in marine systems may consume DOC in excess to primary production, and thus could lead to ecological imbalances (Hopkinson 1985, Findlay et al. 1991). Studies have indicated that excess DOC in estuarine and coastal waters is mainly driven from allochthonous inputs i.e. through riverine runoff, waste discharge etc. As a result uncoupling between bacteria and phytoplankton has been reported (Findlay et al. 1991, Goosen et al. 1997). Studies have indicated that uncoupling due to allochthonous inputs could occur at specific times of the year (Kepkay et al. 1993), or seasonally (Lovejoy et al. 1996) and also due to variations in hydrodynamic conditions (Cho et al. 1994).

Depending on the amount of carbon flow and respiration the trophic status of the system changes. The need for importance of BR estimation in microbial ecology studies is now being felt as it has been found that bacterioplankton account for most of the total community respiration in marine ecosystems. It has been found to have profound effects on the overall carbon and gas balance in aquatic systems (Jahnke and Craven 1995, Sherr and Sherr 1996). Although, a number of approaches have been used to measure DO, most of the researchers needing high accuracy or precision still rely on the titrimetric method of Winkler (Aminot 1988, Carignan et al. 1998). Studies have indicated that respiration measured from oxygen consumption in the dark could be used as an index to estimate the metabolic activity of bacteria in coastal waters, which can

be directly related to the oxidation of organic matter (Hopkinson 1985, Biddanda et al. 1994). It is important to ascertain the role of bacterioplankton in overall community respiration also because nearly half of marine primary production is estimated to flow through bacteria each day (Williams 1981, Cole et al. 1988). The main drawback in most of the studies involving bacteria mediated carbon flux is the lack of methodology for the precise and accurate estimation of bacterial respiration (BR) in aquatic systems. The sources or sinks in marine ecosystem are based on the net ecosystem production (NEP) measurement, which is calculated as the difference between production and respiration. NEP measurements are indicative of trophic status of an ecosystem (Odum 1956) and can be positive indicating net autotrophy and negative indicating net heterotrophy. In the recent years there has been a great debate on the role of planktonic communities as sources (del Giorgio and Cole 1998, Duarte et al. 2001) or sinks (Williams 1998, Williams and Bower 1999) of carbon in marine systems. Studies have indicated that NEP to be highly variable and attributed it to the season, salinity or depth (Nixon and Pilson 1984, Smith and Hollibaugh 1997, Caffrey et al 1998, Serret et al. 1999). Previous studies on this aspect indicate that most of the coastal and estuarine systems are net heterotrophic i.e. oxidation of organic matter is higher than the input (Smith and Hollibaugh 1993, Kemp et al. 1993). Studies indicated that the ratio of inorganic to organic nutrient inputs is very important in controlling eutrophication and oxygen depletion in coastal waters (Billen and Garnier 1997, Nixon 1995). Peierls et al. (1991) showed that the increase in the eutrophication was due to increased inorganic

nutrient loading and tied to changes in human population in Chesapeake Bay. Baines et al. (1994) indicated that understanding the metabolic balance of organic carbon will be useful in predicting POC deposition from pelagic to benthic system in coastal environments which would lead to decreased oxygen levels. The above aspect of study has been the prime focus in several international on going research programs such as Land Ocean Interaction in the Coastal Zone (LOICZ), European Land Ocean Interaction Studies (ELOISE), Joint Global Ocean Flux Study (JGOFS), NSF's Land Margin Ecosystem Research (LMER) program as it deals with carbon metabolism and budgeting. The level of carbon exchange in the biotic systems and the modification of these exchanges by human interventions leading to imbalance in carbon cycling has been an important agenda for the implementation of Kyoto protocol, agreed by parties of the Framework Convention for Climate Change (Canadell and Mooney 1999)

2.3 NATIONAL SCENARIO

Microbiological studies of the tropical marine ecosystems particularly with reference to Indian waters started as early as in the late 1940's with first detailed work on the marine microbes by Velankar in 1950. It was however not until 1970s that the varied potential aspects of the marine microbiology were explored into. While some of the work continued to deal with the microbiology of stored sea food and their shelf life vis-à-vis the microbial attacks, the others concentrated on the distribution of heterotrophs and their physiological groupings. Investigation on diverse groups of organisms such as bacteria, actinomycetes, yeasts, fungi etc. has been carried out both in the east and west coasts of India (Nair and Loka

Bharathi 1977, Nair 1979, Nair and Loka Bharathi 1982). While bacteria have been dealt extensively, studies on fungi and yeasts were restricted to the coastal waters. Apart from bacteria, the ecology and the potential of a novel group of marine protists namely, Thraustochytrids for biotechnological applications in the production of polyunsaturated fatty acid has been dealt in detail in a recent review (Raghukumar 2002). In the east Coast most of the work is concentrated in the Vellar estuary and its adjoining Pitchavaram mangrove areas. These include aspects such as arlysulfatase producing bacteria, chitinoclastic bacteria, luminous bacteria, phosphate solubilzing bacteria, nitrifying bacteria etc. (Dhevendran et al. 1985, Lakshmanaperumalsamy 1983, Venugopalan and Ramesh 1982, Lingaraja et al. 1976, Rajendran 1977). The distribution and role of specialized group of bacteria namely, methanogenic bacteria have been reported by Mohanraju and Natarajan (1992) from the mangrove sediments of Pitchavaram.

Some heterotrophs can exist as epiphytes on the surface of marine fish and crustaceans and this aspect of marine microbiology continues to hold interests. Studies on indicator groups of health significance (Mohandass et al. 2000) and stored sea-food (Karunasagar 1988) have also been carried out.

Microbiological investigation in the Mandovi-Zuari estuarine system started with the study on the distribution of total heterotrophic and coliform bacteria (Row 1981). Studies on the specialized groups of bacteria like phenol-degrading bacteria (Gomes and Mavinkurve 1982) and luminescence bacteria (Ramaiah and Chandramohan 1993) were also carried out. Subsequent studies

included on various physiological groups like sulphate-reducing bacteria (SRB), sulphide oxidizers (SO) and denitrifying bacteria (Loka Bharathi et al. 1991). In addition to understanding of the nature of microbes and their role in culture isolation, attempts have also been made to understand their activity *per se* under *in vivo* conditions. These include the experimental studies on dehydrogenase activity, sulphate-reducing activity, and nitrate reducing activity. The associations of SRB with chemosynthetic sulphur oxidizers like *Thiobacillus* sp. and photosynthetic forms like *Chromatium* sp. (Loka Bharathi 1989), phosphate solubilizers (DeSouza et al. 2000) have been studied in this tropical Mandovi-Zuari estuarine system. Work pertaining to the above in coastal ocean has been very little, until the recently concluded Indo-US project on Trophic Dynamics, which aimed at studying the concept of microbial loop in these waters. Work carried out under this investigation showed variability in abundance and physiological status of particle associated bacteria in these waters (DeSouza 2000). Recently, Ram et al. (2002) indicated BGE to be controlled by substrate supply and grazing activity.

2.4 COASTAL ZONE MANAGEMENT

Applications of the above concept in the management of the coastal and estuarine waters are limited and only a few have been carried out. Studies on the link between human activity and coastal eutrophication in response to changes in land use and water management in the water shed area of Phison river plume was carried out by Billen and Garnier (1997). The impact of terrestrial inputs (Connolly et al. 1992) and riverine discharges leading to massive foam

accumulation (Cadee and Hegeman 1993), and oxygen depletion in bottom waters (Marchetti 1992) in the coastal areas have been well documented. Houde and Rutherford (1993) indicated the importance of the above study in the management of potential fishery resources and harvest. Modeling of survival of enteric bacteria in the coastal waters and relating it to the organic matter concentration like waste discharge have been dealt extensively by a number of workers (Bell et al. 1992).

Recent budget studies at both regional and global scales show that only a limited fraction of nutrients discharged into surface water by human activity ultimately reaches the open ocean, most of them being trapped or eliminated in wetlands, river channels, reservoir estuaries or the coastal zone itself (Howarth et al. 1996).

Several workers defined the above concept in various ways, as mentioned below.

Cairns (1977): "ability of the ecosystem to receive waste discharge without significant alteration of the structure and /or function of indigenous community"

GESAMP (Joint Group of Experts on the Scientific Aspects of Marine Pollution, 1986): "ability to accommodate a particular activity or rate of activity (e.g. volume of discharge per unit time, quantity of dredging dumped per unit time, quantity of minerals extracted per unit time) without unacceptable impact". Definitions similar to the above have been putforth by other workers also (Campbell 1981, Portman and Lloyd 1986). In the above studies the availability of oxygen has been viewed as a prime factor controlling the functional integrity of ecosystem (Cullen 1985).

Stebbing (1992): "ability of a receiving system or ecosystem to cope with certain concentrations or levels of waste discharges without suffering any significant deleterious effects".

Study on this concept started in the late seventies (Cairns 1977, 1981) and later it came into limelight with numerous studies concentrating on it in the mid eighties (Cullen 1985, GESAMP 1986, Portmann and Lloyd 1986). Around this time studies pertaining to assimilative capacity of riverine and stream ecosystems were also conducted (Cullen 1985, Campbell 1981). Later on the importance of applying this concept to the marine environment was considered seriously because of increasing pollution levels in the coastal water body. The assimilative capacity concept with respect to waste disposal for better environmental management was discussed and reviewed by several workers (Preston and Portmann 1981, GESAMP 1986, ICES 1989). In recent times various workers have discussed the concept of assimilative capacity under different heads such as absorptive capacity, environmental capacity, carrying capacity and receiving capacity. The applications of assimilative capacity concept have been dealt by Portmann and Lloyd, (1986) in detail, citing cases of sewage discharge, input of mercury from a sludge dumping operation, discharge of an effluent containing residues from the manufacture of a pesticide, and input of tributyltin from anti-fouling paints applied to boat and ships. The concept of assimilative capacity has been applied widely in the Mediterranean area (Pravdic and Juracic 1988) and in the North Sea (Portmann and Lloyd 1986).

Chadwick and Nilsson (1993) linked the assimilative capacity and critical load concept in environmental management to environmental quality objectives. Contradicting the assimilative capacity concept is another concept termed "precautionary principle" which stresses on the zero percent discharge (Jackson and Taylor 1992). This concept was first developed in Germany and later has been used in the International Treaties and Agreements like North Sea Declaration (1987), United National Environmental Program, (1989), Nordic Council's Conference, (1990), Maastricht Treaty on the European Union, (1994) and Energy Charter Treaty, (1994). Angel and Rice (1996) reviewed the assimilative capacity of the deep ocean with relevance to global waste management. The management of the water bodies for purposes such as navigation, waste disposal, flood control, water abstraction and fisheries can be facilitated by using mathematical models to predict the changes that will result from environmental perturbations. Biochemical oxygen demand (BOD) models have long been established as a management tool and have been used most effectively in monitoring the recovery of a number of grossly polluted estuaries e.g. GEMBASE model.

Though the importance of bacterial compartment in the biological process is well known (Legendre and Rassoulzadegan 1995), studies pertaining to its contribution to assimilative capacity of water body is very much limited even on a global scale. Changes in the environment results in changes in the community structure. Depending on the composition and content of organic matter in an aquatic system, the intensity of biodegradation by bacterial microflora differs as a

function of the biochemical activity of the dominant physiological groups (Rheinheimer and Gocke 1994). The extent of accumulation and transformation of organic matter will be a function of metabolic activity. Estimates of bacterial activity are important in understanding the impact of riverine inputs on coastal processes. Addition of compounds like tannin in the experimental setup resulted in the formation of clumps or tangled chains in gram-negative bacterial forms. Grant and McMurtry (1978) reported the cessation of growth in gram positive and long lag periods in the gram-negative organisms. The fall in the growth rate with the addition of chlorine compounds has also been demonstrated (Carlucci et al. 1989). Pinhassi et al. (1999) studied the short-term changes in the bacterial population using molecular taxonomy with respect to the substrate addition and followed by the changes in the enzyme activity. The potential of the natural bacterial community to degrade refractory substances like chlorinated hydrocarbons with various complexities of the chemical structure was studied by Karthikeyan et al. (1999). Studies pertaining to the changes in the bacterial community structure as a whole or with respect to a single physiological group have been conducted with respect to waste disposal activities like dumping of acid mine drainage (Wassel and Mills 1983), fossil fuel from power plants (Larrick et al. 1981), pharmaceutical wastes (Peele et al. 1981) and coal unloading and sewage dumping activity (Cavari and Colwell 1988). An ongoing programme pertaining to the impact of organic pollutants on microbial community structure is being carried out in the Delaware estuarine ecosystem (Dr. Kirchman, University of Delaware, USA, personal communication) using DNA fingerprinting methods.

The study concentrates on one major class of organic pollutants—polyaromatic hydrocarbons.

In India the concept of assimilative capacity is still in the infant stage and the realization to apply this concept with respect to Indian coastal waters has now been felt. With increasing human activities in our coastal areas it is highly essential to understand the qualitative and quantitative nature of the microbes in our coastal waters. Hence it was appropriate to start a study to assess the waste assimilative capacity of coastal waters and to understand the role of microbes in it. This nature of study on a tropical ecosystem would lead to framing guidelines for better management of aquatic resources. The Department of Ocean Development, Government of India, has initiated studies pertaining to waste assimilative capacity and it is currently in progress on the east coast (Ennore and Tapi estuaries). It deals mainly with modeling.

Different workers have dealt with different approaches in trying to explain the assimilation of organic matter by heterotrophic bacterioplankton. They include

1. determination of bacterial distribution, biomass and its metabolic activity which includes growth rate (Ducklow and Carlson 1992),
2. determination of bacterial growth efficiency (BGE) and the factors involved in controlling or regulating it over spatial and temporal scales (del Giorgio et al. 1997, Roland and Cole 1999, del Giorgio and Cole 2000).
3. role of exo-enzymes involved in the assimilation of high molecular weight compounds by bacteria (Chrost 1991, Hoppe et al. 1998).
4. bacteria-phytoplankton relationship (Conan et al. 1999, Turley et al. 2000)

Being the first of its kind, the present study has integrated all the above four direct approaches for assimilation studies.

Chapter 3

MATERIALS AND METHODS

3.1 STUDY AREA

Three stations were chosen in the tropical Mandovi-Zuari estuarine system located in Goa, central southwest coast of India. They included two in the estuarine (one each in the Mandovi and Zuari estuary) and one in the adjacent coastal waters (Arabian Sea) (Fig. 3.1).

The reasons for choosing the above stations were

1. The Mandovi-Zuari estuarine system is one of the largest riverine network in the westcoast of India. The riverine flow contributes substantial amount of organic matter to the adjacent coastal waters, thus influencing the primary productivity and trophic dynamics of the coastal ecosystem. Understanding this system will help to delineate the importance of riverine inputs on the coastal ecosystem.
2. It is a monsoon driven system
3. Pristine system
4. Logistic criteria

3.1.1 Location and description of the sampling stations

STATION 1: is located at Ribander in the Mandovi estuary at 15° 30.11 N and 73° 52.43 E. The average depth of this station is approximately 4 metres and is mainly influenced by mangrove vegetation. The Mandovi estuary has its origin from the Parwa Ghat of the Karnataka part of Sahyadri hills and joins the Arabian Sea through Aguada Bay after traversing a stretch of about 70 km. Its pre- and post-monsoon flow is regulated by the semi-diurnal tides.

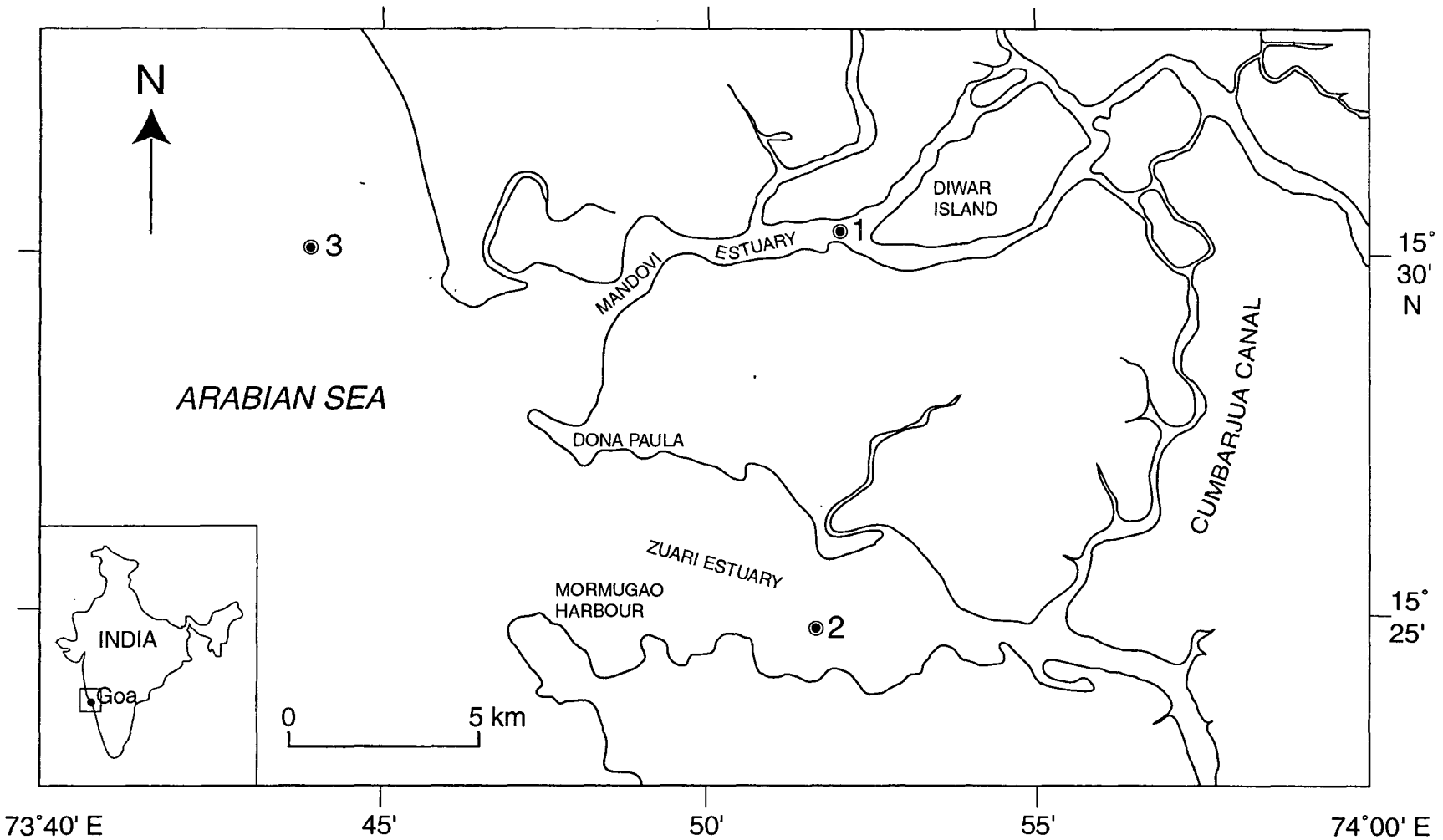

Fig. 3.1. Sampling stations

STATION 2: is located at Chicalim in the Zuari estuary at 15° 25.11 N and 73° 51.47 E. This station is deeper when compared to Mandovi estuary with an average depth of 5.5 metres. Zuari estuary is homogenously mixed throughout, except in the monsoon season when the river become stratified forming a salt wedge. Because this estuary is partially stratified from June to October gravitation circulation plays a role in addition to tidal diffusion and run off (Shetye and Murthy 1987). The riverine inputs during the monsoon season are lower than that of Mandovi. The mouth of Zuari estuary is 20% wider than that of Mandovi estuary, thereby leading to increased salinity.

In both the estuaries, the quantity of freshwater influx or riverine runoff is maximum during the monsoon months with the discharge rate of 175 and 125 m^3 s^{-1} in the Mandovi and Zuari estuaries respectively (Unnikrishnan et al. 1997). In the non-monsoon months the freshwater flow is negligible. The dilution factor in both the estuaries are similar and vary from 1.2 to 0.8; highest values occurring during the monsoon season (Qasim and Sengupta 1981). Based on its environmental characteristics this estuarine system is classified as a tide dominated coastal plain estuary and geo-morphologically identified as drowned river valley estuaries (Murty et al. 1976). The average rainfall in the two river basins varies from 3 metres downstream to 3.73 metres at their uppermost reaches (Anon. 1979). The Mandovi and Zuari estuaries are used extensively for transport of goods (mainly iron ore), for fishing, and for dumping domestic and industrial waste.

STATION 3: is located in the adjacent coastal waters (Arabian Sea) at 15° 30.32 N and 73° 44.02 E. It lies 5 km away from the nearby Aguada coast and is the meeting points of Mandovi and Zuari estuaries. The average depth of this station is 15 metres and lies at a distance of 12 and 16 km from Mandovi and Zuari stations.

Laboratory experiments were performed with water samples collected from Dona Paula Bay located at 15°27'N and 73°48'E in the Mandovi-Zuari estuarine system.

The position of the three stations was determined by Global Positioning System (GPS) (Magellan GPS NAV 5000^{TM}, USA).

3.2 STUDY PERIOD

In the present study, field observations were carried out for a two-year period at the selected stations from January 1998 to December 1999. Sampling was carried out on monthly basis in the first year (1998) (January to October) and once in three months (February, May, August, October and December) in the subsequent year (1999). The study period was grouped according to three distinct seasons, namely Pre-monsoon (February to May), Monsoon (June to September) and Post-monsoon (October to January). Due to navigational constraints, the coastal station (Station 3) could not be sampled during the monsoon seasons due to rough sea conditions and also sand bar formation at the mouth of the estuaries.

3.3 SAMPLING STRATEGIES AND PRE-TREATMENT OF THE SAMPLES

Water samples, both surface and bottom were collected with Niskin water sampler (5-litre capacity, Niskin Corporation, Montana, USA). The water samples were prescreened through 200 μm nylon mesh on board to remove larger organisms like zooplankton, fish larvae etc. The water samples collected were stored in clean plastic carbuoys and transported to the laboratory for analyzing physico-chemical, biological and microbiological parameters taking necessary precautionary measures.

3.4 PHYSICAL PARAMETERS

The depth of the water column was measured with a rope graduated at 0.5m interval suspended with a dead weight. Water temperature was measured on board with the aid of a field thermometer and light penetration (in centimetres) in the water column was determined by using a Secchi disc.

3.5 CHEMICAL PARAMETERS

Salinity was measured using a hand refractometer (ATAGO 2442-W01) calibrated to zero with distilled water. pH was measured using a digital pH meter (Toshnival Digital pH Meter CL-46) after calibrating it with the standard buffers of pH 4, 7, and 9.2 respectively.

3.5.1 Dissolved oxygen (DO)

The dissolved oxygen (DO) concentration in the water samples was estimated using Winkler's titrimetric method (Carpenter 1965). Water samples were collected in 125 ml acid washed (10% HCl) glass-stoppered bottles and fixed immediately on board with 1 ml of manganous chloride (3M) and 1 ml of

alkaline iodide (8M-4M) solution (Winkler's reagents). The samples were mixed and the precipitate was allowed to settle. On arrival at the laboratory, 1 ml of sulphuric acid (10N) was added and the sample was titrated with 0.01N sodium thiosulphate using starch as indicator. The procedure was standardised by using potassium dichromate. Results were expressed as mg l^{-1}.

Percent oxygen saturation (O_s) was calculated from the equation

$$O_s = (O_m/SOC)/ \times 100$$

where O_m is measured dissolved content and SOC is the saturation oxygen content (deduced from salinity and temperature measurements).

3.5.2 Biochemical oxygen demand (BOD)

Water samples filled in 300 ml capacity acid washed standard BOD glass bottles were incubated at 20 °C for five-day period. DO level was estimated by Winklers method at the initial and final incubation time. BOD was calculated by the difference in the values and was expressed as mg l^{-1}. The amount of labile organic material was calculated by multiplying the BOD values with a factor value of 0.7 (Gocke and Hoppe 1977)

3.5.3 Chemical oxygen demand

The estimation of dissolved organic substances in the water samples was carried out following the method of Gocke and Hoppe (1977). The water samples were treated with exact volume of 0.01N potassium permanganate solution (oxidant) followed by 4% sodium hydroxide. After boiling in the water bath for 30 minutes, 4N sulphuric acid followed by 250 mg of potassium iodide was added after the contents were brought to room temperature. The iodide liberated was

titrated against 0.01N sodium thiosulphate solution using starch as indicator. Blank was also maintained in the same manner omitting brackish water and boiling. The difference between the two titers gave the amount of permanganate reduced by the sample water. The results were expressed as mg organic C l^{-1}. The procedure was standardized by repeating with known amount of glucose (1mg l^{-1}) instead of sample water.

3.5.4 Nutrient analysis

Samples for nutrient analysis except ammonia were stored at –20 °C and were later done within 48 hour of sampling.

3.5.4.1 Ammonia

The ammonia in the water sample was estimated by indo-phenol blue method (Koroleff 1969). Ammonia reacts in moderately alkaline solution with trione (sodium dichloroisocyanurate) to form monochloramine, which in the presence of phenol and catalytic amounts of nitroprusside ions gives indophenol blue. Spectrophotometric measurement (Shimadzu 1201V, Japan) was done after six hours of incubation at 630 nm. Freshly prepared distilled water was taken as blank. Samples were analyzed every time with freshly prepared reagents. Ammonium chloride was used a standard. The results were expressed in micromolar (μM).

3.5.4.2 Nitrite

The amount of nitrite in the water sample was estimated following the procedure of Bendschneider and Robinson (1952). The nitrite is allowed to react with sulphanilamide in an acid solution. The resulting diazo compound reacts

with N-(1-naphthyl)-ethylenediamine and forms a highly coloured azo dye. The extinction was read in a spectrophotometer at 543 nm. Standards were run with analytical reagent quality sodium nitrite. Results are expressed in μM.

3.5.4.3 Nitrate

Nitrate in the sample was estimated by passing the water sample through cadmium-copper column, where the nitrate chemically gets reduced to nitrite. The nitrite estimation was carried out as mentioned above. Potassium nitrate was used for standardization.

3.5.4.4 Phosphate

Inorganic phosphate was estimated using the modified method of Murphy and Riley (1962). Phosphate and ammonium molybdate are allowed to react in acid solution to give phosphomolybdic acid, which is reduced by ascorbic acid. Optical density using a spectrophotometer as done after 10 min at 882 nm using a 10 cm quartz cell. Potassium dihydrogen ortho-phosphate was used for standardization. The values were expressed in μM.

3.5.4.5 Total organic carbon (TOC)

For total organic carbon (TOC) analysis, 30 ml of water samples were transferred to clean polycarbonate bottles and immediately acidified with 1 ml of 80% ortho-phosphoric acid (pH=2) and stored in polycarbonate bottles at 4 °C. Samples were analyzed within a month from the date of collection.

TOC content in the water samples was determined with a commercial Shimadzu TOC-5000 organic carbon analyzer (Sugimura and Suzuki 1988), working under the principle of high temperature catalytic oxidation (HTCO). Prior

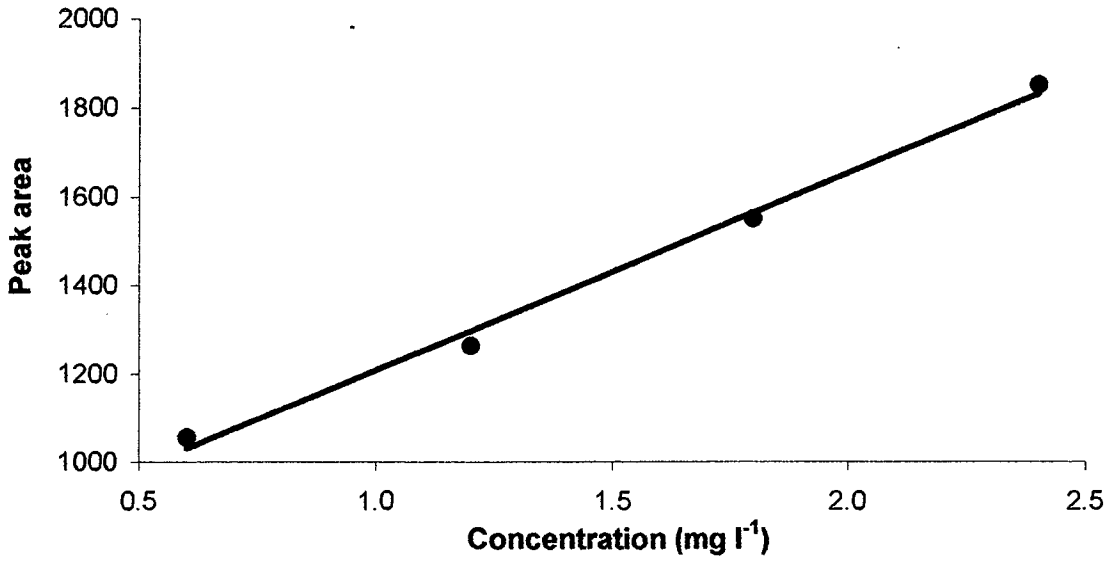

Fig. 3.2. Standard curve for organic carbon

combusted (850 °C, 1 hour) nickel sleeves. Acetanilide standard and blanks (empty Ni leeves) are measured prior to each batch run of samples.

3.5.4.7 Dissolved organic carbon (DOC)

DOC was calculated by subtracting POC from TOC.

3.6 BIOLOGICAL PARAMETERS

3.6.1 Chlorophyll *a* and phaeopigments

Chlorophyll *a* was estimated based on the fluorimetry method as suggested by Yentsch and Menzel (1963). A known amount of water sample (250 ml) containing chlorophyll pigments was filtered in diffused light condition through glass fibre filter (Whatman GF/F). Pigments concentrated on the filter was extracted for $\geq$24 h in ice cold 90% (v/v) acetone and was determined fluorometrically using Turner Designs fluorometer 10-000R at an excitation wavelength of 430 nm and an emission wavelength of 670 nm. Standardization was carried out by using chlorophyll *a* standards (Sigma, USA). Phytoplankton carbon was calculated by multiplying chl *a* value with a factor value of 50 (Cho and Azam 1988, Sherry et al. 1999).

For estimation of phaeopigments, the above sample was acidified with two drops of 1.2M HCl and determined fluorometrically. Both chlorophyll *a* and phaeopigments values were expressed as $\mu g\ l^{-1}$.

3.6.2 Primary Productivity

Primary production was measured by ^{14}C assimilation method (Knap et al. 1996). Three light and one dark 125 ml acid washed bottles were filled with water samples. After inoculation with labeled $NaH^{14}CO_3$ (Activity: 5 $\mu Ci\ ml^{-1}$, BARC,

Mumbai) samples were incubated for four hours at *in situ* light intensity and cooled by running seawater. Following retrieval, the water samples were filtered immediately through 0.45 μm filter (Millipore, GS type) under diffused light and low pumping pressure (<100 mm Hg). Radiolabeled dissolved inorganic carbon ($DI^{14}C$) was removed by exposing the filter to fumes of concentrated HCl for a minute. The filters were then placed in scintillation vials and 5ml of scintillation cocktail in dioxane (Spectrochem, Mumbai) were added. Radioactivity was measured in a liquid scintillation counter (LKB Wallac 1209). The dissolved inorganic carbon (DIC) concentration ranged from 75 to 95 mg CO_2 l^{-1} during the non-monsoon and 15-20 mg CO_2 l^{-1} during the monsoon seasons. The above values were used for the calculation of primary productivity and the results were expressed as μg C l^{-1} h^{-1}

3.6.3 Bacteriological parameters

3.6.3.1 Total bacterial counts (TC)

Samples for total bacterial counts were fixed immediately with buffered formalin on board and stored at 4 °C upon arrival to the laboratory.

Bacterial abundance was determined by acridine orange direct count method (Hobbie et al. 1977). Water samples (2 ml) preserved with 2% (final concentration) buffered formalin were stained with acridine orange (Hi-Media, Mumbai) (final concentration 0.01% w/v) for five minutes before filtering it through 0.22 μm black stained Nucleopore filter (Whatman Asia Pacific, Singapore). Samples were enumerated at 1250x magnification in an Olympus (BH) epifluorescence microscope, using a 515 nm barrier filter and at least 10 fields of

>30 bacteria field^{-1} were counted. Bacterial abundance was expressed as numbers per litre. The bacterial numbers was converted into biomass using a conversion factor of 2 x 10^{-14} g C cell^{-1} (Lee and Fuhrman 1987).

3.6.3.2 Direct viable counts (DVC)

DVC were made following the method of Kogure et al. (1984). Water samples were incubated at 20 °C for 8 h with nalidixic acid (0.02% w/v), piromidic acid (0.001% w/v), pipemidic acid (0.01%w/v) (Sigma, USA) and yeast extract (0.01%). Incubation was terminated by the addition of buffered formalin (final concentration 2%). Samples were stained with acridine orange (as mentioned for TC) and observed under epifluorescence microscope. Only swollen and elongated cells were enumerated as viable bacterial cells and their counts were expressed as numbers per litre.

3.6.3.3 Retrievable counts (Colony forming units, CFU)

For the enumeration of colony forming units (CFU), water samples were serially diluted in autoclaved 50% seawater blanks. 100 µl (10 or 100 fold dilution) of the sample was surface plated on Nutrient Agar medium (NA) (Hi-Media, Mumbai) prepared with 50% seawater. The plates were incubated for 36-48 h at room temperature (28 ± 2 °C). Bacterial colonies were enumerated and expressed as colony forming units (CFU) l^{-1}.

Bacteria strains were isolated at random from distinct colonies. These were transferred to NA plates and upon their growth they were checked for purity on the basis of microscopic examination. The isolates were stored in NA slant tubes at 4 °C for taxonomic identification and characterization.

The bacterial isolates were identified up to generic level following the scheme of Oliver (1982). A total of 44 morphological and cultural tests were carried out for the characterization of the strains. They included cellular morphology, gram staining, colony morphology and colour, production of catalase, oxidiase, gelatinase, amylase, phosphatase, urease, lipase, DNAse, protease; ability to oxidase or ferment glucose, and ability to grow under different temperature (4 and 37 °C), pH (4.1, 7.8 and 9.2) and salinity (0, 35, 50) conditions. The ability to utilize various carbon sources was done using API 20E strips. Sensitivity tests to twelve different antibiotics were also done.

3.6.3.4 Bacterial Productivity (BP)

BP was estimated by the incorporation of the nucleoside ^{3}H-Thymidine into the bacterial DNA (Fuhrman and Azam 1982) and ^{14}C leucine into bacterial protein (Smith and Azam 1992).

3.6.3.4.1 ^{3}H Thymidine incorporation

Saturation experiment

In order to determine the concentration of labeled thymidine required to saturate thymidine uptake, saturation experiments were run by measuring uptake over a thymidine concentration series ranging from 0.5 to 15 nM. The expected Michaelis-Menten function defined saturation was found to occur at 8 nM (Fig. 3.3).

Determination of Conversion factor (CF) for thymidine incorporation

Conversion factor (CF) for thymidine incorporation was calculated according to an equation derived by Kirchman et al. (1982): $CF = (\mu N_0)\ (v_0^{-1})$,

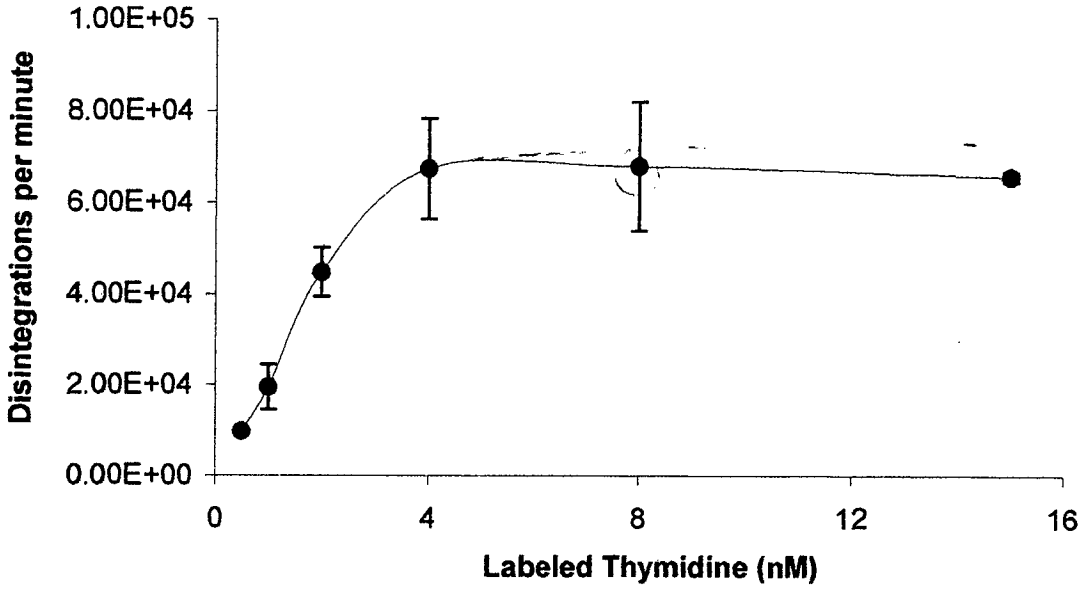

Fig. 3.3. Saturation curve for labeled thymidine. Mean (±SD)

where v_0 was the initial thymidine incorporation rate, μ is the growth rate and N_0 is the initial bacterial abundance. The CF obtained in the pre-monsoon, monsoon and post-monsoon seasons were 1.99 ±0.06, 1.95 ±0.16 and 1.92 ±0.21 respectively. The CF did not vary much (CV%= 5) and so an average CF value of 1.96×10^{18} cells per mole of thymidine incorporated was applied in the present study for calculation of BP.

Productivity estimation

Seawater samples (30 ml) in triplicates were incubated for an hour in the dark with ^{3}H-thymidine with a final conc. of 8 nM (Specific activity =52 Ci mmol^{-1}, BARC, Mumbai). Incubations were terminated with addition of 2% neutral-buffered formalin. The samples were then filtered through 0.22 µm filter (Millipore, GS type) (presoaked with 5% Trichloroacetic acid), extracted in cold 5% TCA and rinsed with 80% ethanol. The dried filters were placed in scintillation vials and filled with 5 ml of dioxane-based scintillator (Spectrochem, Mumbai) and radioactivity was measured in a liquid scintillation counter (Wallac LKB 1209) with external standards. Thymidine incorporation rates were converted to production rates in terms of cells and carbon by using a thymidine conversion factor (CF) of 1.96×10^{18} cells per mol of thymidine incorporated (this study) and a carbon conversion factor of 2×10^{-14} g C cell^{-1} (Lee and Fuhrman 1987).

3.6.3.4.2 ^{14}C-Leucine incorporation

Saturation experiment

In order to determine the concentration of labeled leucine required to saturate uptake, saturation experiment was carried out by measuring uptake over

a leucine concentration series ranging from 1 to 40 nM. The expected saturation concentration of Michaelis-Menten function occurred at about 20 nM (Fig. 3.4).

Productivity estimation

Bacterial production was estimated from the rates of protein synthesis (Kirchman et al. 1985) as measured by the incorporation of ^{14}C leucine (specific activity= 300 mCi $mmol^{-1}$; BARC, Mumbai). To 30 ml of water sample (in triplicates) ^{14}C leucine (final conc. 20 nM) was added and incubated for a period of one hour at an ambient temperature (27 $\pm$1 °C). The activity was stopped by the addition of 50% TCA (5% final concentration.). The samples were then filtered through 0.22 μm membrane filter (Millipore, GS type) and rinsed with 5% cold TCA for 5 minutes, followed by 80% cold ethanol. The dried filter was then placed in scintillation vials filled with 5 ml of dioxane based liquid scintillator (Spectrochem, Mumbai) and the radioactivity was measured in a liquid scintillation counter (LKB Wallac 1209). Bacterial production was calculated using the conversion factor of 3.1 kg C per mol of leucine incorporated (Simon and Azam 1989). The extent of isotopic dilution was not measured during this study but a dilution factor of 2 (Roland and Cole 1999) was used as the average salinity was >25.

For the above study, abiotic absorption (controls) of the labeled substrates was estimated by measuring the incorporation of radiolabeled substrates in samples previously fixed with buffered formalin. All samples were corrected for abiotic incorporation by subtracting the radioactivity in formalin-killed controls.

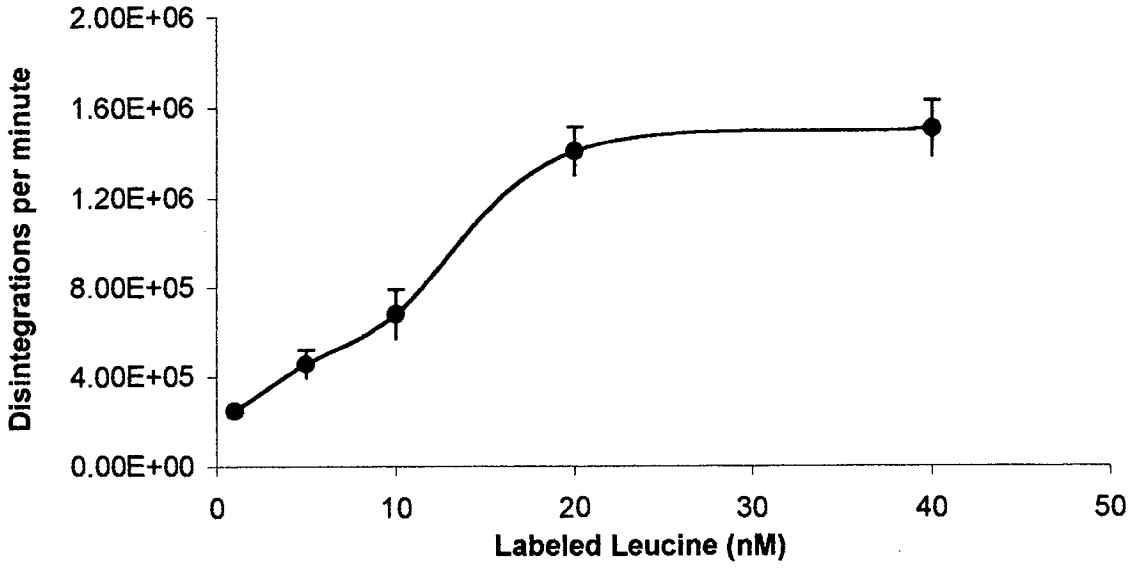

Fig. 3.4. Saturation curve for labeled leucine. Mean (± SD)

3.6.3.5 Bacterial respiration (BR)

BR was estimated from oxygen consumption in the water samples incubated under dark conditions (Hopkinson et al. 1989, Griffith et al. 1990). It was calculated by estimating dissolved oxygen (DO) in BOD bottles using Winkler's method before and after the incubation of water samples. Respiration estimated from oxygen consumption has been used for bacterial respiration. For control (zero hour), the samples were fixed immediately with Winklers reagents. For time series measurements the BOD bottles with the samples were incubated in the dark at ambient temperature for 24-hour period. DO was measured at six hour interval in triplicates. Total respiration was calculated as μg oxygen consumed per litre per hour by subtracting the amount of dissolved oxygen remaining after the incubation time.

Preliminary studies showed that water samples incubated in the dark for 24 h was sufficient to detect a considerable decrease in DO level. Laboratory experiments indicated that the utilization of DO in the unfiltered and filtered (GF/F) water samples were linear up to a period of 36 h (Fig. 3.5). The oxygen used up was converted to carbon respired assuming a respiratory quotient (RQ) of 1 (Biddanda et al. 1994).

3.6.3.6 Bacterial growth efficiency (BGE)

Bacterial growth efficiency (BGE) of the natural bacterial assemblage to natural and added dissolved organic matter was estimated based on productivity rates and respiratory measurements. It was calculated by dividing bacterial

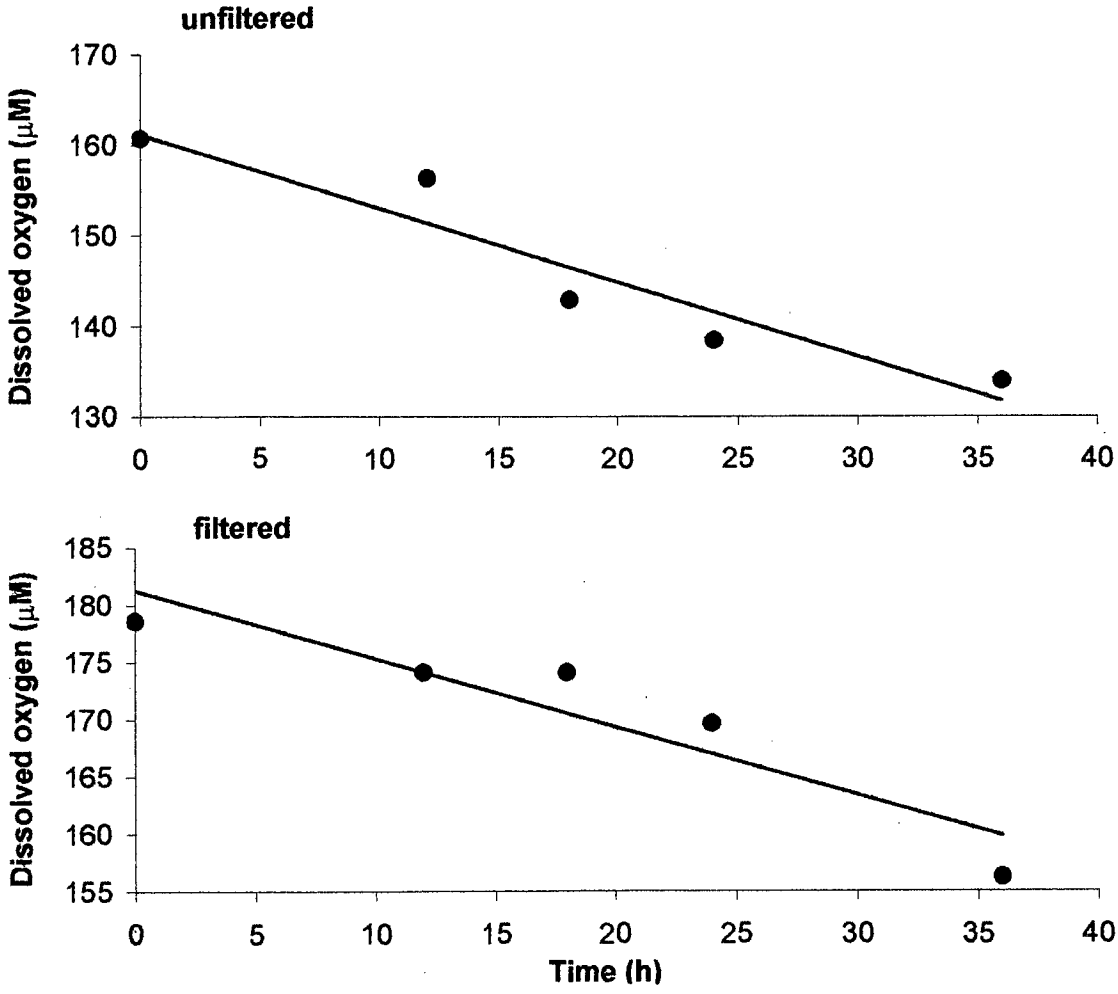

Fig. 3.5. Dissolved oxygen concentration in the unfiltered and filtered water samples with time

production by the sum of bacterial production and respiration (Roland and Cole 1999)

$$BGE\ (\%) = [BP/(BP+BR)] \times 100$$

Respiration and production measures were expressed in $\mu g\ C\ l^{-1}\ h^{-1}$.

3.6.3.7 Specific growth rate (μ)

For field observations, specific bacterial growth rate (SGR) denoted as 'μ' of bacterioplankton was calculated by dividing bacterial production by bacterial biomass and was expressed per day.

For time series laboratory experiments, SGR (μ) was calculated as the slope of the 'ln' transformed bacterial numbers over time during the exponential growth phase. The equation based on exponential growth is expressed as

$$\mu = (\ln N_2 - \ln N_1)/T$$

where 'N_1' and 'N_2' are the bacterial numbers at the beginning and end of an incubation period, 'T'. Linearity of the regression line for time versus logarithm of bacterial cell numbers were always significant, with the regression coefficient being higher than 0.9 for at least 3 samples.

Generation time

Generation time or doubling time was calculated by $\ln 2\ \mu^{-1}$.

3.6.3.8 Heterotrophic activity

The heterotrophic activity of the bacterioplankton was measured by using ^{14}C glucose and ^{14}C glutamic acid isotopic dilution method (Hobbie and Crawford 1969). To 30 ml of each water sample 10, 50 and 100 μl of substrate (^{14}C glucose) at a final concentration of 2.26, 11.3 and 22.6 $\mu g\ C\ l^{-1}$ and ^{14}C glutamic

acid at a final concentration of 0.53, 1.31 and 2.62 μg C l^{-1} was added. A strip of zigzag filter paper (Whatmam No.1) was soaked in 150 μl of ethanolamine and placed in scintillation vials for respiration measurements (for absorbing CO_2). The vials were placed suspended into the flasks containing the water sample with substrate. Incubation was carried out for 2 hours and the reaction was terminated by addition of 0.5 ml concentrated HCl to the water samples. Samples were filtered through 0.22 μm (Millipore, GS type) filter and the dried filter was placed in the scintillation vials for assimilation measurements. A known amount (5 ml) of liquid scintillator in dioxane (Spectrochem, Mumbai) was added to scintillation vials (i.e. for assimilation and respiration measurements) and radioactivity was measured using a liquid scintillation counter (LKB Wallac 1209). The turnover time of the substrate was calculated applying Michaelis-Menton kinetics.

3.6.3.9 Ectoenzymatic activity

For determination of ecto-enzymatic activity in water samples, fluorogenic Methylumbelliferyl (MUF) (Sigma, USA) linked substrates were used (Hoppe 1983). They included MUF-butyrate for lipase activity, MUF- β D glucoside for glucosidase activity, MUF-phosphate for phosphatase activity, MUF-Chitobioside for chitinase activity and MUF-aminopeptidase for protease activity. Stock solutions were prepared in either methyl cellosolve or doubled distilled water depending on its nature of solubility. The prepared solutions were stored at –20 °C.

A known amount (10 ml) of water sample was incubated in the dark with 200 μl of substrate (final conc. 5 μM) for 1 hour. The reaction was then

terminated by the addition of Glycine-Ammonium hydroxide buffer of pH 12.5. 200 µl of Tris HCl buffer (pH-10.3) was placed in the cuvette prior to addition of 1ml of the water sample before noting the observation, as maximum fluorescence has been usually found to be obtained at the above mentioned pH. Fluorescence intensity was measure in a spectrofluorometer (Hitachi 2000, Japan) at an excitation of 364 nm and an emission of 445 nm. Controls were run with water samples boiled for 20 min in a water bath prior to addition of the MUF substrates.

For calibration of MUF (which is liberated from the complex in equimolar concentration), a standard curve with different concentration of methylumbelliferone was prepared (Fig. 3.6).

All statistical analyses like correlation, analysis of variance (ANOVA) regression analysis etc. were performed with using MINITAB software for Windows (Release 12, Minitab Inc., 1999) and Microsoft Excel (Microsoft Office 2000 version). Normalization of the data was carried before processing for statistical analyses

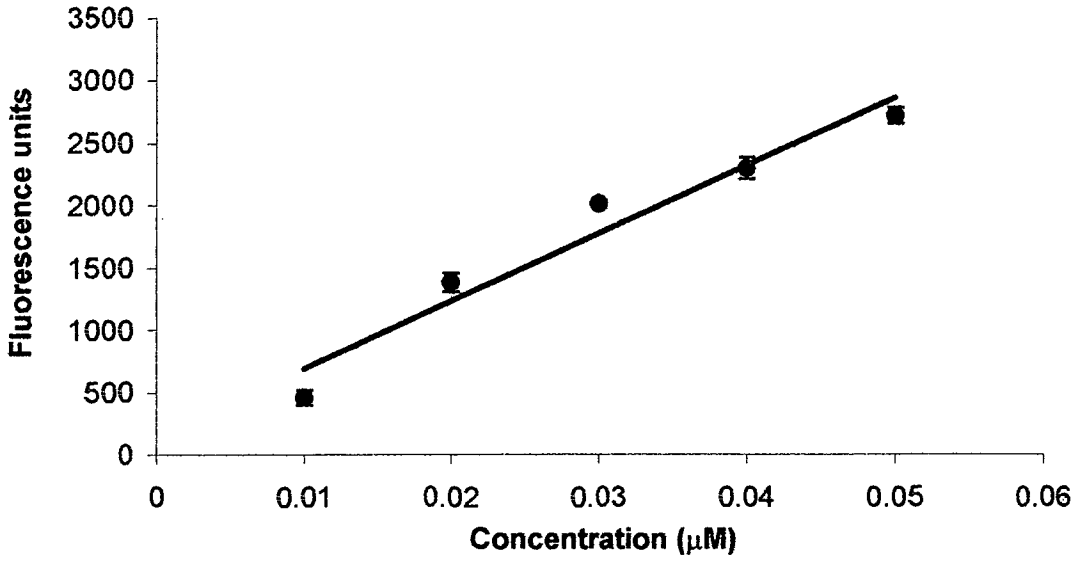

Fig. 3.6. 4-Methylumbelliferone calibration curve

Chapter 4

HYDROLOGICAL CHARACTERISTICS

4.1 INTRODUCTION

The estuary and its adjacent coastal waters referred to as the "coastal ocean" (Holligan and Reiners 1992), display great variability in terms of physical and biogeochemical forcings. Estuarine environments, which have a free connection with, open sea and the river has spatially as well as temporally varying physico-chemical features. They are considered to be the most geochemically and biologically active areas of bioshpere, where large exchange of matter and energy takes place. This exchange between the estuaries and adjacent coastal waters allows rapid changes in salinity, temperature, nutrients and sediment load; this variability has strong effects on both the composition and dynamics of the biota. Many of the estuarine characteristics are shared with the adjacent coastal systems and the combination of characteristics makes each ecosystem unique. Although research has led to the formulation of number of generalizations concerning their structure and function, their uniqueness is largely determined by the set of geomorphological, physical, chemical and biological parameters operating or influencing the system

Inter-relationship between abiotic and biotic variables with bacteria are important to understand the structure and function of an ecosystem, which makes it easier for ecologists, environmentalists to identify ecosystem parts and mechanisms that have to be maintained and protected. Owing to its vast natural resources, coastal environments are greatly under increasing pressure as a result of increasing human population. As our burgeoning coastal population colonises every available ecological niche of the coastal zone, outstripping of

resource is faster, before nature can restore at its best. Increase in human activities like dredging, direct release of industrial effluents, fertilizers and toxic chemicals etc. will significantly influence the hydrological characteristics over time scale. The above activities will thus ultimately have significant impact on the functioning of biogeochemical cycles, which would lead to the lowering of assimilative or recharging capacity of the system. As a result the system will tend to shift its balance from steady state to unsteady state. Study on the above would lead to better understanding of ecobiological processes and relationships, particularly in tropical aquatic systems, which are relatively less known. Such information will also help in understanding the impact of human activity in such ecosystems and thereby predicting changes in them.

This chapter focuses on the hydrological and chemical characteristics observed at the study stations in the Mandovi-Zuari estuarine system over the study period during three distinct seasons. Seasonal variability of different environmental features in the Mandovi-Zuari estuarine system demands an understanding of the freshwater discharge into the system, which is chiefly controlled by the spectacular regime of rainfall during the monsoon, thus making the study period divisible into three distinct seasons: 1) Pre-monsoon (February-May), 2) Monsoon (June-September) and 3) Post-monsoon (October-January).

4.2 RESULTS

4.2.1 Physical parameters

4.2.1.1 Secchi disc or light penetration measurements

The depth of the total water column ranged from 3 to 6 metres at the estuarine stations and from 12 to 17 metres at the adjacent coastal station. On an average Zuari estuary was deeper (4.20 metres) than Mandovi estuary (3.61 metres). Fig. 4.1 shows the monthly variation in secchi disc measurements over the study period at the three stations. In the present study, the Zuari estuary recorded a mean photic depth of 27.16% and 10.48% of the water column during the non-monsoon and monsoon seasons, whereas in the Mandovi estuary the values were 15.40% and 6.57%, respectively (Table 4.1). Although the coastal station was deeper, it did not show any significant difference in light penetration when compared to the estuarine stations during the non-monsoon seasons.

4.2.2.2 Temperature

In the Mandovi-Zuari estuarine system the water temperature mostly ranged from 26 to 32.4 °C during the study period with an annual variation of 5 – 6 °C. Temperature did not differ significantly between the stations. The maximum temperature (33 °C) was observed in the month of May (pre-monsoon) at both the estuarine stations (Fig. 4.2). At the coastal station the difference in temperature from surface to bottom was about 2 °C. In this station a maxima of 33 °C in May (surface) and a minima of 22.4 °C (bottom) in October was recorded. At the estuarine stations there was a progressive increase in temperature from January to May followed by a decline during the peak monsoon

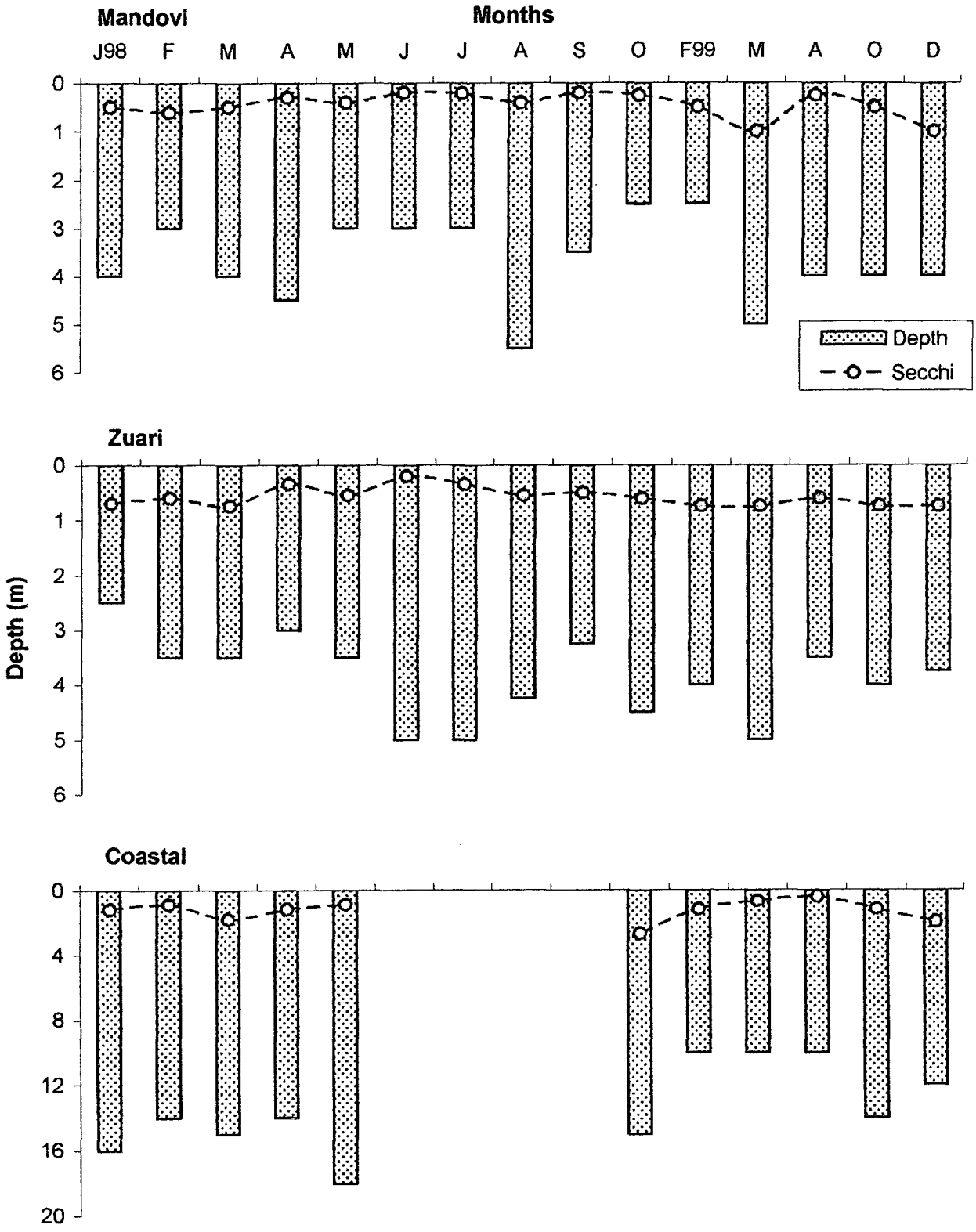

Fig. 4.1. Monthly variation in the water column depth(m) and light penetration(m) (Secchi disc) at the study stations.

Table 4.1. Seasonal variability in the water column depth, secchi depth and light penetration measurements

Station	Period	Depth (m)	Secchi depth (m)	Light penetration (%)
Mandovi	Pre-monsoon	3.40 (± 0.82)	0.55 (± 0.24)	16.17 (± 5.52)
	Monsoon	3.80 (± 0.78)	0.25 (± 0.08)	6.57 (± 0.69)
	Post-monsoon	3.63 (± 0.75)	0.56 (± 0.31)	15.43 (± 6.77)
Zuari	Pre-monsoon	4.33 (± 0.75)	0.63 (± 0.16)	14.55 (± 3.34)
	Monsoon	4.20 (± 0.82)	0.44 (± 0.16)	10.48 (± 5.60)
	Post-monsoon	4.08 (± 0.38)	0.70 (± 0.07)	27.16 (± 6.06)
Coastal	Pre-monsoon	14.50 (± 3.08)	1.12 (± 0.39)	7.72 (± 2.94)
	Monsoon	-	-	-
	Post-monsoon	14.25 (± 1.71)	1.78 (± 0.72)	12.49 (± 6.06)

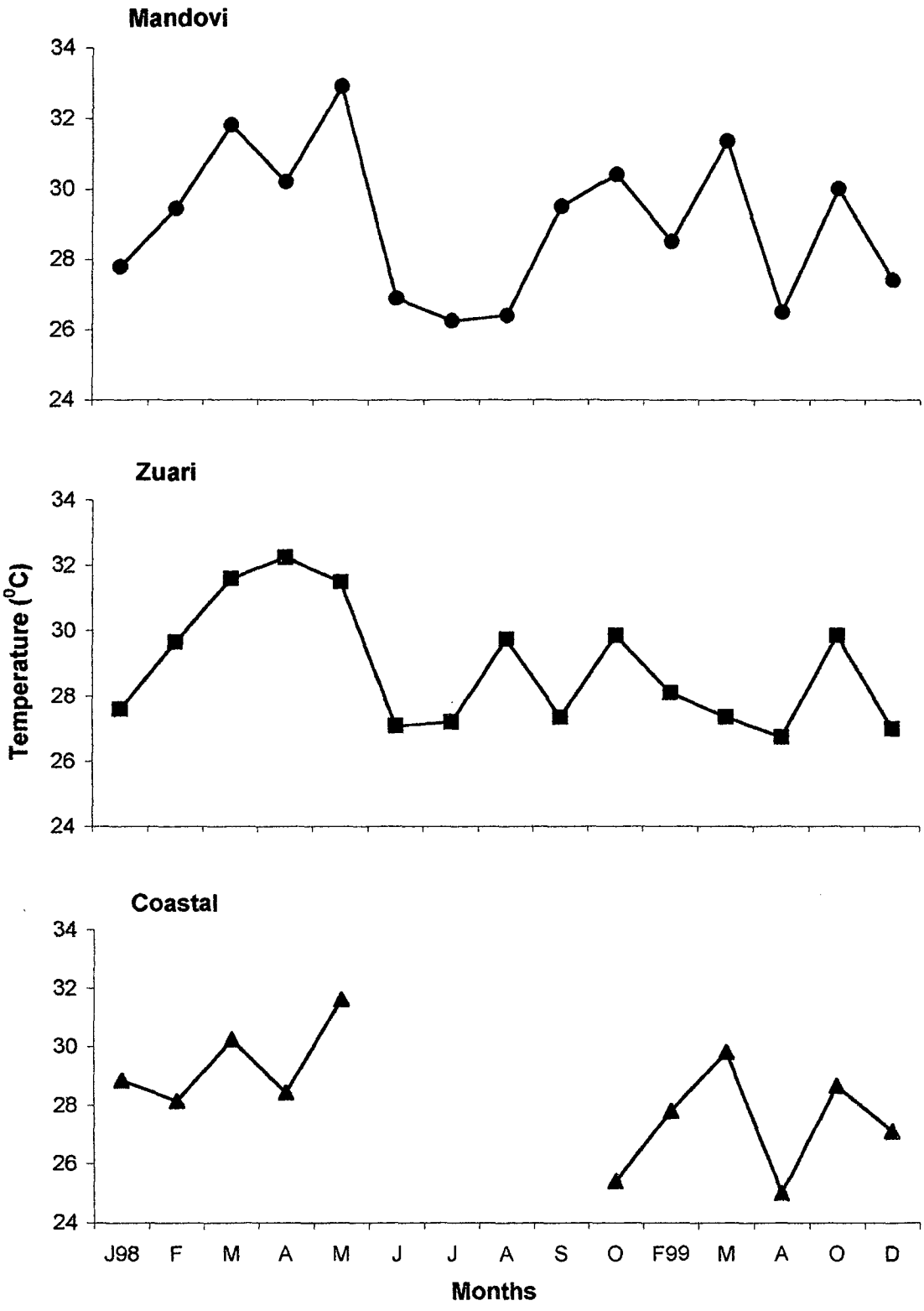

Fig.4.2. Monthly variation in the water temperature (average of surface and bottom)

season. The mean values recorded at the estuarine stations during the monsoon season at surface and bottom is 27.7 and 27 °C respectively (Table 4.2a & b). Analysis of variance (ANOVA) showed water temperature to vary significantly ($p<0.02$) between pre-monsoon and monsoon seasons at both surface and bottom waters in the Mandovi estuary.

4.2.2.3 pH

At the estuarine stations, pH values ranged from 7.28 to 8.14, and did not show any significant variability during the study period. The average values of these estuarine stations are shown in Fig. 4.3. The coastal waters had relatively higher pH with a mean value of 8.09 and 8.05 in the surface and bottom waters. These values were higher and varied significantly ($p<0.006$) with those of the estuarine waters. The seasonal variation in pH values of the three stations is given in Table 4.2a & b.

4.2.2.4 Salinity

Monthly variation in the salinity at the estuarine stations was very large. At both Mandovi and Zuari stations salinity remained fairly high from January to May (Fig. 4.4). It ranged from 29 to 36. With the onset of monsoon the salinity decreased sharply at both the stations. Mandovi estuary did not show any significant variation in salinity between surface and bottom waters. It ranged from 0 to 5.5. In the Zuari estuary, the effect of monsoon on salinity was felt at the surface than bottom waters. At the surface the salinity ranged from 4 to 26, whereas the bottom waters ranged from 25 to 29.2. Seasonal variability was observed between monsoon and non-monsoon seasons. At the coastal station,

Table 4.2a. Seasonal variability in temperature, pH, and salinity measurements in the surface waters

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
Temperature	Range	28.5-33.4	26.0-30.0	27.4-31.2	27.5-30.5	27.2-30.5	27.0-30.5	28.0-33.0	-	27.2-29.0
(°C)	Mean	30.85	27.08	29.15	30.28	28.32	28.80	29.70	-	28.35
	SD	1.73	1.68	1.76	1.35	1.35	1.71	1.91	-	0.81
pH	Range	7.92-8.02	7.36-7.94	7.45-7.90	7.98-8.13	7.64-8.11	7.74-7.97	8.08-8.21	-	8.01-8.1
	Mean	7.97	7.66	7.65	8.06	7.85	7.82	8.12	-	8.05
	SD	0.04	0.24	0.23	0.06	0.19	0.13	0.05	-	0.05
Salinity	Range	23.0-36.0	0-5.5	4.0-31.0	30.0-34.0	4.0-26.0	20.5-34.5	34.0-37.0	-	23.0-35.0
	Mean	30.67	1.50	16.25	32.33	14.40	27.38	35.50	-	30.00
	SD	4.55	2.40	13.33	1.37	9.56	7.65	1.05	-	5.60

Table 4.2b. Seasonal variability in temperature, pH, and salinity measurements in the bottom waters

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
Temperature	Range	28.5-32.4	26.5-29.0	27.4-30.0	27.2-32.0	25.0-29.0	27.0-29.7	27.6-30.2	-	22.4-28.5
(°C)	Mean	30.55	27.14	29.00	30.24	26.94	28.35	28.97	-	25.97
	SD	1.55	0.05	1.40	1.87	1.44	1.30	1.91	-	3.18
pH	Range	7.96-8.02	7.28-7.92	7.63-8.02	7.98-8.14	7.64-8.05	7.89-8.04	8.1-8.21	-	7.73-8.06
	Mean	7.98	7.63	7.81	8.07	7.85	7.96	8.15	-	7.95
	SD	0.03	0.23	0.20	0.07	0.20	0.06	0.04	-	0.19
Salinity	Range	24.0-35.5	0-5.5	10.0-28.0	31.0-34.0	20.5-35.0	29.0-35.0	35.0-36.0	-	23.0-36.0
	Mean	30.92	2.60	20.00	32.40	28.00	32.00	35.33	-	31.66
	SD	4.03	2.53	9.17	1.14	5.71	2.94	0.52	-	7.23

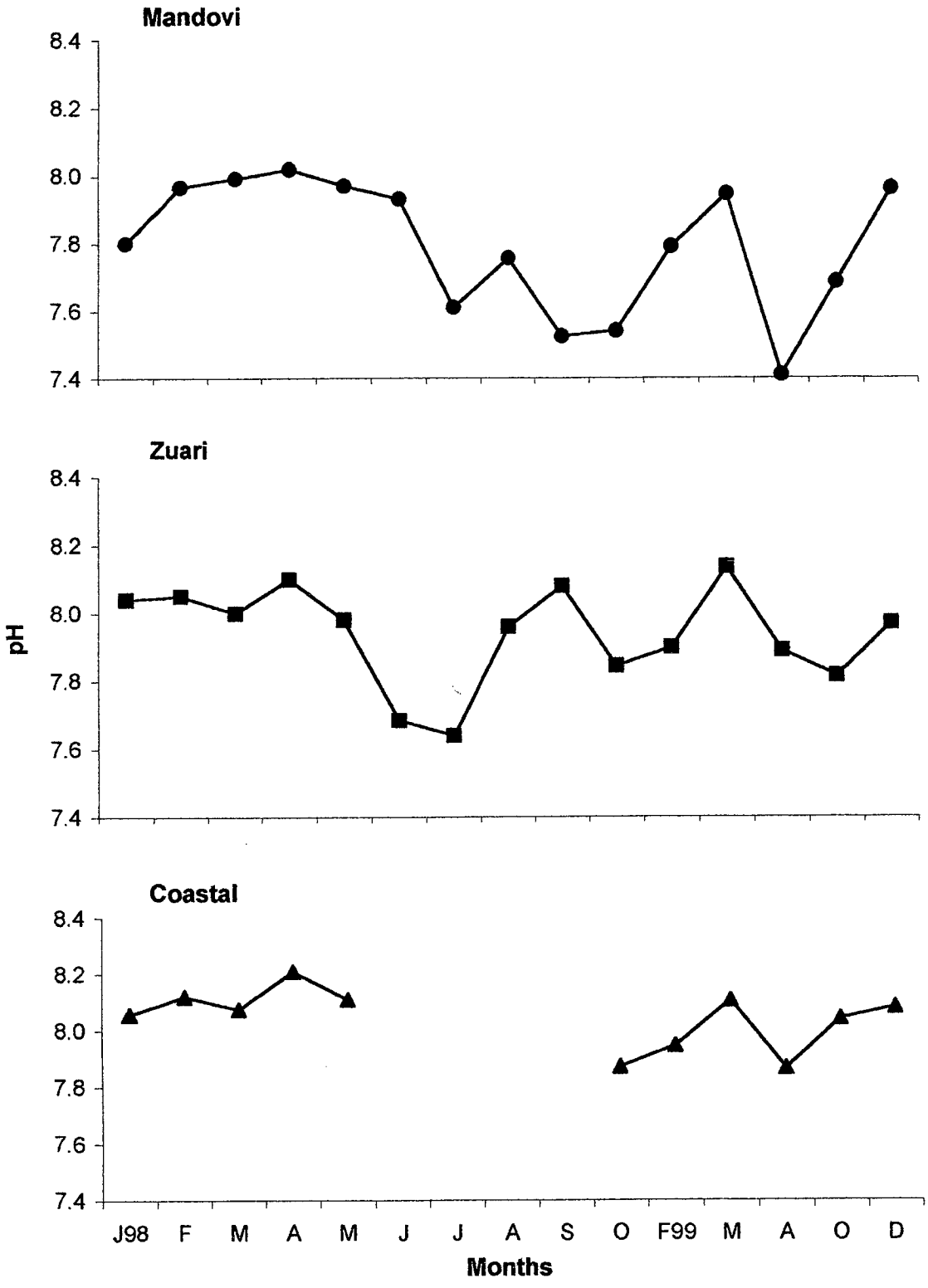

Fig.4.3. Monthly variation in the pH measurements (average of surface and bottom)

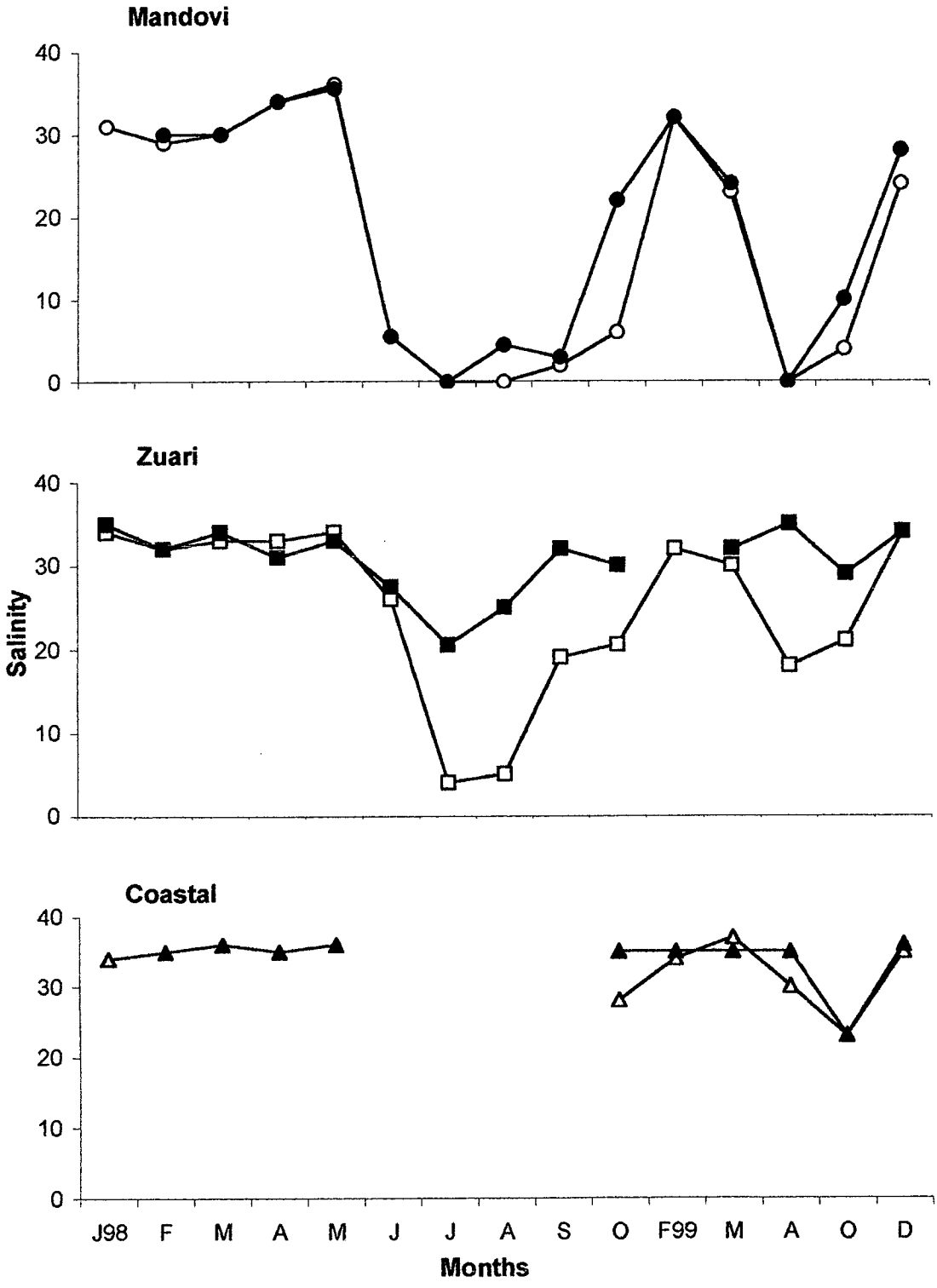

Fig. 4.4. Monthly variation in the salinity measurement at the surface (empty) and bottom (filled) waters.

the average salinity recorded during the pre- and post-monsoon seasons were 35.5 and 30.0, respectively (Table 4.2a & b).

4.2.2 Chemical parameters

4.2.2.1 Dissolved oxygen (DO)

The dissolved oxygen (DO) level in this estuarine system ranged from 3.53 to 7.89 mg l^{-1} at the surface and 2.86 to 7.56 mg l^{-1} in the bottom waters (Fig. 4.5). Although the DO level at the surface was higher than bottom waters throughout season, the values did not differ significantly. The monsoon season was marked by high DO level in Mandovi estuary (both at surface and bottom). In the Zuari estuary significant differences in DO level was observed at the surface and bottom during monsoon season with a mean value of 5.86 and 3.77 mg l^{-1}, respectively (Table 4.3a & b). An exceptionally low DO level of 0.71 and 1.76 mg l^{-1} was observed at the mouth of the Mandovi estuary towards the end of monsoon period with high salinity values (i.e. Oct. '98 and Aug. '99). Significant difference ($p<0.006$) in the DO level was observed at the estuarine stations during the three seasons. On an average surface and bottom coastal waters showed a mean value of 5.16 and 5.07 mg l^{-1} in the pre- and post-monsoon seasons, respectively (Table 4.3a & b).

Percent oxygen saturation calculated from salinity and temperature measurements mostly ranged from 86 to 103% and did not show any seasonal variation between seasons and/or stations (Fig. 4.6). Lower values (60.73-78%) were observed during the pre-monsoon months (Feb-May) at both the estuarine stations and it did not differ significantly.

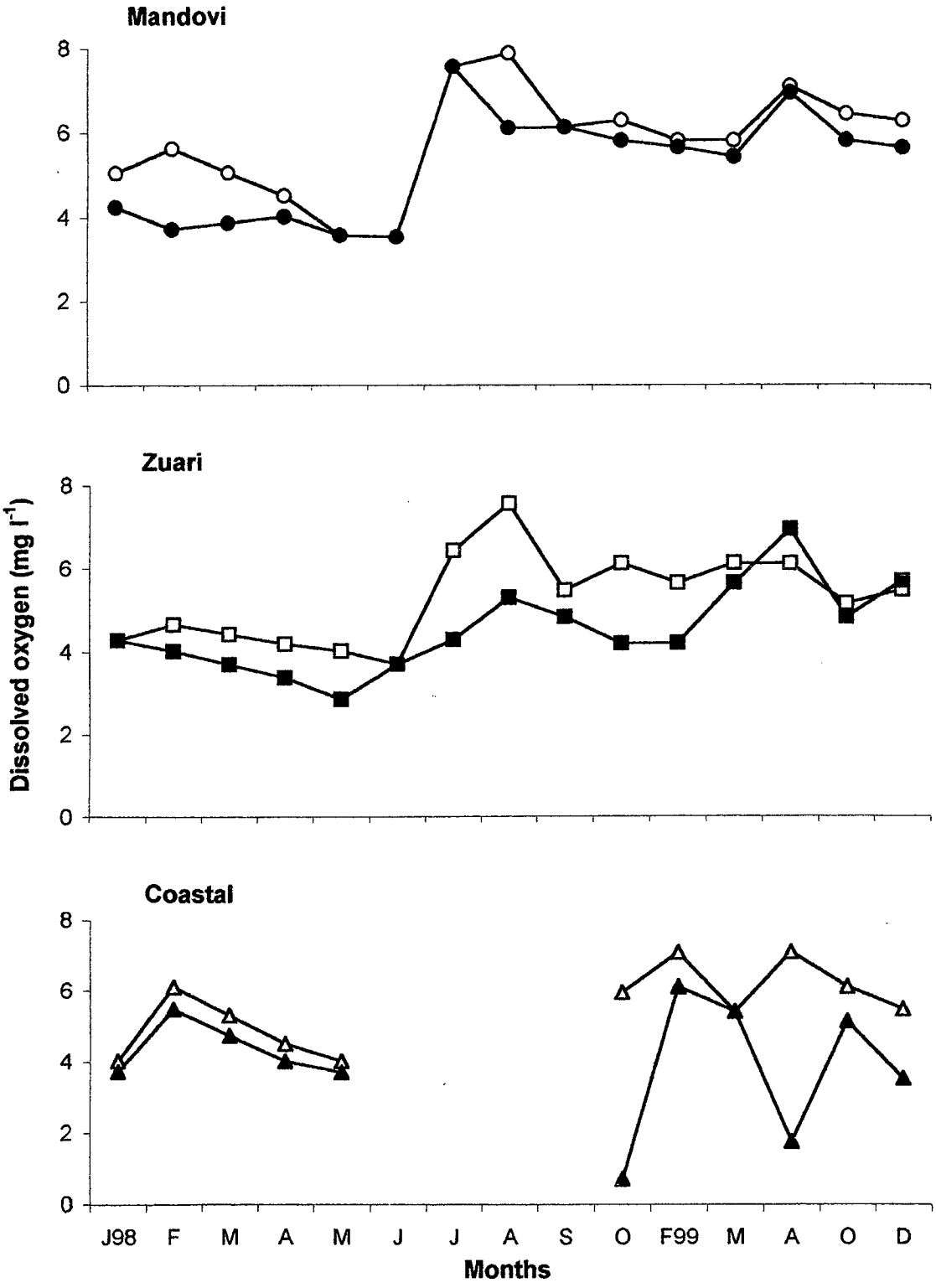

Fig.4.5. Monthly variation in the dissolved oxygen levels at the surface (empty) and bottom (filled) waters.

Table 4.3a Seasonal variation in the dissolved oxygen (DO), biological (BOD) and chemical oxygen demand (COD) in the surface waters

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
DO	Range	3.57-5.80	3.53-7.89	6.27-6.43	4.01-6.12	3.70-7.57	5.14-5.47	4.01-7.08	-	5.47-6.11
($mg\ l^{-1}$)	Mean	5.06	6.44	6.35	4.84	5.86	5.31	5.41	-	5.79
	SD	0.89	1.76	0.11	0.85	1.42	0.23	1.10	-	0.45
BOD	Range	0.80-1.61	0.64-3.00	0.64-1.29	1.13-2.09	0.64-3.54	0.32-1.29	1.29-3.05	-	0.64-2.90
($mg\ l^{-1}$)	Mean	1.21	1.53	0.97	1.47	1.48	0.68	2.15	-	1.63
	SD	0.30	1.04	0.29	0.34	1.18	0.42	0.60	-	1.09
COD	Range	0.33-0.62	0.33-0.78	0.25-0.80	0.38-0.60	0.43-3.46	0.25-0.80	0.17-0.40	-	0.25-0.65
($mg\ C\ l^{-1}$)	Mean	0.42	0.53	0.51	0.50	1.13	0.51	0.22	-	0.44
	SD	0.11	0.17	0.25	0.08	1.31	0.25	0.09	-	0.19

Table 4.3b. Seasonal variation in the dissolved oxygen (DO), biological BOD) and chemical oxygen demand (COD) in the bottom waters

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
DO	Range	3.57-5.63	3.53-7.56	5.63-5.80	2.86-5.63	1.71-5.30	4.83-5.69	3.70-6.12	-	0.71-5.14
(mg l^{-1})	Mean	4.37	6.05	5.71	3.92	3.77	5.26	4.91	-	4.34
	SD	0.91	1.53	0.12	1.05	1.81	0.61	0.93	-	1.14
BOD	Range	0.32-1.44	0.16-3.37	0.32-1.61	0.32-2.09	0.48-2.09	0.32-1.29	0.32-2.89	-	0.64-1.77
(mg l^{-1})	Mean	0.92	1.19	0.91	1.18	1.09	0.76	1.45	-	1.34
	SD	0.40	1.26	0.65	0.68	0.72	0.40	0.97	-	0.61
COD	Range	0.45-1.58	0.17-0.47	0.25-0.84	0.40-2.63	0.42-1.10	0.17-0.98	0.13-0.50	-	0.21-0.80
(mg C l^{-1})	Mean	0.85	0.37	0.54	1.33	0.66	0.52	0.34	-	0.57
	SD	0.42	0.12	0.29	0.91	0.26	0.37	0.13	-	0.31

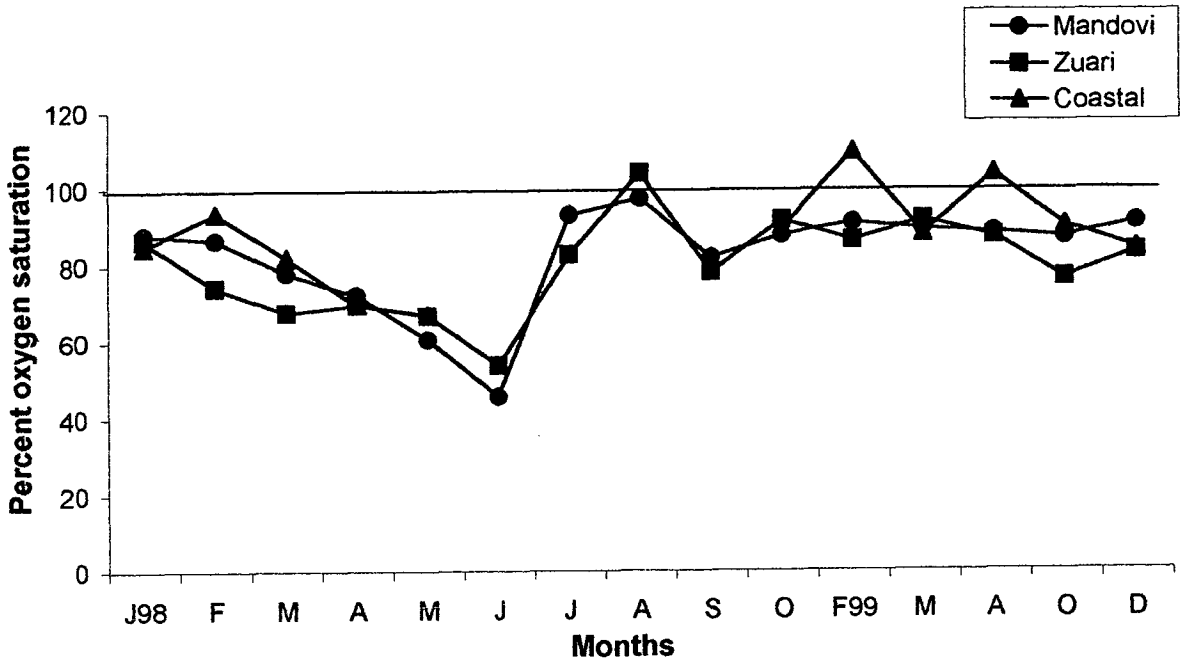

Fig. 4.6. Monthly variation in the percent oxygen saturation at the study stations

4.2.2.2 Biochemical oxygen demand (BOD)

At the estuarine stations the mean biochemical oxygen demand (BOD) values were 1.12 at the surface and 1.11 mg l^{-1} for bottom in Mandovi and Zuari estuaries and did not vary significantly. The bottom waters of Zuari estuary alone showed a significant variation ($p<0.03$) between pre- and post-monsoon seasons. The biochemical oxygen demand (BOD) value in the coastal station ranged from 0.64 to 3.05 mg l^{-1} with a mean value of 1.80 (surface) and 1.49 mg l^{-1} (bottom) in the pre- and post monsoon seasons respectively (Table 4.3a & b).

4.2.2.3 Chemical oxygen demand (COD)

Both Mandovi and Zuari estuaries showed high chemical oxygen demand (COD) values at the surface during the monsoon and bottom during the pre-monsoon season (Table 4.3a & b). In the coastal waters the COD values were significantly lower ($p<0.05$) than Mandovi and Zuari estuaries and ranged from 0.13 to 0.80 mg C l^{-1} in the non-monsoon seasons.

4.2.2.4 Nutrient characteristics

4.2.2.4.1 Ammonia

At both Mandovi and Zuari stations ammonia levels were higher at the bottom waters than surface and did not vary significantly. In the Mandovi estuary ammonia concentration were found to be high in the pre-monsoon season with peak mean value of 4.38 and 4.51 μM at the surface and bottom waters, respectively (Table 4.4a & b). In Zuari estuary high concentration was observed during the pre-monsoon at the surface and post-monsoon season in the bottom

Table 4.4a. Seasonal variation in the nutrient concentrations in the surface waters.

*Bd - below detectable level

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
	Range	1.55-9.21	0.05-7.08	0.61-6.15	0.28-8.01	Bd*-4.21	0.61-6.15	0.05-6.88	-	1.08-4.68
NH_3	Mean	3.39	4.38	2.66	3.27	1.06	2.66	2.87	-	2.91
(μM)	SD	3.27	3.26	2.41	2.96	1.78	2.41	2.95	-	1.80
	Range	1.22-2.26	0.11-0.69	0.30-1.16	0.34-2.58	0.07-1.05	Bd-0.73	0.11-2.12	-	Bd-0.49
NO_2	Mean	1.74	0.64	0.60	1.46	0.47	0.37	1.11	-	0.27
(μM)	SD	0.74	0.46	0.48	1.58	0.37	0.36	1.42	-	0.25
	Range	0.80-14.44	1.93-26.28	0.60-5.46	Bd-9.49	1.93-14.38	Bd-1.12	Bd-8.84	-	Bd-1.92
NO_2+NO_3	Mean	6.67	12.03	3.04	4.07	7.37	0.51	2.30	-	0.62
(μM)	SD	6.54	12.69	2.43	4.70	6.37	0.57	4.36	-	0.90
	Range	1.45-3.73	0.19-1.80	0.13-0.29	1.56-6.54	0.19-1.06	0.14-1.07	0.85-1.98	-	0.37-1.16
PO_4	Mean	2.35	0.93	0.20	3.22	0.56	0.52	1.27	-	0.72
(μM)	SD	1.21	0.82	0.08	2.87	0.44	0.49	0.62	-	0.37

Table 4.4b. Seasonal variation in the nutrient concentration in the bottom waters.

*Bd - below detectable level

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
	Range	1.35-10.88	0.41-8.21	2.21-3.81	0.28-9.81	Bd*-3.88	1.81-10.48	0.05-6.88	-	1.68-4.68
NH_3	Mean	4.17	4.51	2.97	3.79	2.26	4.78	2.87	-	3.37
(μM)	SD	4.02	3.64	0.80	3.71	1.41	3.93	2.95	-	1.54
	Range	1.15-2.27	Bd-0.55	0.30-0.86	Bd-2.47	Bd-1.94	Bd-1.27	0.55-2.10	-	0.30-1.16
NO_2	Mean	1.71	0.96	0.51	0.95	0.65	0.68	1.33	-	0.60
(μM)	SD	0.79	1.50	0.30	1.33	0.79	0.64	1.10	-	0.48
	Range	1.07-14.73	3.33-26.78	3.16-9.60	Bd-9.58	1.65-12.64	Bd-15.30	0.80-8.55	-	Bd-3.84
NO_2+NO_3	Mean	4.72	12.52	5.61	3.67	6.18	4.15	2.95	-	1.46
(μM)	SD	6.68	12.52	3.49	4.57	5.75	7.45	3.74	-	2.08
	Range	1.76-4.27	0.66-5.19	0.53-1.71	Bd-5.90	0.61-3.27	1.51-2.83	2.54-6.00	-	0.26-2.30
PO_4	Mean	3.07	2.22	0.59	2.67	2.07	2.30	3.80	-	1.33
(μM)	SD	1.26	2.58	0.55	2.99	1.35	0.70	1.91	-	0.70

waters (Table 4.4a & b). Ammonia concentration at the coastal station was comparatively lower than that of the estuarine stations.

4.2.2.4.2 Nitrite

In both the estuarine stations nitrite levels showed high values in the pre-monsoon (0.34-2.58 μM) and low value in the post-monsoon months (undetectable to 1.27 μM) (Table 4.4a & b). Unlike ammonia levels the mean nitrite levels were lower at the coastal than estuarine stations.

4.2.2.4.3 Nitrate

The mean nitrate concentration during the monsoon season in both Mandovi and Zuari estuaries were 12.03 and 7.37 μM at the surface and 12.52 and 6.18 μM at the bottom waters, respectively (Table 4.4a & b). The influence of monsoon was observed in the changes of nitrate levels at the estuarine stations and was significantly ($p<0.01$) higher than non-monsoon seasons. Unlike nitrite and ammonia the nitrate concentration at the coastal stations was lower than the estuarine stations.

4.2.2.4.4 Phosphate

Phosphate levels showed similar trend with those of nitrite level at the estuarine stations with higher values in the pre-monsoon months (1.45-5.90 μM) than rest of the months at surface and bottom waters. Coastal waters invariably showed lower values than estuarine stations (Table 4.4a & b).

4.2.2.4.5 Dissolved organic carbon (DOC)

Dissolved organic carbon (DOC) (surface and bottom waters) was higher at coastal when compared to estuarine stations. The mean values at coastal

station in the pre- and post-monsoon seasons were 4.69 and 3.96 mg l^{-1} respectively (Table 4.5a & b). In both Mandovi and Zuari estuaries the DOC (surface and bottom) was higher in the post-monsoon season with a mean value of 4.28 and 4.99 mg l^{-1} respectively.

4.2.2.4.6 Labile organic carbon (LOC)

The percentage of labile organic carbon (LOC) in the coastal waters showed a mean value of 26.58% and was higher than the estuarine waters (Fig. 4.7a & b). At the estuarine stations high value of 30.35 and 28.73% was observed in Mandovi and Zuari estuaries during monsoon than non-monsoon seasons. Similar trend was noticed in the bottom waters except for Mandovi where a high mean value of 14.37% was observed in the pre-monsoon season.

4.2.2.4.7 Particulate organic carbon (POC)

In Mandovi-Zuari estuarine system particulate organic carbon (POC) was generally 1-2 fold lower than DOC. While high POC (1.68 mg l^{-1}) were observed in the surface waters of Mandovi estuary during the monsoon season, Zuari estuary showed a high mean value of 3.19 mg l^{-1} in the bottom waters during the pre-monsoon season (Table 4.5a & b). Variation in POC values was observed in the pre- and post-monsoon seasons in the bottom waters of Mandovi ($p<0.0005$) and Zuari estuaries ($p<0.04$) (Table 4.6).

4.2.2.4.8 Particulate organic nitrogen (PON)

Unlike POC, the trend was similar with respect to particulate organic nitrogen (PON) at both the estuarine stations. PON generally ranged from 0.02 to 0.39 mg l^{-1} in the estuarine and 0.03 to 0.23 mg l^{-1} in the coastal waters. PON

Table 4.5a. Seasonal variation in the dissolved organic carbon (DOC), particulate organic carbon (POC) and nitrogen (PON) level in the surface waters.

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
DOC ($mg\ C\ l^{-1}$)	Range	1.73-5.71	0.30-2.33	1.01-10.85	2.01-3.17	0.72-6.64	1.05-4.76	3.41-6.50	-	2.57-6.26
	Mean	3.89	1.31	4.08	2.50	2.67	3.31	4.69	-	3.86
	SD	1.66	0.77	4.57	0.51	2.69	1.61	1.45	-	1.64
POC ($mg\ C\ l^{-1}$)	Range	1.17-1.32	0.92-3.95	0.06-2.23	1.49-1.95	0.73-2.23	0.48-0.77	0.64-1.06	-	0.47-0.71
	Mean	1.22	1.68	0.87	1.72	1.34	0.62	0.81	-	0.57
	SD	0.23	1.28	0.94	0.23	0.60	0.14	0.22	-	0.10
PON ($mg\ N\ l^{-1}$)	Range	0.17-0.27	0.13-0.54	0.03-0.24	0.1-0.30	0.12-0.39	0.02-0.24	0.06-0.17	-	0.03-0.23
	Mean	0.22	0.26	0.14	0.25	0.26	0.11	0.13	-	0.10
	SD	0.05	0.19	0.11	0.06	0.11	0.12	0.06	-	0.11

Table 4.5b. Seasonal variation in the dissolved organic carbon (DOC), particulate organic carbon (POC) and nitrogen (PON) level in the bottom waters.

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
DOC ($mg\ C\ l^{-1}$)	Range	1.46-6.26	1.05-16.19	3.42-5.78	1.38-5.70	1.78-7.65	3.65-13.83	3.41-6.50	-	2.27-5.15
	Mean	3.57	8.02	4.48	2.84	3.35	6.67	4.69	-	3.96
	SD	2.01	6.12	0.98	1.99	2.44	4.79	1.45	-	1.21
POC ($mg\ C\ l^{-1}$)	Range	1.41-2.71	0.83-4.66	0.37-0.76	2.63-4.18	0.87-4.61	0.96-2.90	0.86-1.64	-	0.45-0.64
	Mean	1.90	2.07	0.62	3.19	1.87	1.58	1.26	-	0.54
	SD	0.71	1.54	0.22	0.86	1.55	0.89	0.39	-	0.10
PON ($mg\ N\ l^{-1}$)	Range	0.21-0.51	0.23-0.80	0.03-0.17	0.41-0.61	0.20-0.73	0.04-0.33	0.13-0.31	-	0.01-0.23
	Mean	0.38	0.36	0.08	0.48	0.35	0.19	0.20	-	0.08
	SD	0.16	0.25	0.08	0.11	0.24	0.15	0.09	-	0.12

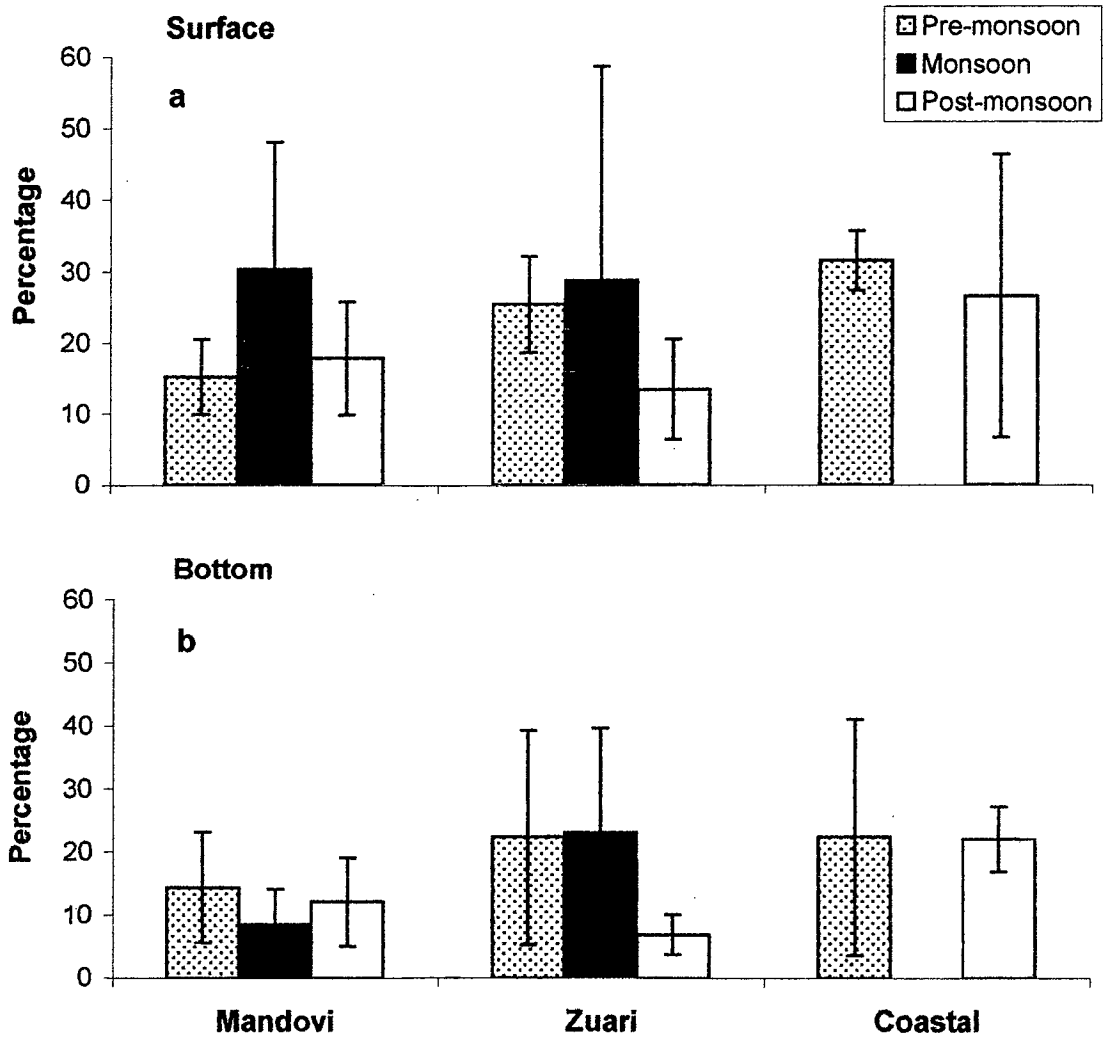

Fig. 4.7. Seasonal variation in the percentage of labile organic carbon in the surface and bottom waters. Standard deviation is indicated as error bars.

Table 4.6. Variation in the particulate organic carbon and nitrogen between seasons and their significance

Parameter	Station	Between seasons	df	F	Fcrit	P value
Particulate organic carbon	Mandovi	Pre and Post (Bottom)	1, 4	8.91	7.71	0.04
	Zuari	Pre and Post (Surface)	1, 4	62.60	6.61	0.0005
Particulate organic nitrogen	Mandovi	Mon and Post (Bottom)	1, 4	14.02	7.71	0.02
	Zuari	Pre and Post (Bottom)	1, 5	7.98	6.61	0.03
	Zuari	Mon and Post (Surface)	1, 6	14.02	7.71	0.01

values were higher in the bottom than at surface waters during the monsoon season. Significant variation in PON values was observed between monsoon and post-monsoon seasons ($p<0.02$) in the surface and between pre and post-monsoon ($p<0.04$) in the bottom waters of Zuari estuary. In Mandovi estuary significant variation was observed in the monsoon and post-monsoon seasons ($p<0.02$) in the bottom waters of Mandovi estuary (Table 4.6).

4.2.2.4.9 POC/PON (C:N ratio)

Particulate C:N ratio was comparatively lower at the coastal station than Mandovi and Zuari estuaries. The present study showed low C:N ratio of 6.4 in the Mandovi estuary (pre-monsoon season) and 6.68 in the Zuari estuary (monsoon season) (Fig. 4.8). At the estuarine stations high C:N ratio were observed during the post-monsoon season and varied significantly with pre- and post-monsoon seasons. At all the three stations the C:N ratio did not vary significantly between surface ad bottom. The data is presented as average of the surface and bottom waters (Fig. 4.8). At the coastal station the C:N ratio was significantly higher than estuarine stations and recorded a mean value of 17.82.

4.2.2.4.10 System variability

The influence of monsoon led to system wide variability in salinity in estuarine and coastal stations leading to significant ($p<0.0001$) difference between them. Similarly BOD values were higher in the coastal than estuarine waters by two folds and differed significantly ($p<0.01$). POC values (both surface and bottom) were lower at the coastal station and varied significantly at Mandovi ($p<0.01$) and Zuari ($p<0.02$) stations (Table 4.7). Likewise, PON also

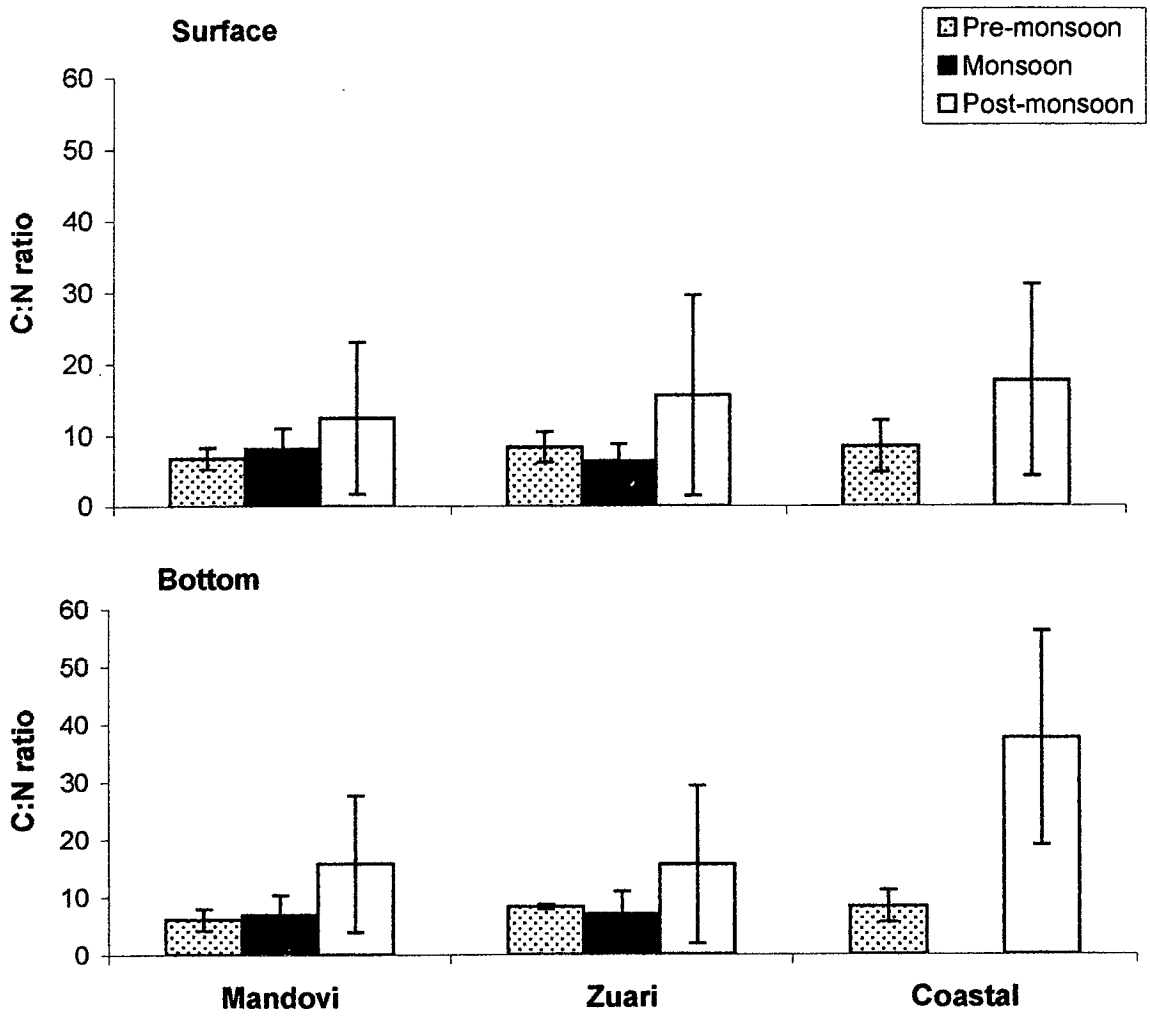

Fig. 4.8. Seasonal variation in the particulate C:N ratio at the surface and bottom waters. Standard deviation is indicated as error bars

Table 4.7. Variation in the particulate organic carbon and nitrogen between the study station and their significance

Parameter	Between stations	df	F	Fcrit	P value
Particulate organic carbon	Mandovi and Coastal (Surface)	1, 22	7.89	4.30	0.01
	Zuari and Coastal (Surface)	1, 22	10.65	4.30	0.003
	Mandovi and Coastal (Bottom)	1, 20	6.09	4.35	0.02
	Zuari and Coastal (Bottom)	1, 20	13.44	4.35	0.001
Particulate organic nitrogen	Mandovi and Coastal (Surface)	1, 20	10.29	4.35	0.004
	Zuari and Coastal (Surface)	1, 20	10.16	4.35	0.004
	Mandovi and Coastal (Bottom)	1, 20	6.96	4.35	0.01
	Zuari and Coastal (Bottom)	1, 20	13.67	4.35	0.001

showed similar trend, with the coastal waters showing significantly lower values than Mandovi (p<0.004) and Zuari (p<0.004) estuaries (Table 4.7).

4.3 DISCUSSION

The coastal ocean which acts as the interface between open ocean and the land, exerts a major control on the exchange of material between these two spheres. Understanding the hydrological and physico-chemical dynamics of coastal oceans would facilitate in studying the distribution, abundance and interactions between organisms and the transformation and flux of energy and matter. This would help to critically understand the role played by the marine biota in important biogeochemical cycles and processes.

Investigation on the general hydrographical and chemical characteristics of the coastal waters requires a good knowledge of the characteristics of the local riverine and estuarine system in order to understand their impact on the near and inshore regions. Observations on the above are very essential for a better understanding of biological processes. Hydrographical properties of the estuarine waters of Goa has been fairly studied extensively in the past and there is information now available on seasonal and diurnal changes in temperature, salinity, dissolved oxygen, current and water flow pattern (Murty and Das 1972, Murty et al. 1976). A review of physical, chemical and biological characteristics of this estuarine system has been earlier presented by Qasim and Sengupta (1981). The chemical properties of the water of these two estuaries has been published by DeSouza et al. (1981), while Verlencar (1982) has studied the hydrographical as well as nutrient cycle of the Mandovi estuary. The most important feature emerging from these studies is that the hydrographical features in this estuarine system of Goa are found to be fairly regular and follow a

seasonal cycle. Intense precipitation and land runoff during monsoon period brings about larger changes in hydrographical features particularly in the salinity, flow pattern and nutrients than during the dry season (i.e. pre- and post-monsoon season).

Study on light penetration in the water column is important to understand problems related to radioactive transfer as well as underwater visibility. The amount of light energy available at any layer in the sea is considered to be an important factor controlling primary production and organic matter dynamics. In the present study the amount of light penetration or secchi reading was generally low in both the estuaries, although Zuari estuary showed more transparency than Mandovi estuary, because of its greater depth. The high turbidity in Mandovi than Zuari estuary was due to its shallowness, which leads to constant suspension of sediments due to churning action of winds, wave and currents, and organic matter and dissolved substances (Satyendranath and Varadachari 1982). In the present study, the Zuari estuary showed a decreasing trend in water transparency with the onset of monsoon, which was possibly due to riverine discharge and land runoff (Fig. 4.1).

Temperature has been identified as an important abiotic factor, which directly influences the distribution, activity and metabolism of the any organism in marine environment. This study showed that the seasonal variation in the water temperature was more significant at Mandovi than at the other stations. High temperature during the peak summer months (April-May) and low temperature during the monsoon months (June-September) observed in the present study

corroborates the findings of DeSouza (1983), which indicated that water temperature is chiefly controlled by climatological conditions. This is because of the shallowness of these estuaries and their proximity to land that permits rapid temperature changes in response to variation in atmospheric temperature as pointed out by Williams (1966). The low temperature recorded during the monsoon period at the study stations could be attributed to intrusion of cold upwelled water, which has been reported from earlier studies carried out in Mandovi and Zuari estuaries from July to October (Verlencar 1982).

A key variable controlling the inorganic carbon system in the marine environment is pH. In the present study pH was found to be higher (>8) in the adjacent coastal waters indicating its influence from the marine or oceanic provinces. However at the estuarine stations the pH generally fluctuated from 7.2 to 7.9. This comparatively lower pH was due to relatively high productivity and intense microbial utilization of organic matter, which can substantially lead to the build up of pCO_2 in sea-water (Sarma 2001).

The most interesting featuring in the Mandovi and Zuari estuaries is the appearance of salinity gradient during the monsoon and its disappearance in the following post-monsoon season. In the present study, salinity gradient was more pronounced in the Zuari than Mandovi estuary due to increased amount of freshwater inflow through riverine runoff leading to stratification in the water column, with less dense freshwater on the surface and denser saline water at the bottom. This resulted in the formation of salt-wedge in this tropical estuarine system as previously reported (Qasim and Sengupta 1982). However

when the flow of freshwater is reduced at the end of monsoon this gradient disappeared and the mixing of water was due to the formation of internal waves and other water forces, surface wind and tidal currents thereby confirming the tidal nature of the estuary (Das et al. 1972). The high salinity observed in the present study during the non-monsoon months particularly during the pre-monsoon summer months was due to high temperature, which resulted in high evaporation rates. Significant positive relationship observed between temperature and salinity ($p<0.01$) at the estuarine stations, substantiates the above findings. pH also indicated a significant correlation with salinity at the estuarine sites. The low average salinity in the Mandovi than Zuari estuary was due to a) the former receives considerable amount of riverine discharge and runoff through a large tributary system (1527 km^2) and b) the latter is 20% larger than the former.

Accurate data on the solubility of gases in seawater is of special importance because departure of ocean/seawater from equilibrium with atmospheric gases provides information on many types of processes. Dissolved oxygen (DO) is governed by the changes in the biological activity of the water column as well as physical properties. As a product of photosynthetic activity and a requirement for respiration the distribution of oxygen has been used to measure biological activity (Williams and Purdie 1991). In the present study high DO level in the estuarine stations especially in the Mandovi during the monsoon season was due to heavy precipitation. Almost freshwater conditions of the two estuaries were associated with low salinity and low temperature of the overlying

waters. DO level related inversely to salinity ($p<0.01$) and temperature ($p<0.01$), which corroborates the findings of DeSouza and Sengupta (1986) in this estuarine system. Relatively high DO values observed during monsoon season might be due to higher solubility of this gases in these less saline waters and low temperature, while the lower oxygen values during pre-monsoon was due to its lower solubility as the salinity increased rapidly in this season. DO level in the bottom (3.77 mg l^{-1}) was significantly ($p<0.02$) lower than surface waters (5.86 mg l^{-1}) in the Zuari estuary. This was due to restricted water exchange and circulation as a result of stratification of water column by salinity gradient in the monsoon season. However, this showed recovery during the post-monsoon season when the salt wedge subsided and the sand bar got disturbed. This study also indicated high oxygen saturation in all the stations suggesting photosynthetic production of oxygen (Kester 1975). An interesting feature observed in this study was the occurrences of very low DO concentration (0.71-1.71 mg l^{-1}) in the bottom waters at Zuari and coastal station during August-October period. This water showed an unusually high salinity (35) which might have been trapped at the bottom due to the shallow sill formed by the sand bar at the mouth resulting in very little mixing as well as stagnant conditions inside the bar mouth (DeSouza and Sengupta 1986). The oxygen flux across the air-sea interface is an important physical factor governing oxygen content in the aquatic systems. The flux of oxygen across air-sea interface is mainly controlled by the oxygen content of the surface waters and the solubility of the oxygen in the surface waters. This parameter is crucial in determining the environmental quality

and health of the ecosystem (Badran and Foster 1998). In this study, the percent oxygen saturation (%) computed from the measured oxygen content and saturation oxygen concentration (calculated from the salinity and temperature) was slightly undersaturated in the most of the months with 90% of the values more than 85%. This undersaturation was due to activity of the heterotrophs in assimilating the available organic matter. High percentage (>100%) of oxygen saturation in the some of the months observed in this study indicated more intense photosynthesis than heterotrophic activity (Fig. 4.6).

Biological oxygen demand (BOD) or dissolved organics, which determines the amount of labile organic carbon available to the microbial community for assimilation, has often been used as an indicator for determining the nutritional status of the ecosystem. In the present study the coastal station showed higher BOD values than estuarine stations indicating the occurrence of higher percentage of labile organic carbon. Jonas (1997) showed BOD values to range from 1-4 mg l^{-1} and sometimes up to 7 mg l^{-1} during plankton bloom in Chesapeake Bay. This high value is indicative of high availability of labile organic matter for bacterial assimilation. Chemical oxygen demand (COD) is a practical measure of organic contamination and high values are indicative of eutrophic conditions. In contrast to BOD, COD values were higher in the estuarine stations than coastal and were lower than BOD by an order. Significant variation in the COD values was observed between coastal and Mandovi stations at the surface ($p<0.03$) and coastal and Zuari stations in the bottom waters ($p<0.05$). The present study indicated low COD with values mostly ranging from 0.13 to 1.10

mg l^{-1} when compared to high COD values of 2.1 to 4.5 mg l^{-1} in Tokyo Bay (Kawabe and Kawabe 1997).

Nutrients are the material currency for energy and structural form in biological system. The concentration of these nutrients greatly determines the distribution and activity of the marine biota and has often been used to classify water bodies based on its levels. In the present study, high concentration of ammonia in the pre-monsoon (summer) months at the estuarine stations particularly in the Mandovi estuary was due to high microbial degradation of organic matter. The relatively high BOD and low DO levels during this period supported the above observation. Similarly high nitrite values in the pre-monsoon season could be due to degradation of organic matter, although a possibility of nitrification by nitrifying bacteria cannot be underestimated (Iriarte et al. 1997). The high nitrate values in the monsoon season could be due to high riverine discharge and precipitation (Qasim and Sengupta 1981). In the present study phosphate concentration was higher at the bottom than at the surface waters indicating its origin from the exchange between mud water interface and water exchange with the sea (DeSouza 1977).

Dissolved organic carbon (DOC) is the major form of organic carbon in almost all aquatic ecosystems. It not only plays a significant role in aquatic food webs (Findlay et al. 1986), but also mediates the availability of dissolved nutrients (Carlson et al. 1994, Chio et al. 1999), and modifies the optical properties of water bodies (Wetzel 1992). In the present study comparatively high DOC values were recorded at the coastal than at estuarine stations. This DOC

could be both refractory and labile. Hence the availability of labile DOC (L-DOC) value to bacteria is of more significance than refractory. The L-DOC ranged from 0.23 to 2.1 mg l^{-1} and 0.45 to 2.14 mg l^{-1} at the estuarine waters and coastal waters respectively. In this study, L-DOC was relatively higher in the coastal than estuarine stations with a mean percentage value of 25.58. The sources of high L-DOC could either come from phytoplankton photosynthesis, riverine sources, sloppy feeding by zooplankton, grazing activity and dissolution of particles in the marine environment. Microbial processes of L-DOC are dependent on the supply of mineral nutrients and physical factors such as temperature that determine the growth rate and uptake capacity. L-DOC was higher in surface than bottom waters. Zweifel (1999) showed L-DOC to vary from 3.4 to 17% in the Gulf of Riga. The low percentage of DOC is indicative of refractory nature, hence not available for heterotrophic utilization. It has been reported that the percentage of labile organic carbon to total organic carbon generally ranged from 0.4 to 40% (Moran et al. 1999). The present study showed DOC values to range from 1.05 to 6.50 mg l^{-1} and was close to those reported in Texas estuaries, USA (4.4 – 20.9 mg l^{-1}), Pontchartrain estuary, Louisiana (5.3 - 8.5 mg l^{-1}), Gulf of Riga, Spain (4.68 – 8.06 mg l^{-1}), Gulf of Mexico (4.7 mg l^{-1}), and Ross Sea (1.2 – 14.4 mg l^{-1}).

The abundance and composition of particulate organic matter can change substantially in response to variations in the biological and physical processes. Physical processes are influenced by the transport and delivery of particles while the biological processes are by primary productivity and respiration, fluctuation over a range of temporal scales in response to the availability of light, nutrient

and temperature regime. In the present study, both particulate organic carbon (POC) and nitrogen (PON) showed parallel trends in the pre-monsoon and monsoon seasons, as indicated by highly significant correlation ($p<0.001$) between the two, indicating its biogenic nature. Interestingly in contrast to estuarine stations the coastal station did not record any relationship between POC and PON in both surface and bottom waters and showed comparatively high C: N ratios. High C:N ratio of particulate matter (17.82 ±13.92) observed in the coastal station indicates poor nutritional quality of substrate for microbial assimilation. Similarly Lui and Dickhut (1998) reported high POC: PON ratio of 19.40 ±9.30 caused due to increased terrestrial input, atmosphere deposition and urban runoff in the southern Chesapeake Bay. The POC and PON values observed in the present study is similar to the values reported in other estuaries like Chinhae Bay, Korea (4.42 and 0.79 mg l^{-1}) and Caete estuary, Brazil (2.88 and 0.41 mg l^{-1}). The average C: N ratio observed in the present study at the estuarine stations was 7.41, which was close to the Redfield ratio. In the post-monsoon season the mean C: N ratio was found to be high (12.5 ±1.65) probably due to settling of detrital particles from the terrestrial sources after the monsoon. On comparison the C: N ratio of estuarine stations was lower than that of coastal stations. Doval et al. (1999) reported a POC: PON ratio of 8.6 ±0.6, similar to those observed in the present study in the Catalan coast in the NW Mediterranean and showed a significant relationship between POC and PON. Low POC:PON values of 5-11 similar to the present study have been reported by Sara et al. (1999) in the Mediterranean . In most natural water bodies the size of

DOC pool is approximately 5-15 times and 2-5 times than that of POC in the oligotrophic and eutrophic environments respectively (Wetzel 1992). However this ratio varies with respect to system, where oligotrophic oceans are characterized by higher DOC:POC ratio when compared to eutrophic systems. In the present study, DOC dominated POC by 4-5 folds in the coastal and 2-3 folds in the estuarine stations suggesting that the dissolved organic resources are high in low productive environments and particulate resources increase in high productive environments. Similar dominance is also reported from south East China Sea (Hung et al. 2000) and Chesapeake Bay (Fisher et al. 1998).

4.4 CONCLUSION

The above observations can be summarized as follows:

Estuary

The estuary is well mixed throughout the year, except in the monsoon season wherein the stratification caused by salinity gradient is more prominent in Zuari than Mandovi estuary. High DO values (>4 mg l^{-1}) indicated that the system is well oxygenated. The estuarine waters recorded a high nutrient level when compared to the adjacent coastal waters. Low particulate C: N ratio at the estuarine stations during the pre- and monsoon seasons is indicative of biogenic source where high C: N ratio in the post-monsoon season is indicative of non-biogenic or terrestrial sources.

Coastal

The influence of salinity was not observed at this station. This station did not show any seasonal variability in the physico-chemical parameters and was less dynamic than the estuarine waters. Nutrients were low and the particulate C:N ratio was higher than estuarine waters. DOC values were significantly higher (by two fold) than POC throughout the year.

Chapter 5

BIOLOGICAL PARAMETERS

5.1 INTRODUCTION

Bacteria are the most abundant and important biological component involved in the transformation and mineralization of organic matter in aquatic systems (Cho and Azam 1988, Pomeroy et al. 1991). Heterotrophic bacteria contribute to the cycles of nutrients and carbon in two major ways: by the production of new bacterial biomass and by the remineralization of organic carbon and nutrients. Understanding this dual role of planktonic bacteria in marine ecosystem is a central paradigm of contemporary microbial ecology (Pomeroy et al. 1991, Ducklow and Carlson 1992). Autotrophs and heterotrophs, which represent a dominant community in aquatic ecosystems, are fundamental and complementary functional units and therefore play an important role in exerting their influence on the chemistry and geology of aquatic systems (Azam et al. 1994). In ecosystems the main control process is generally attributed to the heterotrophs, especially the bacteria, which forms an integral part and exerts a major role in the aquatic ecosystem food web. However, the distribution and activity is largely regulated by the quality and quantity of organic matter. Most of the dissolved and particulate organic matter in aquatic systems has polymeric structure. This polymeric structure upon hydrolysis with the surface bound exo-enzymes is broken into simpler units and later assimilated into its biomass. The ability to degrade high molecular weight compounds is dependent upon the metabolic capabilities of the bacterial community. They exhibit tremendous versatility not only in surviving extreme environments but also in exhibiting invaluable commercial potential.

Bacteria respond quickly to biotic and abiotic changes in their environment. Because of their ubiquitous distribution and short generation time they have been used as valuable biological indicators for characterization of different water bodies (Rheinheimer and Gocke 1994). Previous studies have shown that bacterial metabolism can change very rapidly in response to changes in environmental conditions, probably more rapidly than other planktonic organisms (Straskrabova and Fuksa 1982). Thus measurements within shorter time intervals are useful in revealing bacterioplankton functions and lead to an analysis of the mechanisms involved in their control and regulation (Jugnia et al. 1998). Investigation on temporal and spatial variation in microbial populations and its relationship with environmental factors is very important for better understanding of biogeochemical cycling of elements and for effectively managing coastal ecosystems.

The major aim of biological oceanography is to identify the major processes that control the dynamics of aquatic ecosystems. This information is a pre-requisite for predicting the changes in systems that occur in response to natural and anthropogenic environmental changes and also for the management of marine resources. In order to understand the functioning of an aquatic ecosystem it is relevant to know the microbial component, how microbes are distributed and what regulates their abundance and biological activity. The coupling of bacterial abundance and activity is an important approach to understand the eutrophication in tropical estuaries submitted to pollution. Only very few detailed studies concerning the microbiology of an ecosystem by

comparative analysis have been presented worldwide and none from tropical region.

In the present study, endeavors were made to utilize all methods (combination of both modern and classical methods) and apparatus to study the inter-relationships between bacteria and other biotic and abiotic parameters in determining their assimilation potential to take up organic substrates. Extensive microbiological (both field and laboratory) investigations were carried out in this tropical estuarine system and compared with hydrographic, chemical and planktonological data.

5.2 RESULTS

The findings of the present investigation in the tropical Mandovi-Zuari estuarine system led to a better understanding on the role of the microorganism and its relationship with biotic and abiotic factors over seasonal scale (both spatially and temporally).

5.2.1 Phytoplankton productivity (PP)

Primary productivity (PP) at the surface waters ranged from 5.63 to 67.69 $\mu g\ C\ l^{-1}\ h^{-1}$ (mean value = 31.39 $\mu g\ C\ l^{-1}\ h^{-1}$) in the Mandovi estuary and 9.59 to 59.39 $\mu g\ C\ l^{-1}\ h^{-1}$ (mean value = 35.85 $\mu g\ C\ l^{-1}\ h^{-1}$) in Zuari estuary (Fig. 5.1). No significant variation in PP was observed between the two estuaries. At both the estuarine stations PP was higher in the pre-monsoon season and varied significantly ($p<0.001$) with the monsoon season. The average PP in the non-monsoon and monsoon seasons at the estuarine stations was 41.62 and 20.81 $\mu g\ C\ l^{-1}\ h^{-1}$ respectively. At the coastal station, the surface water PP ranged from 7.84 to 30.49 $\mu g\ C\ l^{-1}\ h^{-1}$ (mean value = 15.89 $\mu g\ C\ l^{-1}\ h^{-1}$) (Fig. 5.1). These rates were 2-3 fold lower when compared to estuarine station and varied significantly ($p<0.001$ for Mandovi estuary and $p<0.006$ for Zuari estuary). At the coastal station, PP was significantly ($p<0.01$) related to dissolved organic carbon.

5.2.2 Chlorophyll *a* and Phaeophytin pigments

The chl *a* values of the surface waters ranged from 0.02 to 6.94 $\mu g\ l^{-1}$ (mean value =1.39 $\mu g\ l^{-1}$) in the Mandovi estuary and 0.03 to 4.74 $\mu g\ l^{-1}$ (mean value = 1.46 $\mu g\ l^{-1}$) in the Zuari estuary (Fig. 5.2). Mandovi estuary showed significant seasonal variation in chl *a* values between pre- and monsoon seasons

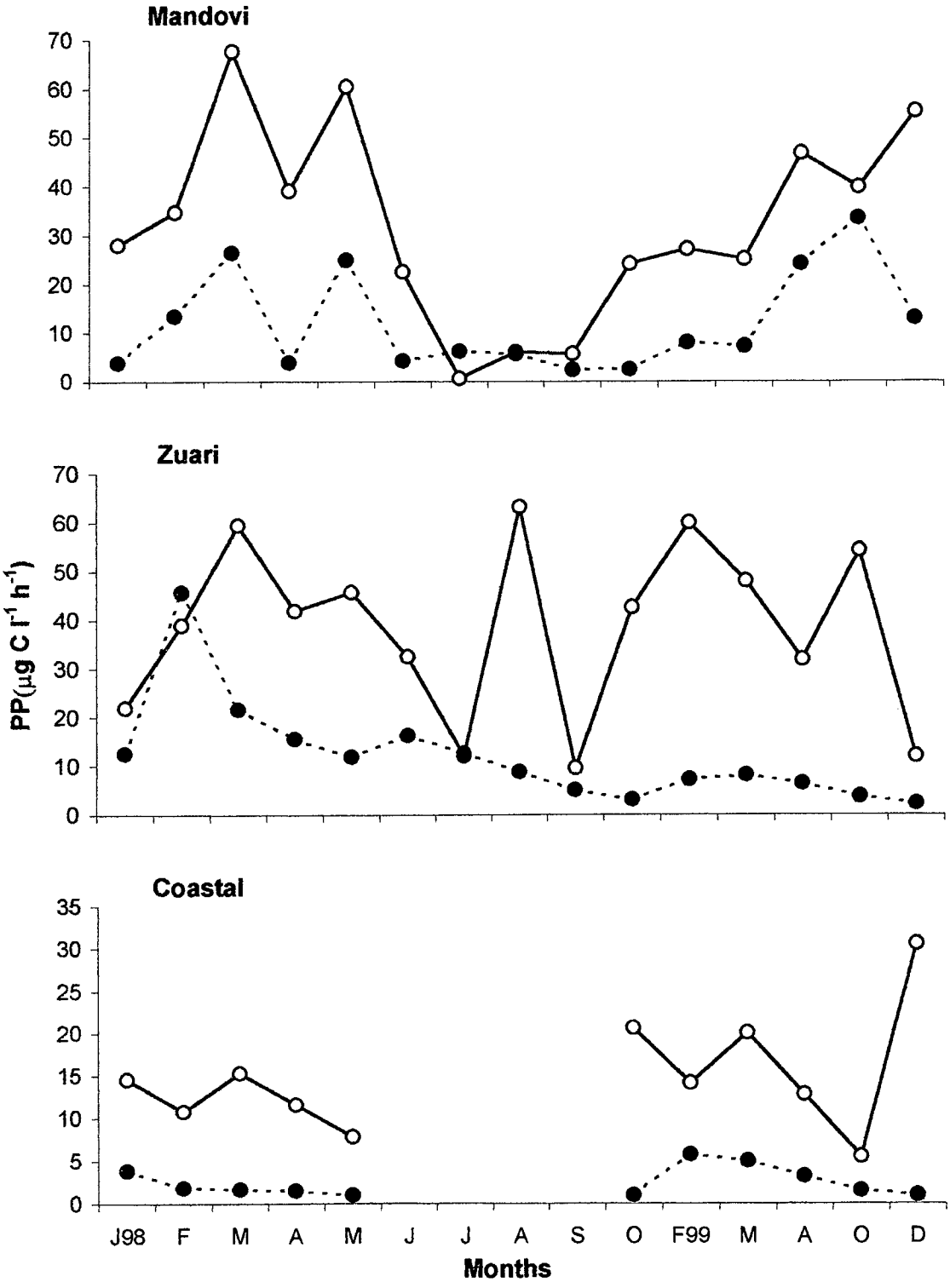

Fig. 5.1. Monthly variation in the primary productivity (PP) in the surface (empty) and bottom (filled) waters at the study stations

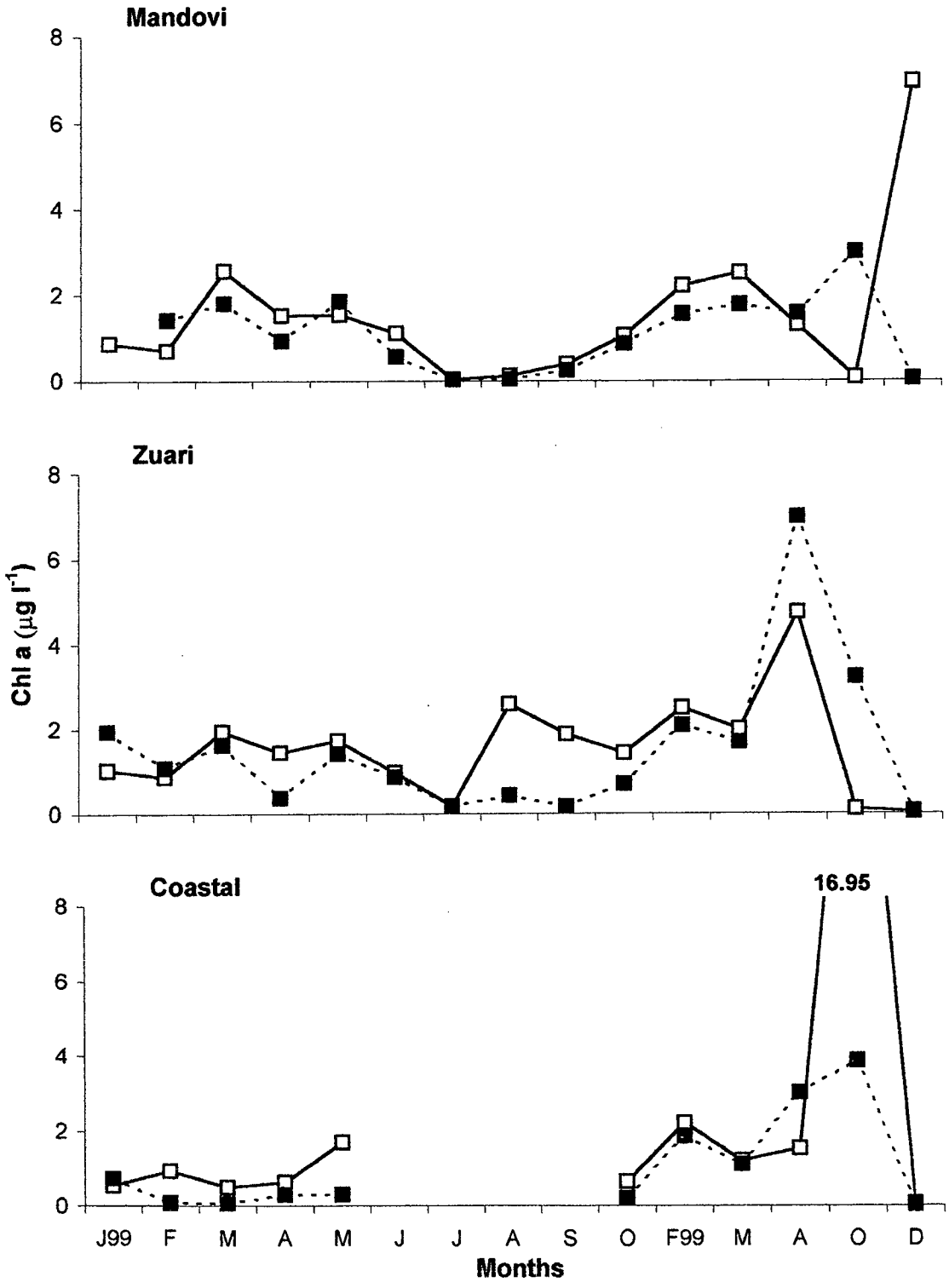

Fig. 5.2. Monthly variation in the chlorophyll *a* (Chl *a*) concentration in the surface (empty) and bottom (filled) waters at the study stations

both at the surface ($p<0.04$) and bottom ($p<0.002$) waters. Significant variation ($p<0.01$) in chl *a* values between surface and bottom was observed only in the coastal station. The mean chl *a* values at the bottom waters in Mandovi and Zuari estuaries were 1.02 and 1.46 μg l^{-1} respectively. The coastal waters showed a mean value of 2.61 and 0.95 μg l^{-1} at the surface and bottom waters respectively. Unlike PP the chl *a* concentration or the algal biomass was higher by two folds in the estuaries than coastal waters.

Phaeophytin pigments, derogative form of chlorophyll, ranged from undetectable level to 0.92 μg l^{-1} (mean value=0.15 μg l^{-1}) at the estuarine stations and undetectable level to 1.99 μg l^{-1} (mean value= 0.25 μg l^{-1}) at the coastal station at both surface and bottom waters. Significant difference ($p<0.04$) in phaeopigments was observed between the estuarine stations.

5.2.3 Bacteriological parameters

5.2.3.1 Total bacterial counts (TC)

Total bacterial counts (TC) did not significantly differ between estuarine and coastal stations. TC ranged from 1.86 to 19.3 x 10^9 cells l^{-1} in the Mandovi estuary and from 1.78 to 21.66 x 10^9 cells l^{-1} in Zuari estuary (Fig. 5.3). In the Mandovi estuary, TC significantly differed ($p<0.01$) between non-monsoon and monsoon seasons, whereas in the Zuari estuary TC varied significantly ($p<0.03$) between pre- and monsoon seasons. At the coastal station, TC mostly ranged from 1.31 to 11.87 x 10^9 cells l^{-1} (Fig. 5.3). TC was negatively related ($p<0.05$) to salinity and C:N ratio at the Mandovi station (Table 5.1a). At the coastal station TC was positively ($p<0.01$) related to PON (Table 5.1b).

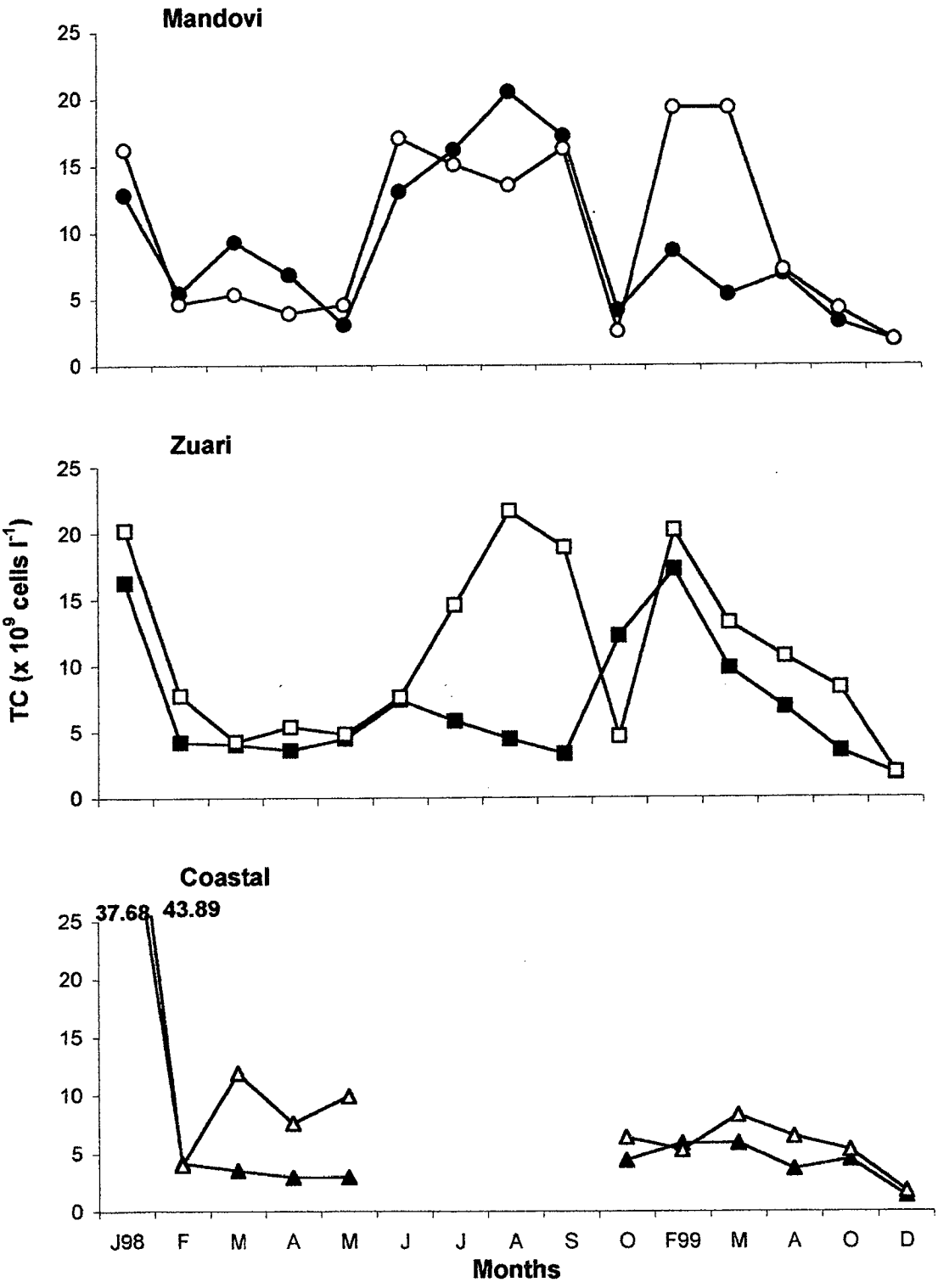

Fig. 5.3. Monthly variation in the total bacterial counts (TC) in the surface (filled) and bottom (empty) waters

Table 5.1a. Significant relationship between physico-chemical, biological and bacteriological parameters in the Mandovi estuary

Level of significance - * = p<0.05, ** = p<0.01, *** = p<0.001

Variable (n=30)		r
x	y	
Total bacterial counts	Salinity	-0.41*
Total bacterial counts	Temperature	-0.41*
Total bacterial counts	C:N ratio	-0.38*
Total bacterial counts	Labile organic carbon	0.43*
Direct viable counts	V_{max} (Glucose)	0.66***
Colony forming units	Particulate organic carbon	0.49**
Colony forming units	Particulate organic nitrogen	0.39*
Bacterial productivity	C:N ratio	0.70***
Turnover time (glutamic acid)	Particulate organic nitrogen	0.57**
V_{max} (glutamic acid)	Dissolved organic carbon	0.76***

Table 5.1b. Significant relationship between physico-chemical, biological and bacteriological parameters in the coastal waters

Level of significance - * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Variable (n=24)		r
x	y	
Total bacterial counts	Total viable counts	0.74***
Total bacterial counts	Particulate organic nitrogen	0.65**
Colony forming units	Particulate organic nitrogen	0.62**
Bacterial productivity	pH	0.52*
Bacterial productivity	Particulate organic nitrogen	0.46*
Bacterial productivity	C:N ratio	0.81***
Bacterial productivity	Primary productivity	0.76***
Bacterial productivity	Labile organic matter	0.61**
Bacterial growth rate	Primary productivity	0.47*
Bacterial growth rate	Particulate organic nitrogen	0.45*
V_{max} (glutamic acid)	Dissolved organic carbon	0.69**
Kt+Sn (glutamic acid)	Particulate organic nitrogen	0.69**

5.2.3.2 Direct viable counts (DVC)

On an average the direct viable counts (DVC) was less than TC by 1-3 folds and ranged from 0.29 to 5.13 x 10^9 cells l^{-1} and 0.16 to 8.74 x 10^9 cells l^{-1} in the Mandovi and Zuari estuary respectively (Fig. 5.4). Significant ($p<0.05$) variation in DVC was observed only during the pre- and monsoon season at Mandovi station. At the coastal station the DVC ranged from 0.19 to 3.72 x 10^9 cells l^{-1}. DVC related significantly to TC at both coastal ($p<0.001$) and Zuari ($p<0.01$) stations (Table 5.1b & c).

5.2.3.3 Retrievable plate counts (colony forming units, CFU)

The retrievable plate counts (CFU) were 1-3 orders lower than that of the total bacterial counts and ranged from 0.006 to 0.78 x 10^9 cells l^{-1} (mean value= 0.12 x 10^9 cells l^{-1}) in the Mandovi estuary and 0.004 to 0.35 x 10^9 cells l^{-1} (mean value= 0.07 x 10^9 cells l^{-1}) in the Zuari estuary at both surface and bottom waters (Fig. 5.5). At the coastal station the counts were lower than the estuarine stations and ranged from 0.0025 to 0.25 x 10^9 cells l^{-1} (Fig. 5.5). Though the counts were higher at the bottom than surface waters, it did not differ significantly. CFU related significantly to PON ($p<0.001$) at all three stations and to POC ($p<0.05$) at the Mandovi station only (Table 5.1a).

5.2.3.4 Ratio of bacterial biomass: phytoplankton biomass (BB:PB)

Bacterial biomass (calculated using 20 fg C $cell^{-1}$) exceeded phytoplankton biomass (calculated using a conversion factor of 50) at all the three stations in this tropical estuarine system with higher ratios in the bottom than surface waters (Table 5.2a & b). The BB: PB ratios were particularly higher in the monsoon

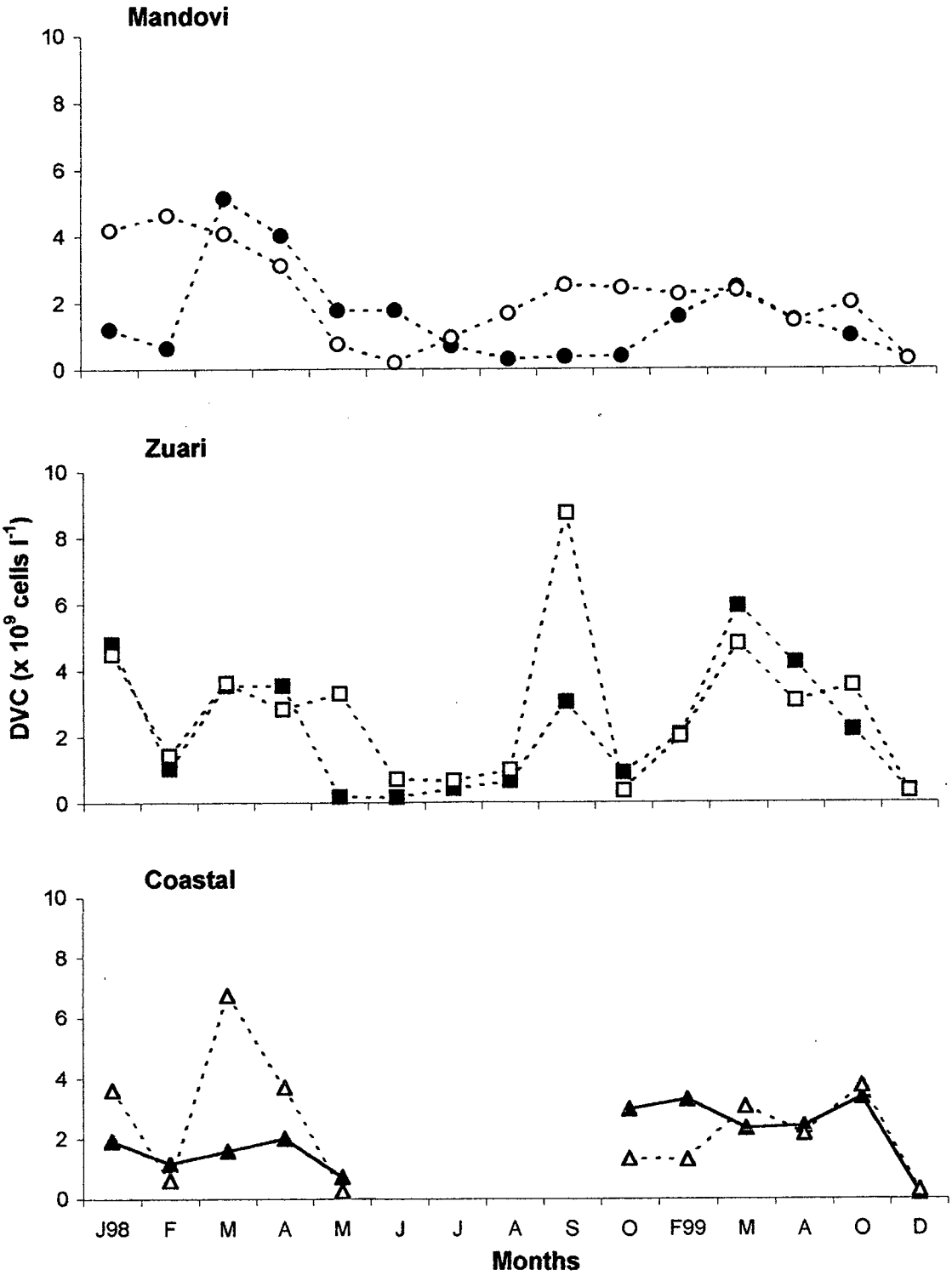

Fig. 5.4. Monthly variation in the total direct viable bacterial counts (DVC) in the surface (filled) and bottom (empty) waters

Table 5.1c. Significant relationship between physico-chemical, biological and bacteriological parameters in the Zuari estuary

Level of significance - * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Variable (n=30)		r
x	y	
Total bacterial counts	Total viable counts	0.50**
Direct viable counts	Turnover time (glutamic acid)	0.59**
Colony forming units	Particulate organic nitrogen	0.66***
Primary productivity	Labile organic carbon	0.54**
Bacterial productivity	C:N ratio	0.75***
Bacterial productivity	Primary productivity	0.45*
Turnover time (glutamic acid)	Total organic carbon	0.69***

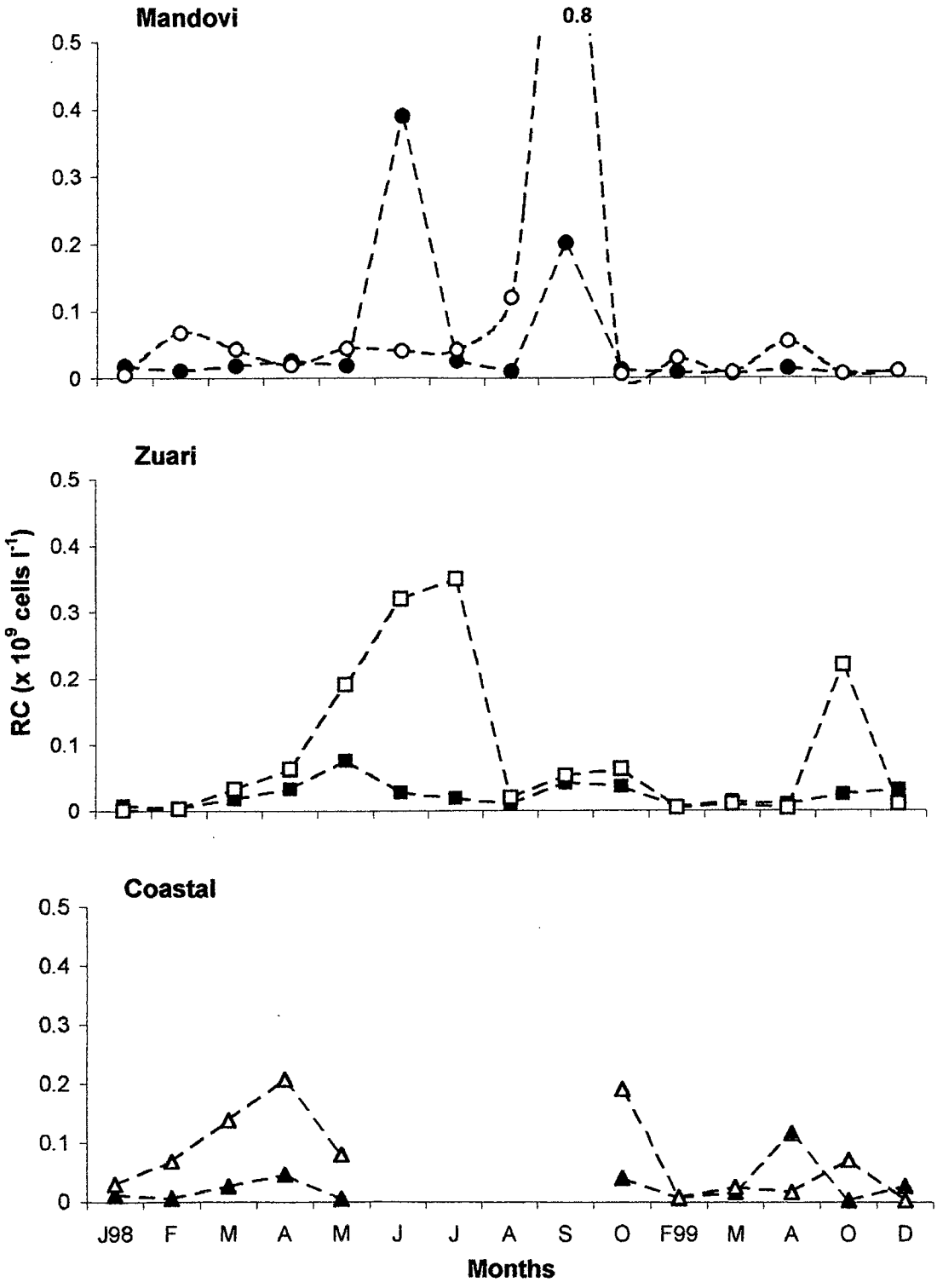

Fig. 5.5. Monthly variation in the retrievable counts (RC) in the surface (filled) and bottom (empty) waters

Table 5.2a. Seasonal variation in the phytoplankton biomass (PB), bacterial biomass (BB), and BB:PB ratio in the surface waters

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
PB ($\mu g\ C\ l^{-1}$)	Range	35.0-127.5	55.0-64.5	3.2-52.50	43.5-97.5	49.0-130.0	1.6-72.0	24.5-84.0	-	27.0-32.50
	Mean	78.88	59.75	33.50	75.13	91.17	32.63	46.50	-	29.75
	SD	37.86	6.72	26.28	23.44	40.60	34.68	26.65	-	17.86
BB ($\mu g\ C\ l^{-1}$)	Range	61.0-186.0	137.0-261.4	37.2-82.6	72.0-89.8	65.6-148.0	35.8-70.2	8.0-81.6	-	26.2-89.1
	Mean	123.10	199.20	61.44	81.65	111.01	52.99	51.41	-	67.43
	SD	52.24	87.96	22.86	7.43	33.83	24.34	30.99	-	35.72
BB:PB	Range	0.79-3.11	2.1-4.8	1.6-5.9	0.83-1.93	0.57-3.02	0.69-6.32	0.33-1.88	-	2.68-27.91
	Mean	1.56	3.33	1.83	1.08	1.21	1.62	1.11	-	2.26
	SD	1.37	1.86	0.80	0.50	1.19	0.70	0.77	-	2.00

Table 5.2b. Seasonal variation in the phytoplankton biomass (PB), bacterial biomass (BB), and BB:PB ratio in the bottom waters

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
PB ($\mu g\ C\ l^{-1}$)	Range	46.0-92.0	27.5-78.0	1.81-43.0	18.5-81.5	9.0-43.50	2.6-161.0	3.5-20.25	-	1.8-192.0
	Mean	74.63	52.75	22.41	56.13	26.25	74.02	15.00	-	68.25
	SD	21.41	35.71	29.12	27.51	20.12	70.11	10.37	-	36.67
BB ($\mu g\ C\ l^{-1}$)	Range	78.6-106.8	142.8-341.6	38.0-84.46	85.0-155.2	152.4-378.0	37.5-404.0	79.8-150.8	-	34.9-877.9
	Mean	92.35	242.21	57.81	110.95	222.0	174.81	115.30	-	89.03
	SD	11.53	140.56	23.99	30.88	114.01	161.54	50.20	-	48.13
BB:PB	Range	0.99-1.71	1.83-12.42	0.57-20.93	1.04-5.81	1.04-5.81	1.03-14.64	10.05-67.83	-	0.55-11.55
	Mean	1.23	4.59	2.58	1.97	8.54	2.36	7.69	-	1.30
	SD	0.30	3.93	0.82	1.12	4.03	2.30	4.84	-	1.31

season at Mandovi station and post-monsoon season at Zuari and coastal stations (Table 5.2a & b). Significant inverse relationship ($p<0.01$) was observed between ratio of bacterial to phytoplankton growth rate (Bμ : Pμ) to BB:PB at both estuarine and coastal stations (Fig. 5.6). Seasonal variation in BB, PB and their ratios for both surface and bottom waters are shown in Table 5.2a & b.

5.2.3.5 Bacterial productivity (BP)

Bacterial productivity (BP) generally ranged from 0.24 to 5.34μg C l^{-1} h^{-1} in the Mandovi estuary and 0.57 to 5.47μg C l^{-1} h^{-1} in the Zuari estuary (Fig. 5.7). High values of 66.74 and 84.50 μg C l^{-1} h^{-1} were recorded in both Mandovi and Zuari estuaries in the monsoon season. Significant ($p<0.01$) seasonal variation in the BP was observed between non-monsoon and monsoon seasons at both Mandovi and Zuari estuaries. At the coastal station, BP mostly (90%) ranged from 0.56 to 5.98 μg C l^{-1} h^{-1}. A high value of 114.40 μg C l^{-1} h^{-1} was observed in Oct. '98 (Fig. 5.7). In the coastal waters significant variation in BP rates was observed between pre- and post-monsoon seasons ($p<0.008$). BP related well with ammonia ($p<0.01$) in the Mandovi estuary (Table 5.1a) and with PP ($p<0.05$) in the Zuari estuary (Fig. 5.8). At the coastal station, BP was significantly related to pH ($p<0.05$), PON ($p<0.05$) and LOC ($p<0.01$) (Table 5.1c). Positive significant relationship ($p<0.001$) between BP and PP was observed at this station (Fig. 5.8). At all the stations, C:N ratio related inversely to BP (Fig. 5.9). No significant relationship was observed between BP and bacterial biomass.

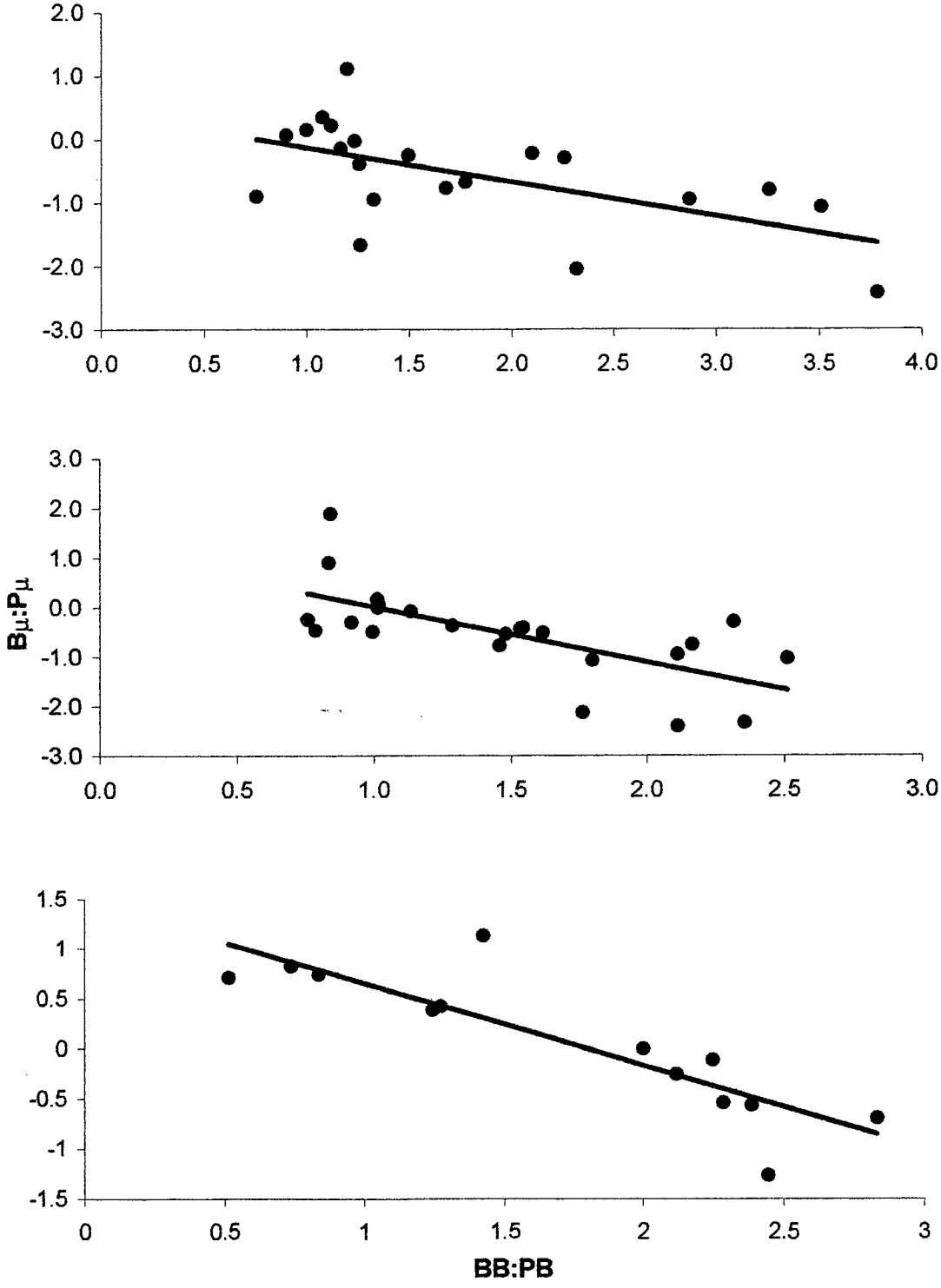

Fig. 5.6. Scatter plot between bacterial growth rate:phytoplankton growth rate and bacterial biomass:phytoplankton biomass (log transformed) at Mandovi ($n=21$, $r=0.52$, $p<0.001$), Zuari ($n=24$, $r=0.67$, $p<0.01$) and coastal ($n=13$, $r=0.87$, $p<0.001$) stations

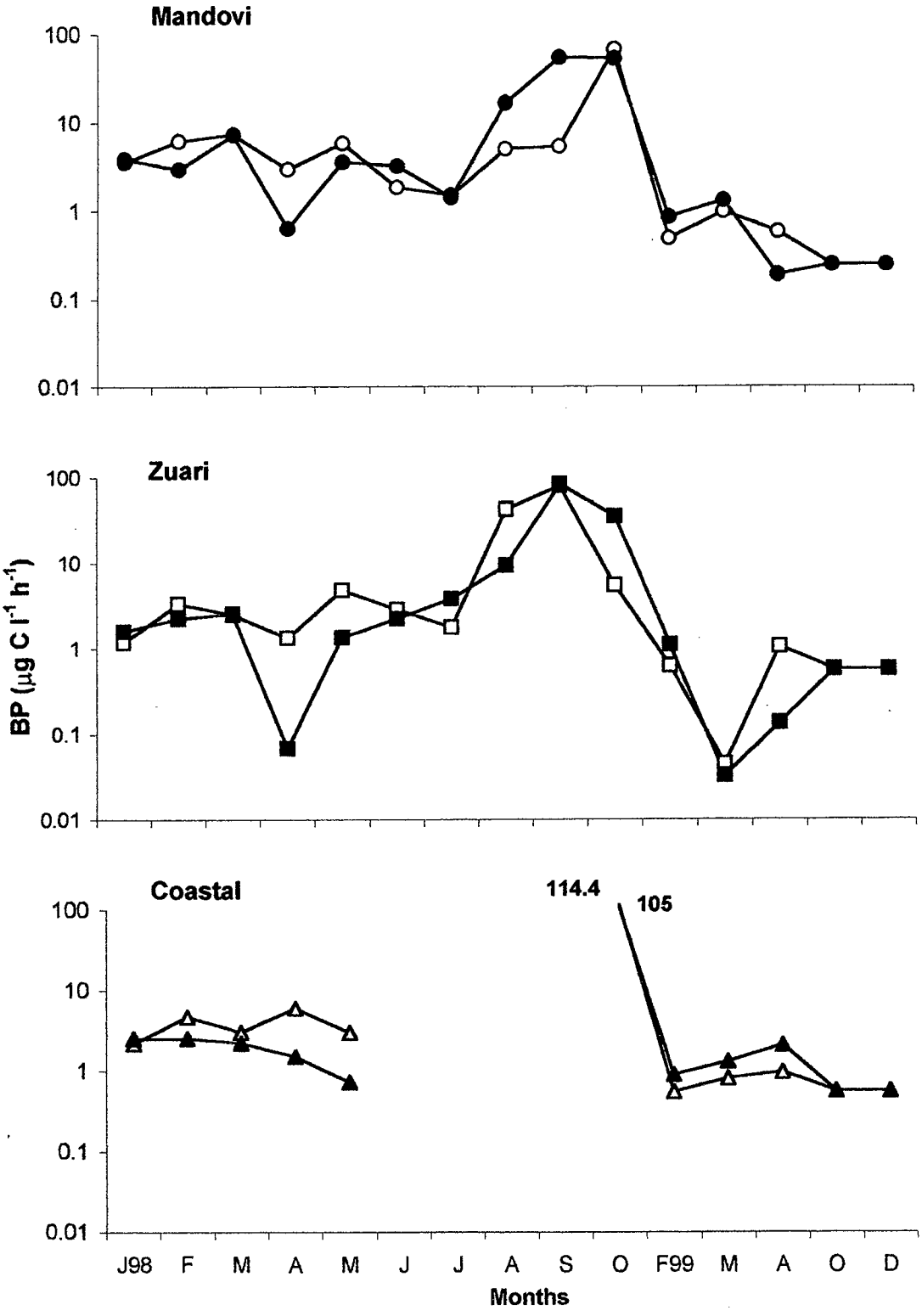

Fig. 5.7. Monthly variation in the bacterial productivity (BP) in the surface (empty) and bottom (filled) waters

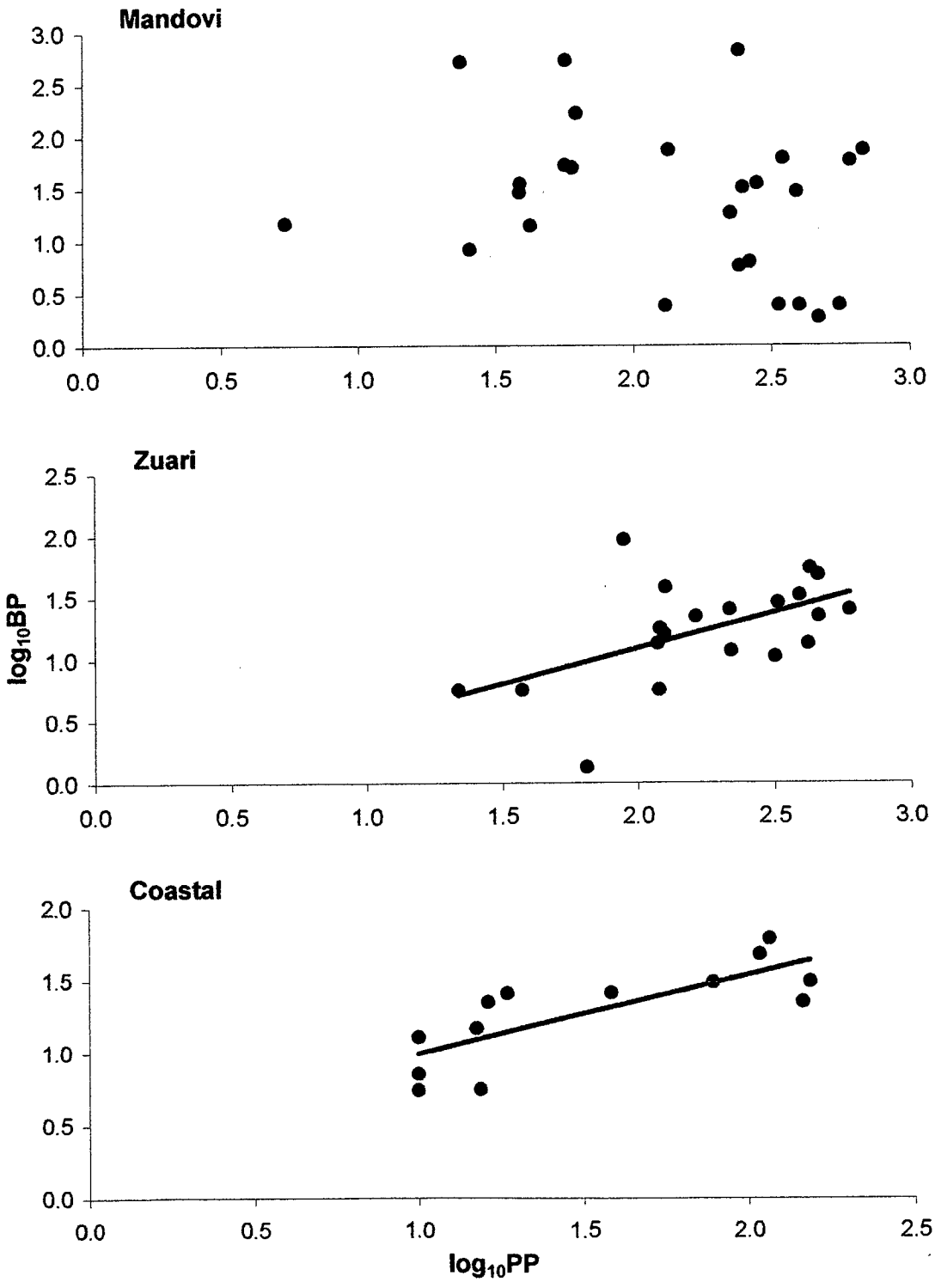

Fig. 5.8. Relationship between log transformed (log $_{10}$) bacterial productivity and primary productivity at Mandovi (not significant), Zuari (n=20, r=0.54, p<0.01) and coastal (n=13, r=0.78, p<0.001) stations

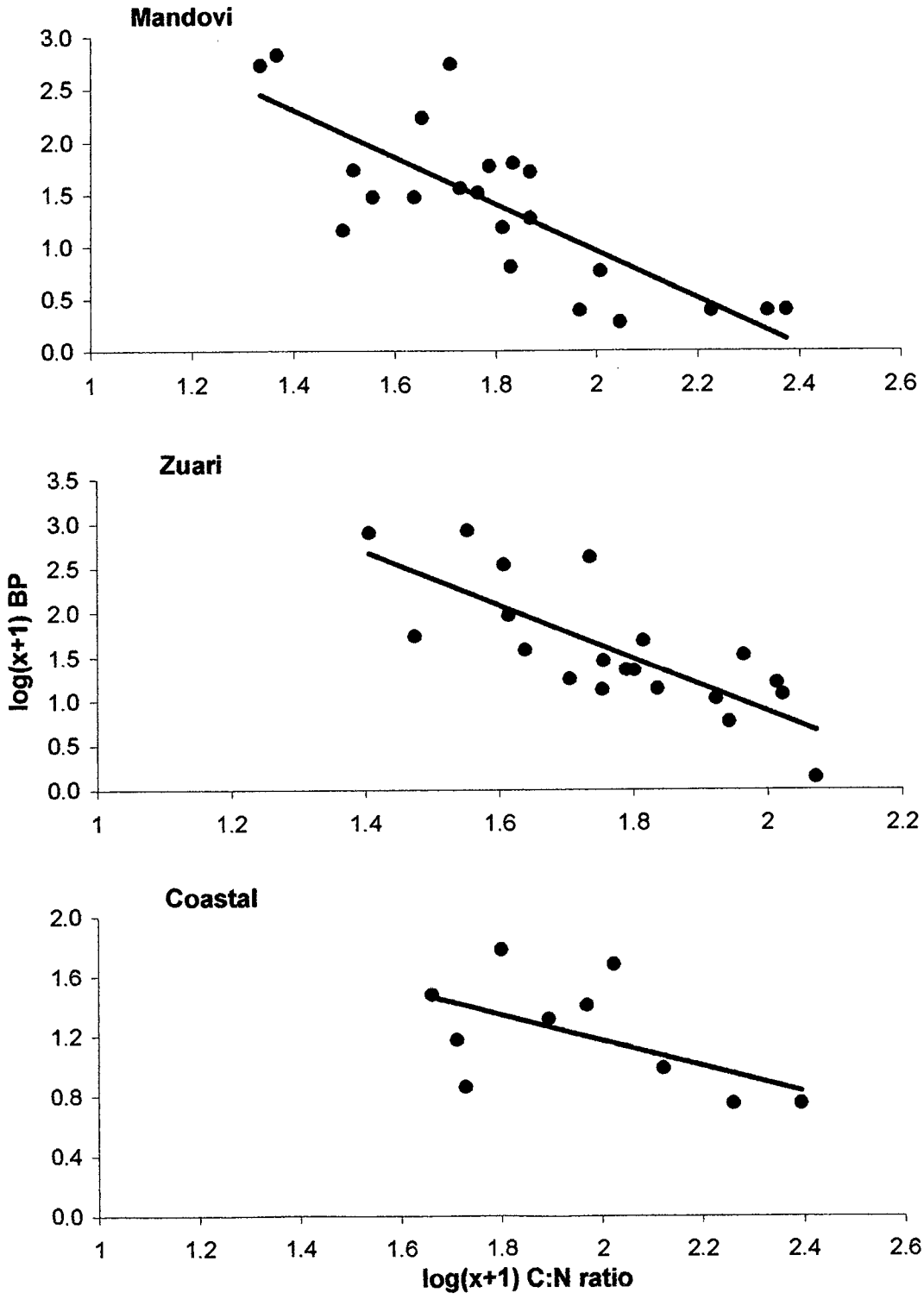

Fig. 5.9. Relationship between C:N ratio and bacterial producitivity at Mandovi (n=22, r=0.79, p<0.001), Zuari (n=20, 0.78, p<0.001) and coastal (n=11, r=0.60, p<0.05) stations

5.2.3.6 Specific growth rate (μ) and generation time

Specific growth rate was higher at the estuarine when compared to coastal station. Bacterial specific growth rate (SGR) at the estuarine stations ranged from 0.05 to 3.70 d^{-1} (mean value= 0.52 d^{-1}) at Mandovi and from 0.01 to 7.79 d^{-1} (mean value= 0.80 d^{-1}) in the Zuari estuary (Table 5.3a & b). Their doubling time mostly ranged from 0.19 to 7.92 days with a mean value of 3.43 and 2.76 days for Mandovi and Zuari estuaries. Although SGR was higher in the pre-monsoon than other seasons, it did not significantly vary. SGR did not show any significant variation among stations nor between surface and bottom waters. The coastal station showed SGR to range from 0.07 to 2.35 d^{-1}(mean value =0.44 d^{-1}) (Table 5.3a & b). Their corresponding doubling time ranged 0.09 to 9.96 days. Seasonal variation of SGR and generation time for the three stations is given in Table 5.3a & b. SGR significantly related to PP ($p<0.05$) at the coastal station.

5.2.3.8 Heterotrophic activity

Glucose

Turnover time (Tt)

The Tt of bacterial population with glucose as substrate mostly ranged from 2 to 208.54 h in the Mandovi estuary and 5 to 320 h in the Zuari estuary (Fig. 5.10). Significant variation in Tt was observed in the pre- and post-monsoon seasons at estuarine stations with Zuari estuary showing higher values than Mandovi estuary. However in the monsoon season the reverse was observed with Mandovi estuary showing higher (1-4 fold) Tt. The Tt at the coastal station

Table 5.3a. Seasonal variation in the specific bacterial growth rate (SGR) and generation time (T_d) of the bacterial community in the surface waters.

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
SGR	Range	0.22-2.19	0.10-0.37	0.16-0.33	0.44-1.26	0.19-0.46	0.09-0.53	0.11-2.35	-	0.15-0.51
(d^{-1})	Mean	1.04	0.21	0.24	0.84	0.34	0.30	1.04	-	0.33
	SD	0.77	0.12	0.12	0.34	0.14	0.20	0.93	-	0.25
T_d	Range	0.32-3.16	1.88-6.88	2.10-4.33	0.55-1.57	1.51-3.64	1.31-7.70	0.29-6.30	-	1.35-4.62
(d)	Mean	0.67	3.3	2.89	0.83	2.03	2.31	0.67	-	2.1
	SD	0.50	2.24	1.18	0.44	1.50	1.18	0.75	-	2.36

Table 5.3b. Seasonal variation in the specific bacterial growth rate (SGR) and generation time (T_d) of the bacterial community in the bottom waters

Parameters		Mandovi			Zuari			Coastal		
		Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
SGR	Range	0.19-1.59	0.11-3.70	0.12-0.15	0.02-0.71	0.31-0.52	0.08-0.36	0.19-0.75	-	0.07-0.38
(d^{-1})	Mean	0.87	1.37	0.14	0.35	0.39	0.18	0.32	-	0.19
	SD	0.58	1.67	0.02	0.28	0.11	0.16	0.24	-	0.18
T_d	Range	0.44-3.64	0.18-6.30	4.62-5.78	0.98-34.65	1.33-2.24	1.93-8.66	0.92-3.64	-	1.82-9.90
(d)	Mean	0.80	0.51	4.95	1.98	1.77	3.85	2.17	-	3.65
	SD	0.35	0.54	2.92	0.61	0.93	3.47	1.10		2.57

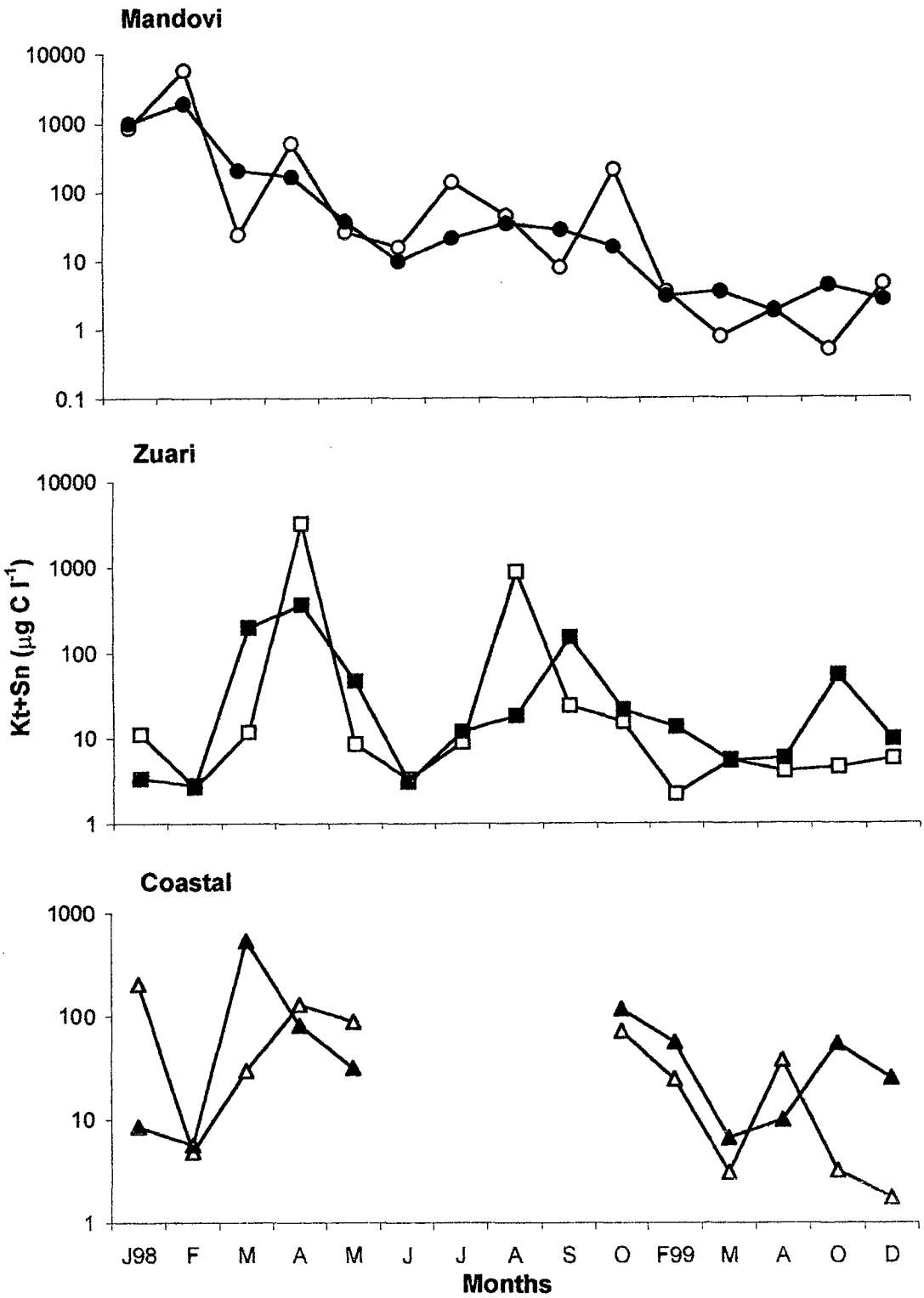

Fig. 5.10. Monthly variation in the turnover time (Tt) of ^{14}C-glucose by bacterial population at surface (empty) and bottom waters (filled).

ranged from 3.5 to 221.01 h (Fig. 5.10). No significant difference in Tt was observed between surface and bottom waters at the stations.

Uptake velocity (V_{max})

The uptake velocity for glucose substrate ranged from 0.25 to 212.81 μg C l^{-1} h^{-1} in the Mandovi estuary and 0.02 to 55.67 μg C l^{-1} h^{-1} in the Zuari estuary. At both the stations, the V_{max} values seasonally varied by 2-3 orders of magnitude. Zuari recorded high values in the monsoon than other seasons. Highest value of 55.67 μg C l^{-1} h^{-1} was recorded in August (1998) at Zuari estuary (Fig. 5.11). Although the bottom waters showed higher V_{max} than surface by 1-2 folds, the values did not vary significantly. Like Zuari station the coastal station also showed a high V_{max} of 7.09 μg C l^{-1} h^{-1} in October (1998). The V_{max} values were lower than that of Mandovi estuary. Significant relationship ($p<0.01$) was observed between V_{max} and DVC in Mandovi estuary (Table 5.1a).

Transport constant and natural substrate concentration (Kt+Sn)

The Kt+Sn ranged mostly from 0.5 to 208.76 μg C l^{-1} with a high value of 5770.56 μg C l^{-1} in February (1998) in the Mandovi estuary (Fig. 5.12). Zuari estuary showed less variability in Kt+Sn values and ranged from 2.20 to 15.45 μg C l^{-1} (CV=74%) except for a high value of 3246.46 μg C l^{-1} in April (1998). At the coastal station the values ranged from 3.50 to 221.01 μg C l^{-1} with post-monsoon season showing significantly ($p<0.02$) lower values than pre-monsoon season. In both the surface and bottom waters of Zuari estuary, Kt+Sn related significantly ($p<0.05$) with temperature (Table 5.1b).

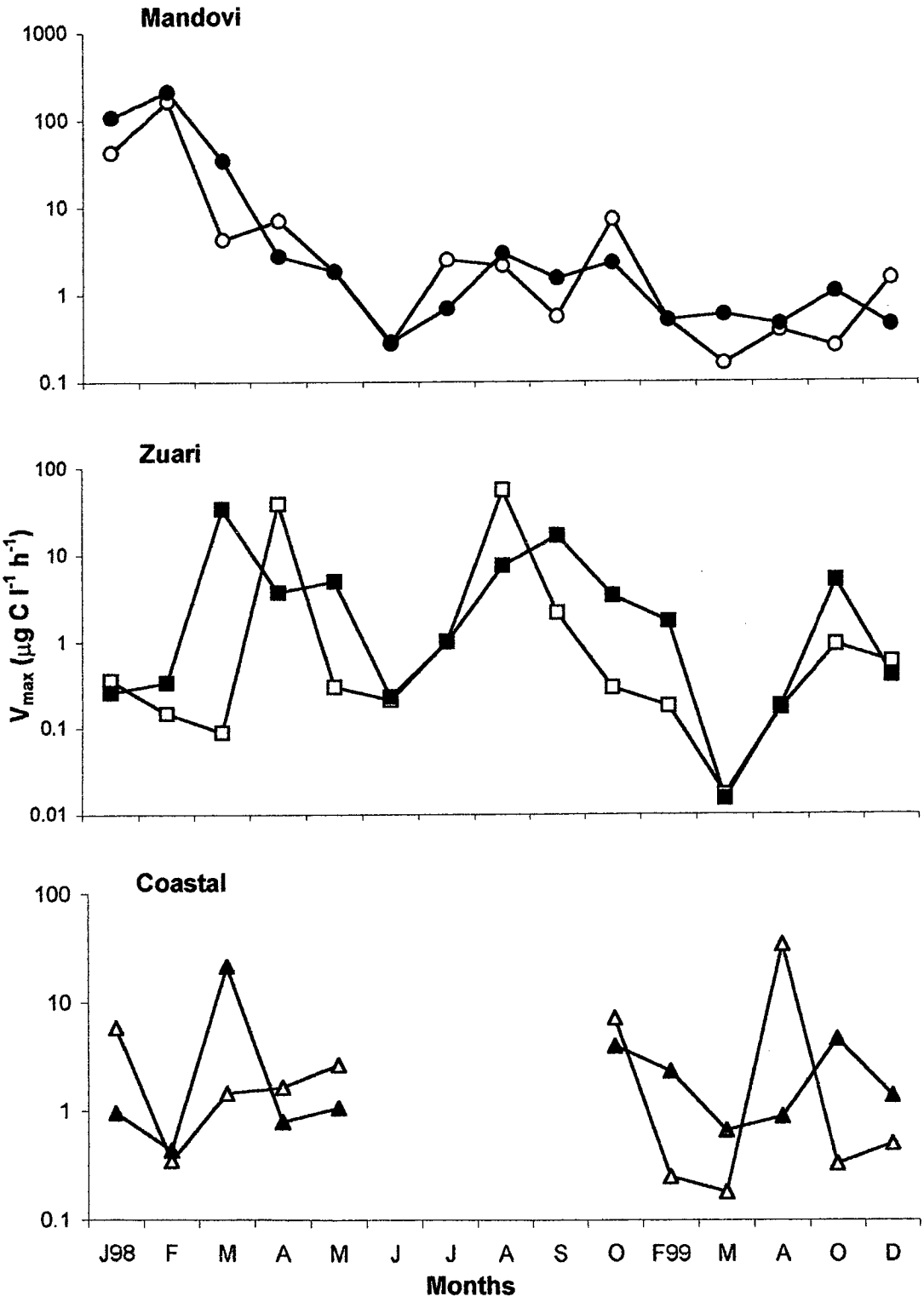

Fig. 5.11. Monthly variation in the uptake velocity (Vmax) of ^{14}C-glucose by bacterial population at surface (empty) and bottom waters (filled)

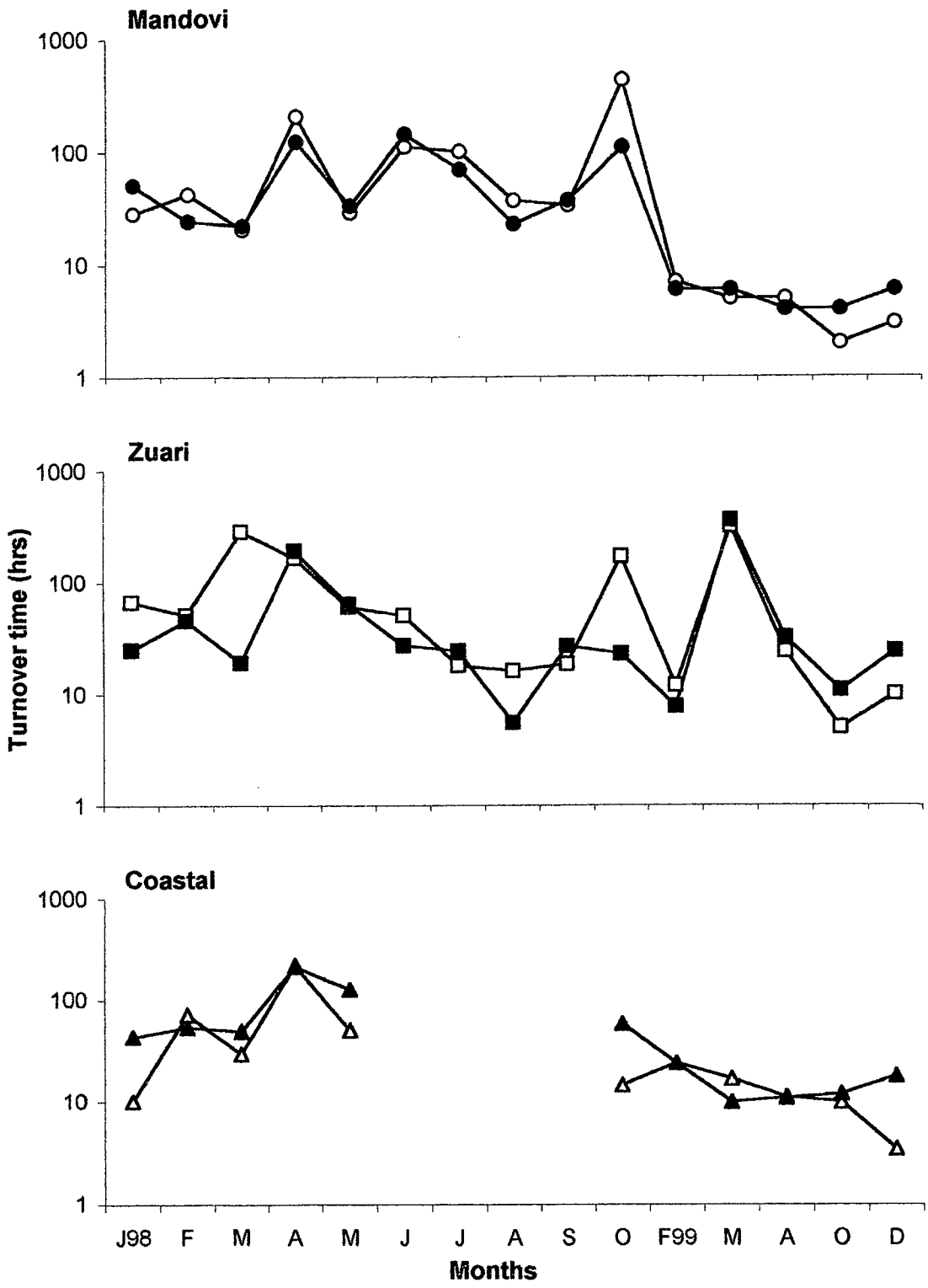

Fig. 5.12. Monthly variation in the of transport constant and natural substrate concentration (Kt+Sn) of glucose at surface (empty) and bottom waters (filled)

Glutamic acid

Turnover time (Tt)

The Tt of bacterial population utilizing glutamic acid ranged from 3.2 to 690.61 h (Fig. 5.13) and did not show any seasonal variation. Similarly in Zuari estuary the values mostly ranged from 2 to 333.00 h and was lower than that of Mandovi. Tt was highest in the adjacent coastal waters, which ranged from 4 to 573.49 h (Fig. 5.13). At all the stations the surface and bottom waters did not differ significantly.

Uptake velocity (V_{max})

The maximum uptake velocity was highest in the Zuari estuary than at Mandovi and coastal stations. In the Zuari estuary the V_{max} ranged from 0.01 to 1.78 μg C l^{-1} h^{-1} and 0.07 to 2.46 μg C l^{-1} h^{-1} respectively (Fig. 5.14). In both the surface and bottom waters of Mandovi estuary, V_{max} related significantly ($p<0.01$) with total organic carbon (Table 5.1a).

Transport constant and natural substrate concentration (Kt+Sn)

Kt+Sn did not follow any seasonal pattern at both the estuarine and coastal stations. The Kt+Sn ranged from 3.61 to 386.95 μg C l^{-1} at the estuarine and 1.2 to 104.01 μg C l^{-1} at the coastal stations (Fig. 5.15). The coastal station showed significant variation ($p<0.02$) between pre- and post-monsoon seasons. At all the three stations there was no significant variation between surface and bottom waters.

Seasonal variation in the ratio between bacterial productivity and heterotrophic activity at the all the study stations are shown Table 5.4a & b.

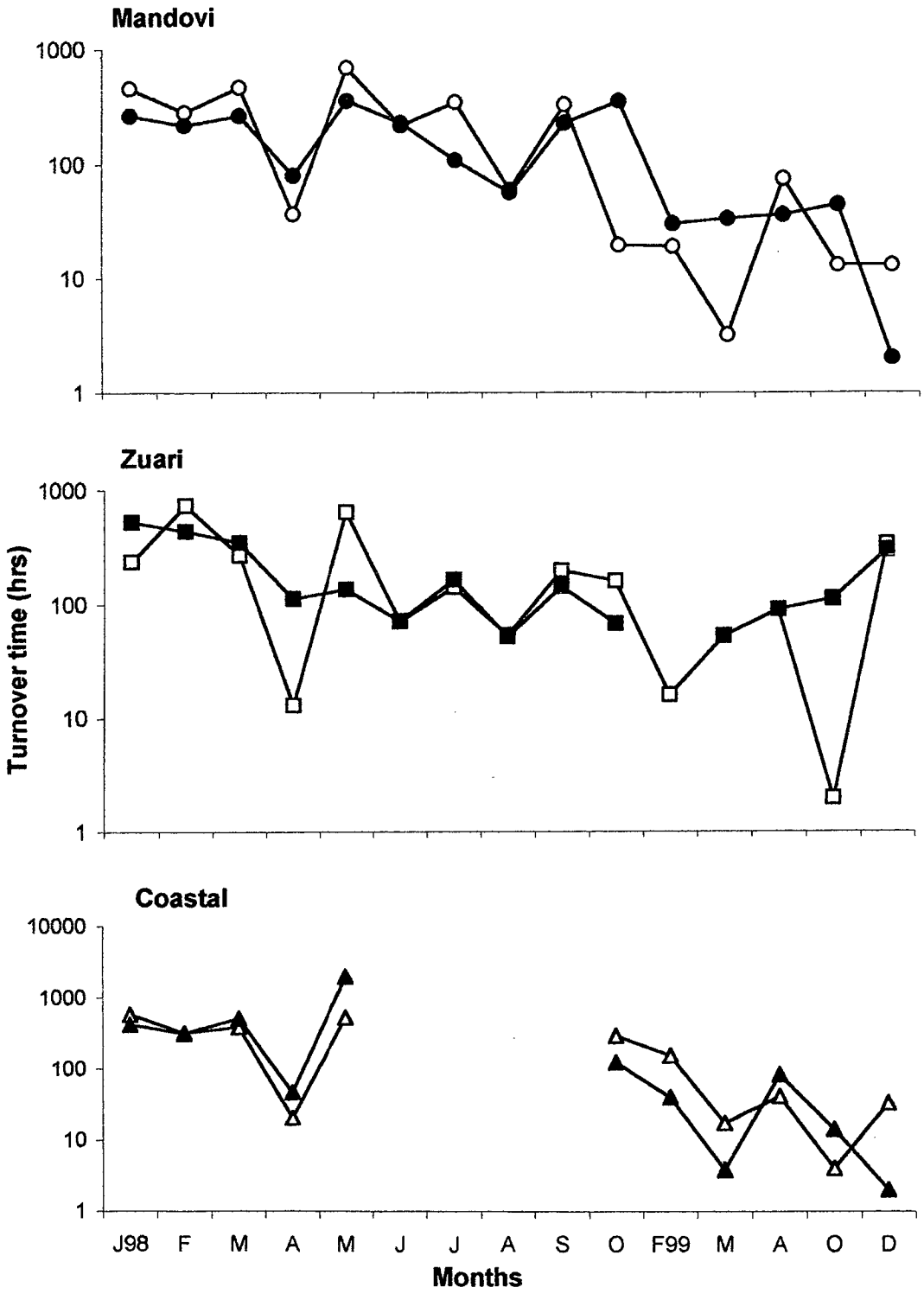

Fig. 5.13. Monthly variation in the turnover time (Tt) of ^{14}C-glutamic acid by bacterial population at surface (empty) and bottom waters (filled).

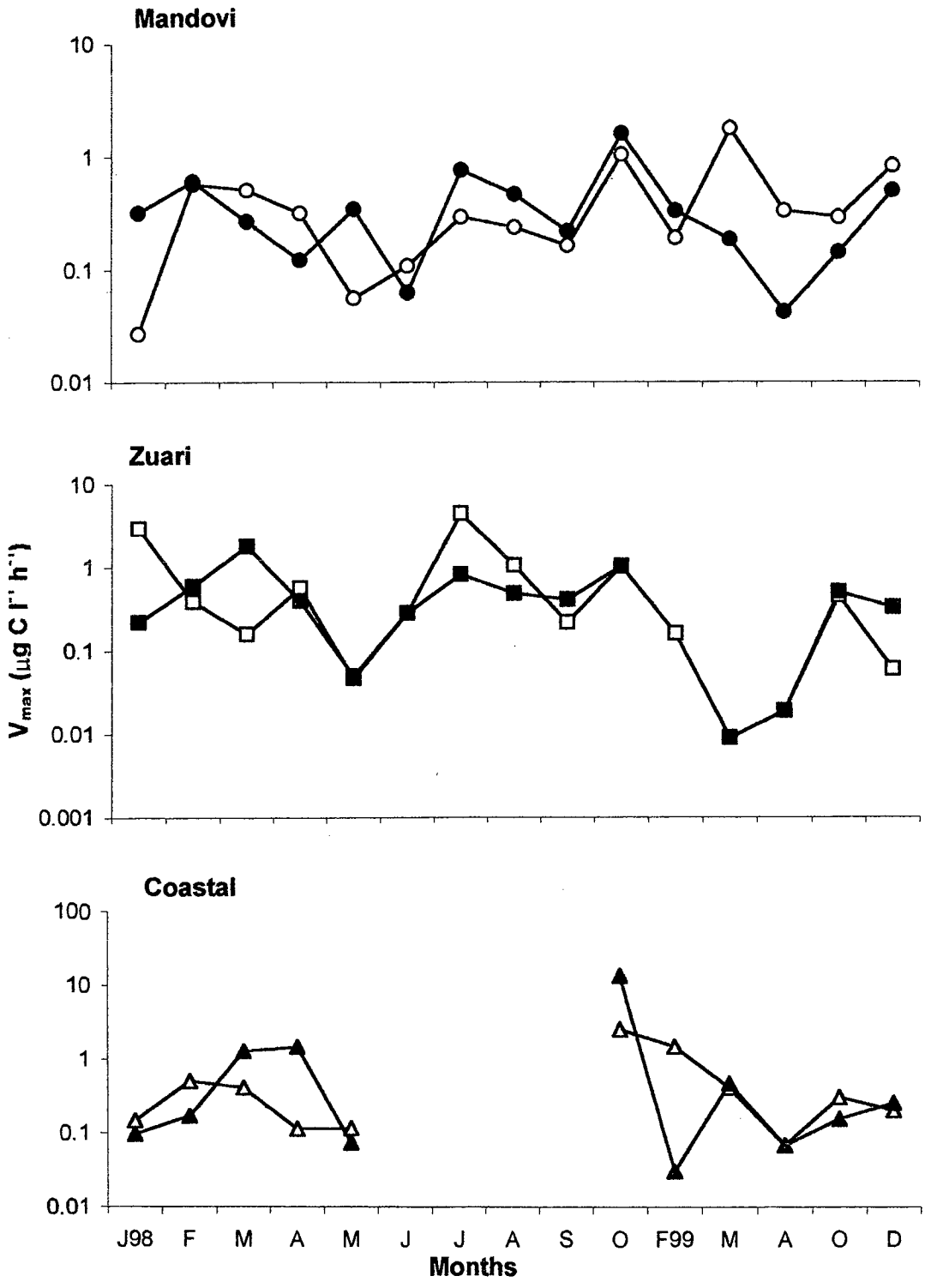

Fig. 5.14. Monthly variation in the uptake velocity (Vmax) of ^{14}C-glutamic acid by bacterial population at surface (empty) and bottom waters (filled)

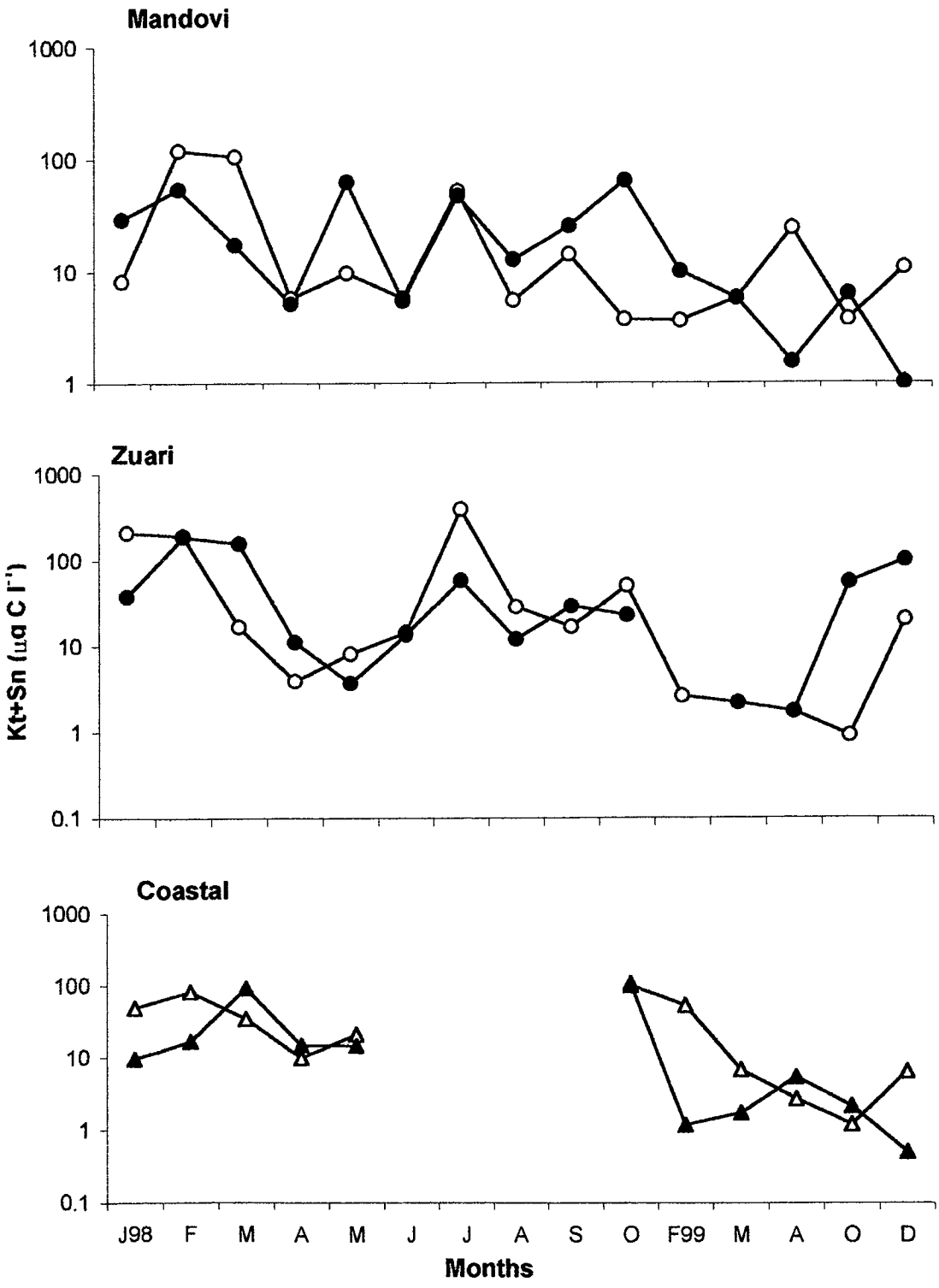

Fig. 5.15. Monthly variation in the of transport constant and natural substrate concentration (Kt+Sn) of glutamic acid at surface (empty) and bottom waters (filled)

Table 5.4a. Seasonal variation in the ratio between productivity and activity in the surface waters

Stations	Pre-monsoon	Monsoon	Post-monsoon
Mandovi	5.16*	0.60	4.83
	0.42**	0.18	1.16
Zuari	0.16	0.43	0.74
	0.18	0.53	0.9
Coastal	0.40	-	1.06
	0.57	-	0.25

* glucose

** glutamic acid

Table 5.4b. Seasonal variation in the ratio between productivity and activity in the bottom waters

Stations	Pre-monsoon	Monsoon	Post-monsoon
Mandovi	2.12*	0.64	2.07
	0.18**	0.16	0.69
Zuari	4.38	0.74	2.43
	1.44	0.11	0.41
Coastal	2.49	-	2.74
	0.35	-	0.22

* glucose

** glutamic acid

5.2.3.9 Exo-enzymatic activities

Lipase

Exo-enzymatic lipolytic activity was higher in the monsoon at surface waters (63.42 nmol h^{-1}) in the Mandovi estuary. Whereas in Zuari estuary and coastal waters, high values were observed at the surface waters of pre-monsoon season with a mean value of 25.29 and 25.44 nmol h^{-1} respectively (Table 5.5a & b). Overall at both surface and bottom waters, lipolytic activity was low in the post-monsoon seasons.

β-glucosidase

The β-glucosidase activity ranged from 0.11 to 15.59 nmol h^{-1} in the estuarine and 0.15 to 11.85 nmol h^{-1} in the coastal station respectively (Table 5.5a & b). The activity was significantly ($p<0.01$) lower than lipolytic activity. Glucosidase activity showed similar pattern like lipolytic activity being higher in the pre-monsoon season than in the monsoon.

Phosphatase

In this tropical ecosystem phosphatase activity was found to vary significantly with seasons. Mandovi estuary showed high phosphatase activity at both surface (mean= 89.10) and bottom (mean=71.01) waters in the monsoon season, whereas Zuari estuary showed a high value in the post-monsoon season. The mean activity was 111.07 and 147.19 nmol h^{-1} respectively (Table 5.5a & b). At the coastal station, phosphatase activity showed higher values in the surface waters of pre-monsoon season (38.44 nmol h^{-1}) and bottom waters of

Table 5.5a. Extra cellular enzymatic activity expressed as maximum rate of hydrolysis (V_m=nmol h^{-1}) in the surface waters.

Extracellular enzyme	Mandovi			Zuari			Coastal		
	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
Lipase	25.33 (±22.92)	63.42 (±6.38)	0.19 (±0.02)	25.29 (±22.78)	6.17 (±4.86)	0.27 (±0.07)	25.44 (±22.84)	-	0.17 (±0.05)
Glucosidase	5.87 (±4.18)	3.19 (±2.69)	0.82 (±0.20)	7.48 (±6.04)	3.07 (±2.80)	1.03 (±0.83)	5.98 (±4.17)	-	0.95 (±0.12)
Phosphatase	21.30 (±8.61)	89.10 (±55.66)	65.09 (±52.34)	19.70 (±4.47)	45.55 (±43.90)	111.07 (±46.09)	38.44 (±23.20)	-	18.40 (±0.40)
Chitinase	9.38 (±5.83)	10.65 (±3.98)	9.19 (±5.29)	10.30 (±5.20)	10.58 (±3.60)	12.19 (±1.13)	11.01 (±7.03)	-	12.83 (±0.92)
Aminopeptidase	12.35 (±3.00)	1.84 (±1.60)	0.62 (±0.12)	9.07 (±3.26)	2.14 (±1.32)	0.57 (±0.28)	4.98 (±4.04)	-	0.77 (±0.24)

Table 5.5b. Extra cellular enzymatic activity expressed as maximum rate of hydrolysis (V_m=nmol h^{-1}) in the bottom waters.

Extracellular enzyme	Mandovi			Zuari			Coastal		
	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
Lipase	36.52 (±11.60)	6.39 (±6.38)	0.35 (±0.24)	25.66 (±22.86)	6.87 (±6.38)	0.21 (±0.17)	23.53 (±21.82)	-	0.14 (±0.06)
Glucosidase	9.18 (±7.91)	2.52 (±2.42)	2.93 (±0.48)	7.63 (±6.34)	3.30 (±3.07)	1.09 (±0.72)	6.12 (±5.64)	-	0.52 (±0.35)
Phosphatase	19.08 (±5.45)	71.01 (±57.94)	71.82 (±42.82)	20.55 (±8.37)	34.76 (±25.98)	147.19 (±4.99)	17.25 (±5.89)	-	92.33 (±101.44)
Chitinase	11.31 (±6.88)	9.94 (±4.50)	9.61 (±5.40)	8.94 (±5.87)	9.86 (±4.39)	5.79 (±5.26)	6.35 (±1.44)	-	9.50 (±5.10)
Aminopeptidase	8.28 (±3.81)	1.87 (±1.48)	1.08 (±0.62)	9.85 (±8.48)	2.54 (±1.41)	1.79 (±1.21)	6.73 (±2.87)	-	0.93 (±0.38)

post-monsoon season (92.33 nmol h^{-1}). The enzyme hydrolysis rates were significantly higher (p<0.01) than other enzymes.

Chitinase

Of all exo-enzymes chitinase showed the least activity. Estuarine stations generally showed higher rates in the monsoon seasons and did not differ significantly with other seasons. Coastal station showed a mean value of 11.01 and 12.83 nmol h^{-1} at the surface than bottom waters of pre-monsoon and post-monsoon seasons and did not vary significantly with the estuarine stations (Table 5.5a & b).

Leucine Aminopeptidase

At all the three stations aminopeptidase activity was higher in the pre-monsoon than other seasons. The high values observed at the surface waters of Mandovi, Zuari and coastal stations are 12.35, 9.07 and 4.98 nmol h^{-1} respectively (Table 5.5a & b).

5.2.3.10 Community classification and characterization

In the present study a total number of 144 isolates were isolated during the field investigation and was subjected to morphological, physiological and biochemical characterization. In the process of sub-culturing, 15% of the isolates were lost and could not be retrieved either due to their inability of isolates to grow or due to contamination. Biochemical characterization and identification of the isolates were carried out following the scheme of Oliver (1982) up to genus level.

In this study the common bacterial groups encountered were *Pseudomonas, Alcaligenes, Flavobacterium, Cytophaga, Moraxella, Bacillus,*

members of Enterobacteriaceae and *Acinetobacter.* Of these, *Pseudomonas* was the dominant genus in both estuarine and coastal waters. High percentage of pigmented bacteria was observed during the monsoon season. Most of the isolates were gram negative, and oxidase and catalase positive. Although members of Enterobacteriaceae were found in this tropical estuarine system, they were less abundant.

Individual isolates were screened for their catabolic potentialities, growth under different physiological conditions and for antibiotic sensitivity.

The catabolic potentialities of the bacterial community were expressed in terms of exo-enzymatic average index (EAI). Seasonal variation in EAI of the bacterial isolates is shown in Table 5.6. In the present study, high EAI was observed for lipase, phosphatase, protease and urease enzymes in monsoon than other seasons. Amylase and gelatinase showed high percentage of 51.3 and 5.9% in the post-monsoon season respectively (Table 5.6). Irrespective of the seasons, the ability of isolates to utilize gelatin was higher (51.7%) than for any other substrates. The increasing order of EAI observed in the present study is as follows

Gelatinase>amylase>lipase>urease>phosphatase>DNAse>caesinase

Apart from the biochemical traits, the ability of the bacterial isolates to grow and respond to various physiological conditions like pH (4.2, 9.2), temperature (10°C, 37°C), and salinity (0, 35, 50) were tested. It was observed that the bacterial isolates showed better growth at pH 9.2 (67%) than at 4.2 (37%). Isolates grew better when they were incubated at 37°C (54%) than when

Table 5.6. Seasonal variation in the ecto-enzymatic average index (EAI%) in the Mandovi-Zuari estuarine system.

Enzymes	Pre-monsoon	Monsoon	Post-monsoon	Overall
Amylase	33.1	47.1	51.3	43.8
Caesinase	15.8	29.4	10.0	18.4
Gelatinase	46.2	50.1	59.2	51.7
Lipase	30.8	47.1	35.5	37.8
Phosphatase	25.4	41.7	17.2	28.1
Urease	38.5	47.1	24.1	36.6
DNAse	36.4	20.1	13.8	23.4

compared to 10°C (40%). Although 40% of the isolates grew at 10°C their growth was very slow (7-10 days) when compared to isolates incubated at 37°C (24-30 hours). Bacterial isolates showed high percentage of growth at 35 (66%) than at 0 (59%) and 50 (52%) salinity.

Antibiotic sensitivity tests on 15 different types of antibiotics were carried out for all the 125 isolates and overall percentage or response to the dose of antibiotics is shown in Table 5.7. Over 75% of the isolates showed the ability to grow in nutrient media amended with heavy metal (cadmium and mercury) separately at 10 ppm concentration.

Table 5.7. Percentage response of bacterial isolates to different antibiotics

Antibiotics	Percentage
Penicillin G (10 units/disc)	25.93
Nystatin (100 units/disc)	8.64
Polymyxin B (300 units/disc)	66.66
Chloramphenicol (30 mcg/disc)	99.00
Rifampicin (5 mcg/disc)	89.55
Streptomycin (10 mcg/disc)	66.66
Gentamycin (10 mcg/disc)	85.20
Tetracycline (30 mcg/disc)	93.44
Nalidixic acid (30 mcg/disc)	59.25
Oleanodomycin (15 mcg/disc)	62.96
Erythromycin (15 mcg/disc)	70.76
Chlortetracycline (30 mcg/disc)	94.20
Neomycin (30 mcg/disc)	87.65
Kanamycin (30 mcg/disc)	93.54
Doxycycline (30 mcg/disc)	24.19

5.3 DISCUSSION

Estuaries are subjected to a great deal of environmental stress and these 'stress habitats' form an unique and fascinating environment by being dynamic, complex and constantly changing through the interactions of freshwater with sea-water. This has considerable influence on the hydrography and productivity of the adjacent coastal waters. Thus knowledge of the environmental features is very essential for understanding the fluctuation in the distribution of organisms.

The total bacterial abundance (counts,TC) were in the order of 10^9 cells l^{-1}, which is in agreement with those reported in other particle-dominated estuarine systems like Hudson river estuary (Findlay et al. 1991), Mississippi river plume (Chin-Leo and Benner 1992), Georgia Bight (Biddanda et al. 1994) in USA. The bacterial abundance was mainly controlled by the quality of the substrate than quantity as inverse relationship ($p<0.05$) between TC and particulate C:N ratio was noted. This was further corroborated by the fact that particulate organic carbon did not show any relationship to bacterial abundance. Negative relationship ($p<0.05$) between salinity and total bacterial abundance at the estuarine station could be due to the physical processes. Such observations have been observed in the St. Lawrence estuary, Canada (Painchaud et al. 1996) and in NE Pacific Ocean of Oregon coast, USA (Sherr et al. 2001). DVC was 1-3 folds lower than TC, and varied seasonally by an order of magnitude. DVC was 3.01 to 6% of TC and these values were close to the values reported in other mesotrophic bays and estuaries such as Tokyo Bay, Sagami Bay, Kurushio Bay (Japan), where it ranged from 0.7 to 7.9% (Kogure et al. 1980). Significance

of above relationship has been reported in Guanabara Bay (Brazil) (Parahanos et al. 2001). It was interesting to observe that in estuarine stations DVC related significantly to chlorophyll concentration than TC, indicating DVC to be a better predictor and often be used as a indicator of eutrophic status of water bodies (Quinn et al. 1985). The percentage of DVC:TC observed in the present study is lower than those of other coastal waters (Kogure et al. 1980, Bing and Huaishu 1992). Interestingly, retrievable counts showed more significant relationship with POM at the coastal ($p<0.001$) than at estuarine stations ($p<0.05$). Such relationship was attributed to upwelling in Ria de Vigo estuarine system, Spain which brought nutrient rich water to the surface, stimulating phytoplankton growth and consequently leading to synthesis of POM (Zdanowski and Figueiras 1999). The coastal waters of this area are subjected to upwelling due to monsoon driven physical forces. The frequent occurrence of pigmented bacteria in the monsoon season could have been from the terrestrial material introduced by aeolian transport (Rheinheimer and Gocke 1994). There was the dominance of bacterial biomass (BB) over phytoplankton biomass (PB) at all the three stations, indicating the importance and significance of the role of bacteria in the nutrient turnover in this tropical ecosystem. This high abundance of different groups of bacteria was due to increased photosynthetic activity (release of DOC) and increase in nutrients from trapped sediments due to wind action, particle dissolution etc. The possibility of higher growth rate of bacteria over phytoplankton would have contributed to this dominance as reported in Sargasso Sea by Fuhrman et al. (1989) and Ducklow and Carlson (1992). The same has

also been reported in southern East China Sea (Shiah et al. 2000) and in sub tropical waters east of New Zealand. Similar conclusion could be drawn from this study (Fig. 5.5), as the ratio of bacterial growth rate: phytoplankton growth rate ($B\mu$: $P\mu$) increased, BB: PB decreased at a logarithmic scale in the estuarine stations.

Studies have indicated CF to range from 0.6 to 61x 10^{18} cells $mole^{-1}$ in aquatic systems. Since there is a large variation in the reported factors, CF derived in this study (1.96 x 10^{18} cells $mole^{-1}$) was used for calculation of BP. this CF was closer to the one commonly being used i.e. 2 x 10^{18} cells $mole^{-1}$ (Kirchman et al. 1985). Comparison of CF with those reported in the literature is shown in Table 5.7. Thus accuracy in the estimation of BP rates is largely dependent on conversion factor (CF). Bacterial productivity (BP) in this system is largely regulated by primary productivity (PP) as significant relationship was observed in the Zuari estuary ($p<0.05$) and coastal waters ($p<0.001$). Pelagic primary productivity in this estuarine system was high and ranged from 5.63 to 67.69 $\mu g\ C\ l^{-1}\ h^{-1}$. These rates differed significantly with adjacent coastal waters. The variation in the PP between the two systems was due to difference in inorganic nutrients, particularly with the nitrate concentration as it showed a significant relationship. At the coastal station PP was the prime source of organic matter, which support bacterial carbon demand (BCD). This was indicated by a strong positive significant relationship between BP and PP. Stronger relationship of BP with PP than with bacterial abundance indicated BP to be a better indicator of bacterial activity. This is could be due to dormant or inactive nature of the

bacterial cells. Specific growth rate (SGR) in this estuarine system is low with the estuarine system showing comparatively higher rate than coastal with a generation or doubling time of 0.51-4.95 days. These growth rates were significantly related to PP and particulate organic nitrogen indicating they could be limited by substrate quality and/or quantity. Doubling time observed in this study (0.54-5.4 days) has been reported in Gulf of Mexico (Bianchi et al. 1988), Chesapeake Bay (Kroer 1993), Louisiana Shelf (Biddanda et al. 1994) in USA and Schelde estuary (Goosen et al. 1997) in Netherlands. Low growth rate could also be due to the activity of the grazers. In order to study how this high biomass replenishes itself, estimation on the rate of biomass production are important to determine their assimilation efficiency and the amount of biomass potentially available to the grazers. It has been reported that grazing rates are higher in marine system where the bacterial population is above the threshold level of 1x 10^6 cells ml^{-1} (Nakano 1994). In order to substantiate the above, the abundance versus activity (log bacterial abundance versus log bacterial production) concept was used to assess relative importance of bottom up (substrate limitation) and top down (any sort of removal) process (Billen et al. 1990). No relationship pertaining to the above was observed in this study; thereby indicating that top down control could play a role in regulating bacterial abundance. Results of the laboratory experiments also proved that grazing was one of the factors controlling bacterial growth activity and efficiency (Chapter 6). Moreover, high percentage of labile organic carbon (L-DOC) was observed in the coastal than estuarine waters. This high percentage of L-DOC further substantiated that the

particulate matter supported the BP. Significant relationship between PP and dissolved organic carbon values in the coastal waters also corroborated the fact that the planktonic community was significant producers of organic carbon in the water column, available for bacterial assimilation. On the other hand, Zuari estuary showed low significant ($p<0.05$) relationship indicating partial (minor fraction) utilization of allochthonous apart from the autochthonous organic matter to support. Similar relationship between BP and PP has also been reported elsewhere (Bird and Kalff 1984, Cole et al. 1988, Azam et al. 1993).

In-spite of high PP, Mandovi estuary did not show any relationship indicating uncoupling between BP and PP. This could be either due to the super saturation of organic carbon and/or limitation of bacterial growth by inorganic nutrients. The uncoupling and lack of relationship in the Mandovi estuary indicated that allochthonous matter (release of organic material from sediments by constant wave action, particle dissolution, high turbidity and/or churning process), especially in the monsoon season could have supported BCD. Uncoupling between BP and PP can also lead to mass aggregation and sinking of phytoplankton as reported in the Arabian Sea (Azam et al. 1994). Absence of significant relationship and uncoupling between BP and PP has been reported in systems like Hudson River estuary (Findlay et al. 1991), St. Lawrence estuary (Painchaud and Therriault 1989), Schelde estuary (Goosen et al. 1997) where allochthonous inputs dominate. Inverse relationship between BP and C:N ratio also indicated the limitation of bacterial growth by substrate quality. Moreover significant relation between ammonia and phosphate with BP indicated that

inorganic nutrients could limit bacterial growth. High phosphatase activity further substantiated the above. In the present study, the ratio between alkaline phosphatase and aminopeptidase (APA: AMA) ranged from 1.72 to 105 in both surface and bottom waters indicating that bacterioplankton could be phosphate limited. Sala et al. (2001) brought about the importance of APA: AMA ratio in assessing the nutrient limitations in microbial population in marine environments. Microcosm experiment carried out with water collected from Catalan coast (north west Mediterranean sea) indicated a high ratio ranging from 0.89 to 2.3 which suggested that phosphate could limit bacterial growth and activity. This ratio could highly vary to physiological adaptation or to changes in species composition (Sala et al. 2001). Obernoseter and Herndl (1995) observed low β-glucosidase activity in the bacterial utilization of phtyo extracellular release (PER) indicating P limitation in laboratory experiments. However, further study is needed to extend the application of this ratio in this system. Lower beta glucosidase activity than phosphatase activity observed in the present study further indicates the importance of inorganic nutrients particularly P was limiting in this system. Such low beta glucosidase activity was reported in Rimov reservoir (Czech) (Vrba 1992).

Heterotrophic activity of bacterioplankton, which is assumed to be the representative of total metabolism, depends on the kinetic parameters like maximum uptake velocity (V_{max}), turnover time (Tt) and natural substrate concentration and transport constant (Kt+Sn) (Wright and Hobbie 1966). At Mandovi and coastal stations, high V_{max} was for glucose whereas at Zuari station

it was for glutamic acid. High V_{max} and low Tt indicated that the heterotrophic process was dominant in these waters. Ramaiah et al. (1996) reported heterotrophic activity of 0.96-2.8 μg C l^{-1} h^{-1} in the east coast of India (Madras and Cuddalore regions). V_{max} has always been equated to determine the extent of eutrophication in aquatic system (Gocke 1977). Higher the V_{max} greater are the chances of eutrophication. In this study, heterotrophic activity was low when compared to other polluted estuaries and bays like Shimoda Bay, Tokyo Bay (Japan), indicating the pristine nature of Mandovi-Zuari estuarine system. Less number of DVC could be accounted for low V_{max} as compared to other estuaries, which accounted for high V_{max} (Hobbie and Crawford 1969, Seki et al. 1975). A higher V_{max} in the monsoon season at both the estuarine and coastal stations coincided with high BP, which was due to the riverine input of labile organic matter of low C: N ratio. In the Mandovi-Zuari estuarine system, the overall Tt of glutamic acid was much higher (1-3 folds) than glucose suggesting the difference in the availability of the substrate leading to the physiological and genotypical adaptation of community (Gocke 1977). The two substrates may be used differently for biosynthesis and energy requirement at different seasonal situation. Stevenson and Erkenbrecher (1976) in a review on estuarine bacteria found that heterotrophic potential of these bacteria was greater than those of the sea or freshwater environment. In the present study, discrepancy existed between V_{max} and total numbers in the estuarine stations, which suggested that the saprophytic bacteria could play a prominent role than expected from their

small share with in the total population. CFU related well ($p<0.001$) with the V_{max} in both the surface and bottom waters at the coastal station.

Wide range of Kt+Sn for glucose has been reported (Wright 1974, Gocke 1977). The values reported in the present study are much higher than what has been previously reported value of 51.18 and 41.18 μg C l^{-1} for glucose and glutamic acid in the Kavratti lagoon of Lakshadweep islands (Chandramohan and Ramaiah 1987). High values ranging from 62 to 284 μg C l^{-1} has been reported in the several eutrophicated bays like Tokyo Bay, Shimoda Bay (Japan) etc. Of the three stations, Mandovi station showed a seasonal shift in the productivity: heterotrophic activity ratio with the bacterial community being active in the non-monsoon seasons and productive in the monsoon season, when compared to Zuari station which showed the productivity to dominate over activity throughout. Like Mandovi, the coastal station also showed a shift with the pre-monsoon season being productive and post-monsoon season being active. As mentioned previously the difference in the productivity and activity could be attributed mainly to the physiological characteristics and bacterial community structure (Gocke 1977).

Bacterial assimilation of organic matter is dependent on the community structure. Overall, the response of the bacterial population to seven enzymes tested was 34.25%, and is much higher than the reported value of 15% in the Arachon Basin (France) (Van Wambake et al. 1984). But however the average index of the carbonaceous compounds utilization (26.38%) was less when compared to those reported in the above basin (49%). In this study bacteria,

which could hydrolyze protein and starch, were fairly numerous. High percentage of bacteria exhibiting proteolytic (72%) and amylolytic (54%) is reported in the Koninski channel waters, which receive large quantities of allochthonous organic matter. The ability of the present isolates to grow at various pH (7.5, 9.2), temperature (20°, 28°, 35°C) and salinity (15, 35, 50) indicated their versatility and adaptability in this continuously changing environment. As a supplementary approach in this study, preliminary analysis of metabolic or community fingerprinting carried out on BIOLOG (GN microplate) test panels showed that the bacterial community was found capable of using more than 80% of carbonaceous compounds. This approach of studying the community metabolic fingerprinting has been adopted in variety of aquatic ecosystem (Schultz and Ducklow 2000, Sinsabaugh and Foreman 2001).

In the present study, most of the bacteria isolated belonged to the division of Proteobacteria, subdivision C3: Gama class. They included *Pseudomonas, Bacillus, Moraxella, Acinetobacter, Cytophaga, Aeromonas.* In the present study *Pseudomonas* sp. dominated the group (68%) followed by *Bacillus* (7.2%), while others contributed the minor fraction. Studies have indicated that cultivated pseudomonads has been typically characterized as metabolically versatile and capable of degrading a wide range of low molecular weight compounds (Kersters et al. 1996) and due principal mediators of dissolved organic carbon. The contribution of *Pseudomonas* in nutrient cycling and biodegradation including recalcitrant organics has been well known (Palleroni 1992). Zdanowski and Figueiras (1999) indicated *Pseudomonas* to be an important group in the

colonization and decomposition of POM in Ria de Vigo, Spain. In the present study, members of the *Cytophaga* group were found to be less probably due to absence of marine macro aggregates (Riemann et al. 2000). Austin et al. (1979) indicated the presence of pseudomonas in pristine environments. The dominance of *Pseudomonas* particularly has also been reported in number of estuarine environments like the Chesapeake Bay, Tokyo Bay etc. where they are present throughout the year irrespective of the season.

5.4 CONCLUSION

1. Bacteria were a significant component of the tropical Mandovi-Zuari estuarine system.
2. Seasonal variability in the bacterial biomass and activity was more pronounced in the estuarine than in the adjacent coastal waters.
3. While the Mandovi estuary and coastal waters showed preference to glucose, Zuari estuary showed preference to glutamic acid, which was indicated by the high V_{max} value.
4. Phytoplankton photosynthesis is the major source of labile organic matter for supporting bacterioplankton growth in the coastal waters.
5. In the estuary, the bacterial growth was nutrient limited.
6. Like hydrological characteristics, biological variables also indicated that the estuary was more dynamic than the adjacent coastal region.

Chapter 6

BACTERIAL GROWTH EFFICIENCY

6.1 INTRODUCTION

In aquatic systems the fate of organic carbon arriving from phytoplankton productivity (autochthonous) and external (allochthonous) sources is dependent on the bacterial metabolic activity or assimilation potential. Numerous studies have indicated that bacteria utilize a large fraction of the carbon that flows within aquatic systems (Cole et al. 1988). It is important to understand how efficiently bacteria metabolized this total dissolved organic matter (DOM) pool. This efficiency termed as the bacterial growth efficiency (BGE), calculated with bacterial production (BP) and respiration (BR) measurements is defined as the ratio of biomass produced to substrate assimilated (A), where A is the sum of bacterial production and respiration, so that BGE=BP/(BP+BR) = BP/A.

BGE is a fundamental attribute of microbial metabolism, which adequately determines the ecological, and biochemical roles of bacteria played in the microbial food webs and in aquatic ecosystems (del Giorgio and Cole 1998). The efficiency with which bacteria utilize the organic matter largely depends upon the metabolic capability of the residing bacterioplankton community.

In most studies although the conversion of bacterial production to gross carbon flux with a constant efficiency factor has often been attempted (Azam et al. 1983, Riemann and Sondergaard 1986, Baines and Pace 1991), published efficiencies with a range covering 2 decades have made this exercise somewhat elusive. These values (BGE) are now widely regarded as overestimates of the real growth efficiency of natural bacterioplankton utilizing natural substrates (Jahnke and Carven 1995, del Giorgio and Cole 1998). BGE seems to vary in

time and space (Kroer 1993, Middelboe and Sondergaard 1993, Russel and Cook 1995), with single species (Ho and Payne 1979) and over diel cycles (Coffin et al. 1993).

The magnitude and variation of BGE are not well understood (del Giorgio and Cole 1997), mainly due to the difficulty in simultaneously covering all the spatio-temporal scales involved in the dynamics of pelagic physics and biology (McManus and Fuhrman 1988). Depending on the composition of the organic pool and the ratio at which the various sources contribute to it, the activity and growth efficiency will vary widely. Studies on the BGE values and the factors controlling them in tropical ecosystem are scarce. With this backdrop the present study was undertaken to:

(i) determine the annual and seasonal variations of BGE both temporally and spatially and

(ii) determine the factors involved in controlling or regulating BGE in this tropical estuarine system.

In this chapter, spatial-temporal the variation in BGE in this tropical system and the factors regulating BGE will be discussed. The station location and methodology adopted have been presented in detail in Chapter 2.

6.2 RESULTS

Bacterial growth efficiency (BGE), calculated from the rates of bacterial production (BP) and respiration (BR) ranged from 2.22 to 92.56% at the surface and 3.35 to 92.58% at the bottom waters in Mondovi estuary (Fig. 6.1). The BP rates mostly (70%) ranged from 0.58 to 7.36 μg C l^{-1} h^{-1} with a mean value of 1.80 μg C l^{-1} h^{-1}. In the Zuari estuary, the BGE values ranged from 4.98 to 88.19% at the surface and 9.64 to 84.06% at the bottom waters (Fig. 6.2). At this site BP rates mostly (85%) ranged from 0.57 to 5.47 μg C l^{-1} h^{-1} with a mean value of 2.18 μg C l^{-1} h^{-1} at the surface and 0.57 to 9.46 μg C l^{-1} h^{-1} with a mean value of 2.76 μg C l^{-1} h^{-1} at the bottom waters. BGE at the coastal station ranged from 4.71 to 27.12% with a mean of 11.36% at the surface and 3.64 to 21.19% with a mean of 11.12% at the bottom waters (Fig. 6.3). The BP ranged from 0.54 to 5.98 μg C l^{-1} h^{-1} with a mean value of 1.80 μg C l^{-1} h^{-1}. Generally on comparison the BP values at the coastal station were lower (1-2 folds) than estuarine stations. BR rates were higher at the coastal station and varied significantly ($p<0.05$) with the estuarine stations. The BGE at this station was significantly lower than that of estuarine stations ($p<0.01$).

Relationship between BP and BR, which indicates the physiological flexibility of the bacterial population showed weak relationship in the Mandovi estuary ($p<0.05$) and thus was not linearly related (Fig. 6.4). Zuari estuary did not show any significant relationship between BP and BR (Fig. 6.4). On comparison the coastal station showed good positive correlation between BP and BR ($p<0.01$) than the estuarine stations (Fig. 6.4).

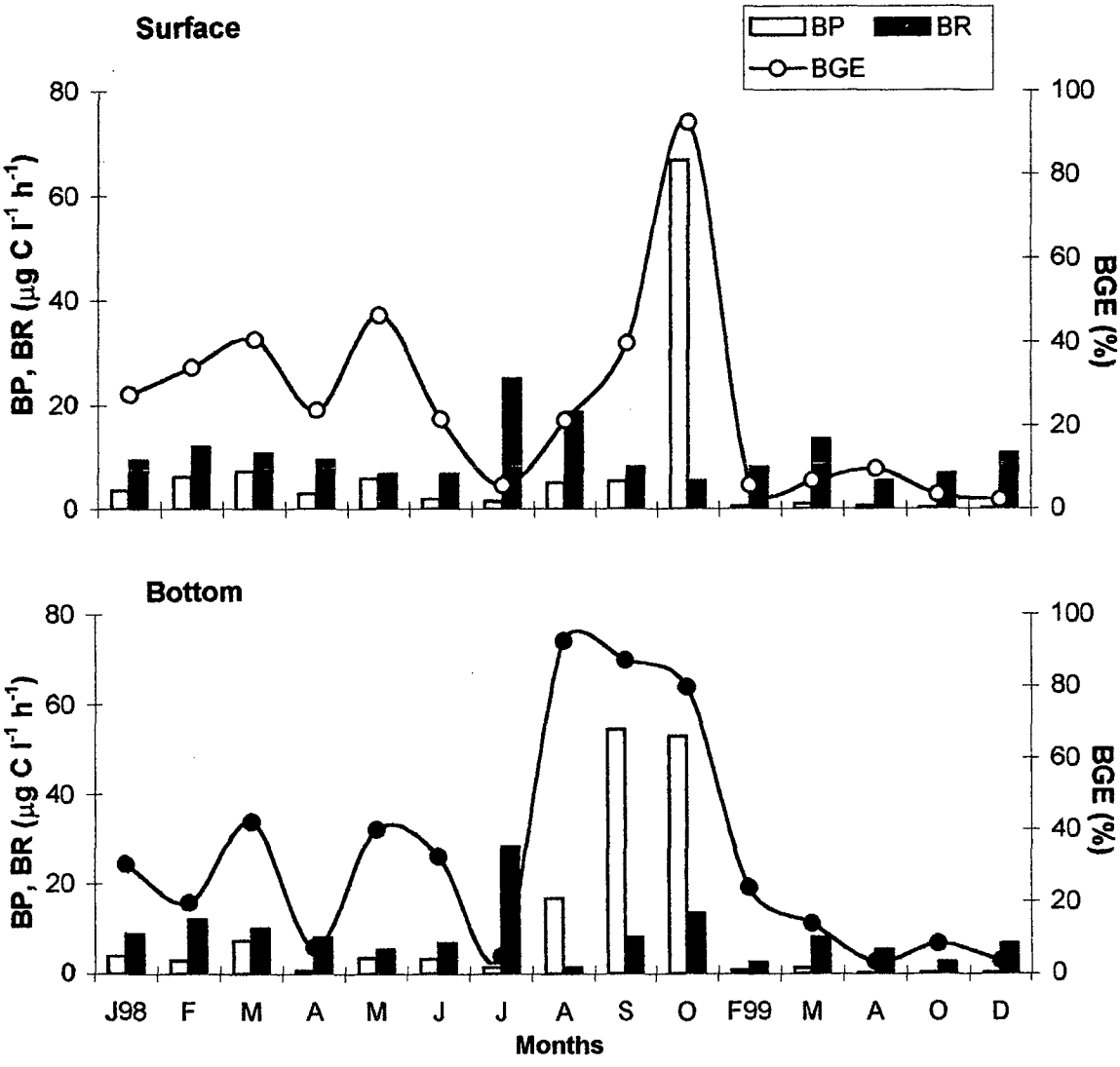

Fig. 6.1. Monthly variation in bacterial productivity (BP), respiration (BR) and bacterial growth efficiency (BGE) in the Mandovi estuary

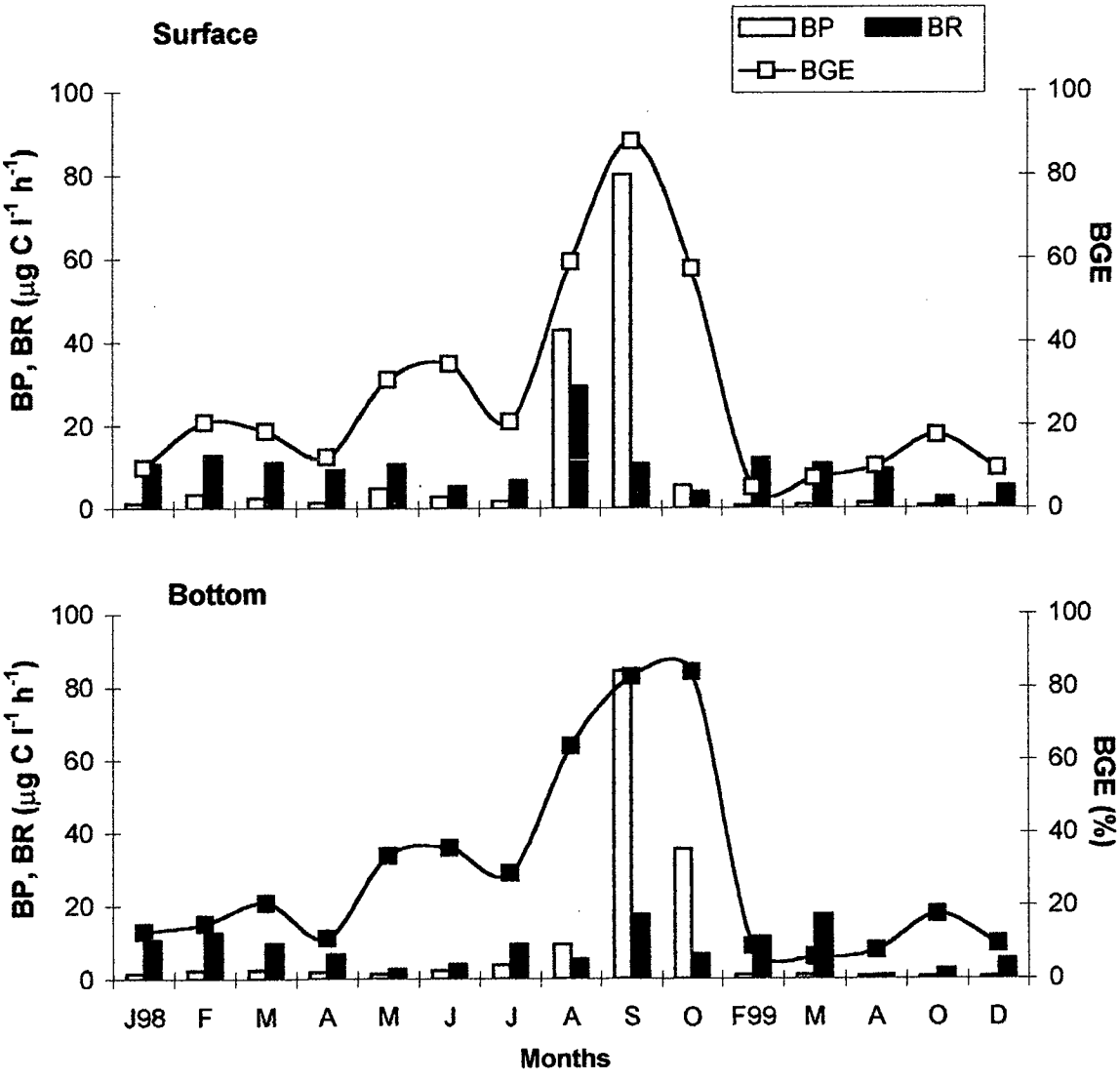

Fig. 6.2. Monthly variation in bacterial productivity (BP), respiration (BR) and bacterial growth efficiency (BGE) in the Zuari estuary

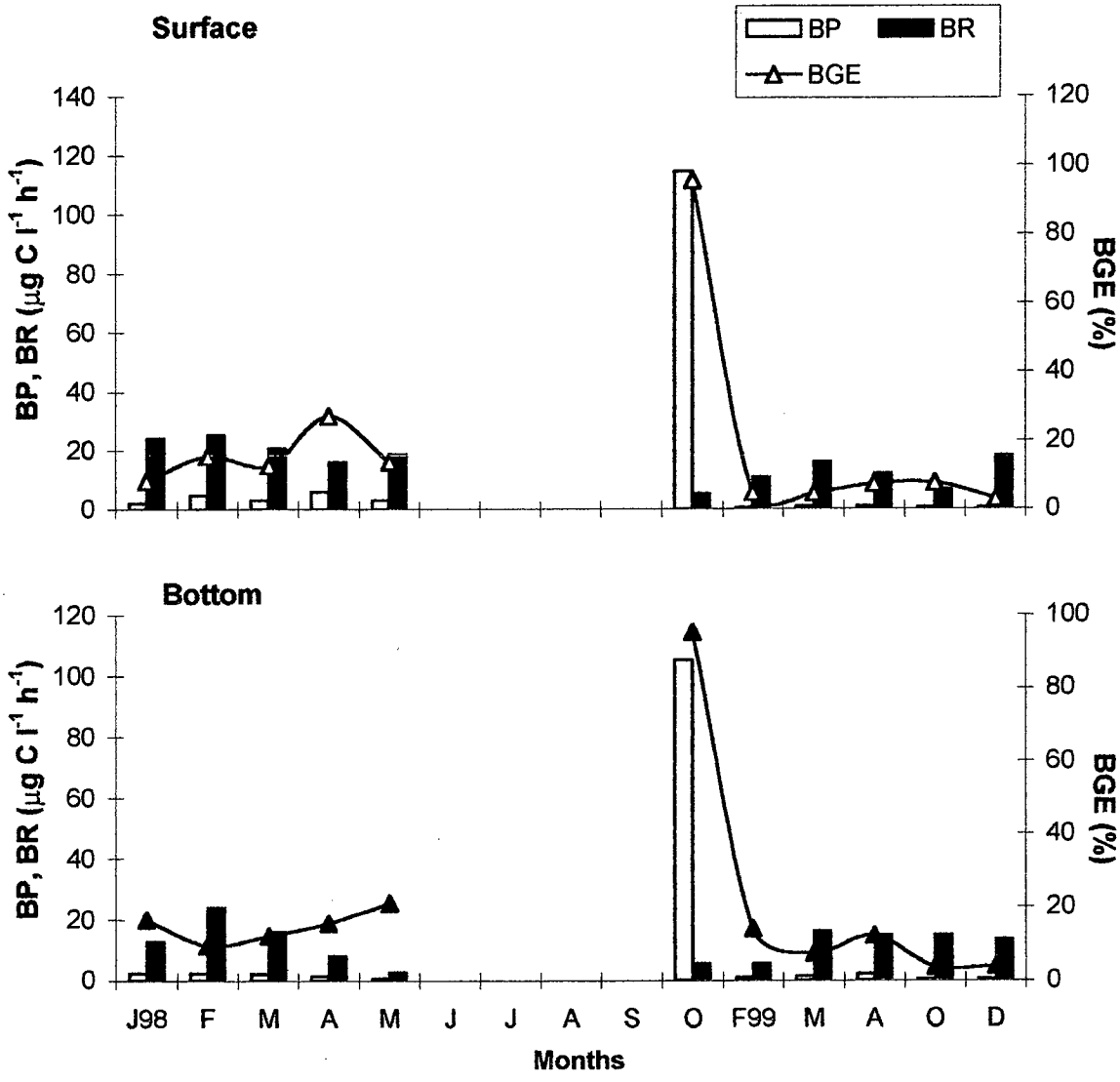

Fig. 6.3. Monthly variation in bacterial productivity (BP), respiration (BR) and bacterial growth efficiency (BGE) in the Coastal station

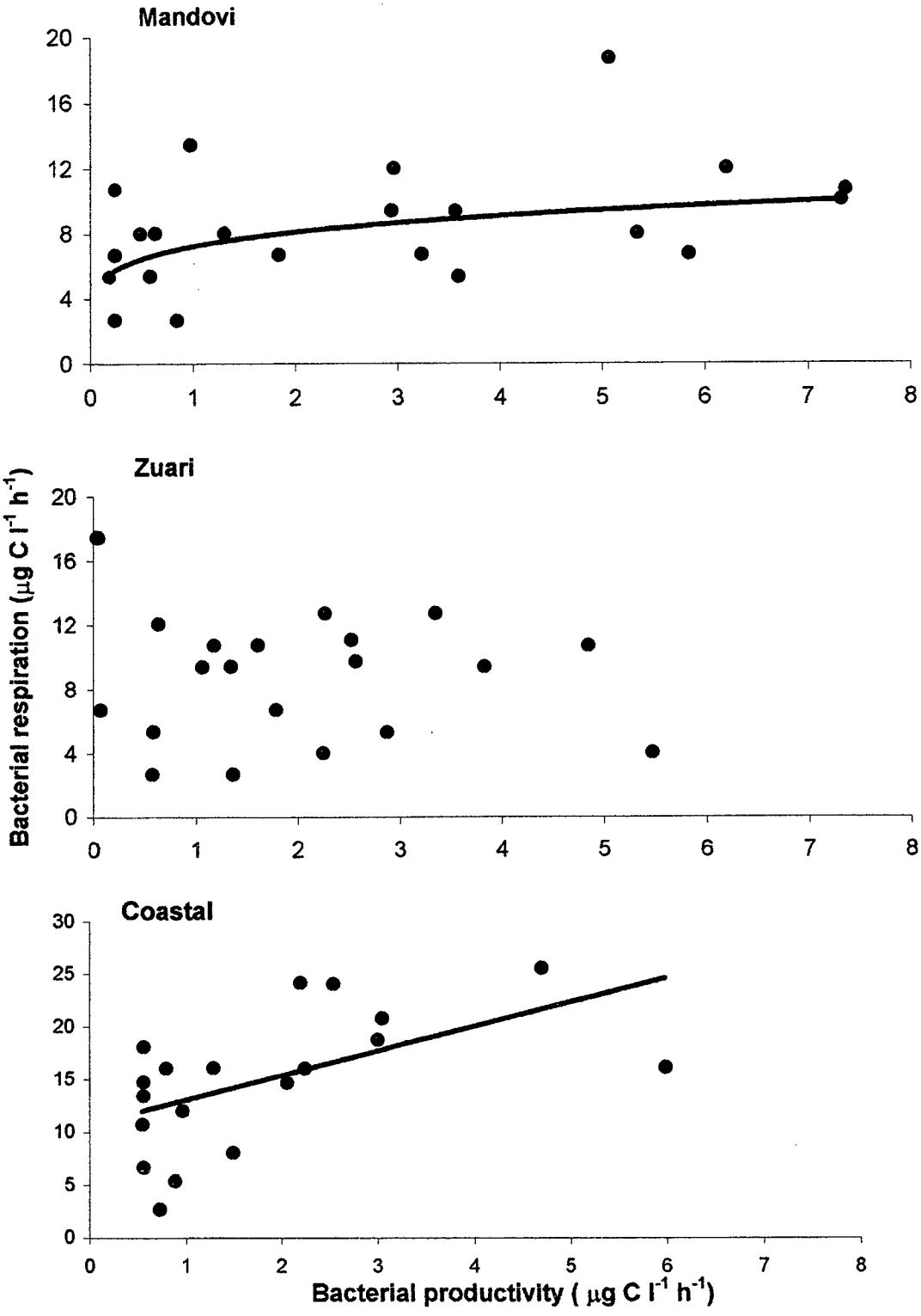

Fig. 6.4. Relationship between bacterial productivity and respiration at Mandovi (n=23, r=0.46, p<0.05), Zuari (not significant) and coastal (n=19, r=0.5, p<0.01) stations

While the BGE differed significantly ($p<0.01$) between monsoon and non-monsoon seasons, no seasonal variation in BGE was observed between surface and bottom waters in the estuaries. Similarly, BP rates also differed significantly ($p<0.01$) between non-monsoon and monsoon seasons. High BGE observed during the monsoon season coincided with high BP and low BR rates (Fig. 6.5). In contrast to BP and BGE, BR rates did not differ significantly at Mandovi station. Higher values were obtained in the pre-monsoon than in other seasons. The mean respiration values observed in the pre-monsoon, monsoon and post-monsoon are 8.88, 9.07 and 7.82 $\mu g\ C\ l^{-1}\ h^{-1}$ respectively (Fig. 6.5) The mean BR rates recorded in the pre-monsoon, monsoon and post-monsoon seasons are 10.23, 10.7 and 6.04 $\mu g\ C\ l^{-1}\ h^{-1}$ respectively (Fig. 6.5). Unlike Mandovi estuary, Zuari estuary also showed similar characteristics of high values in the monsoon season and significantly ($p<0.01$) high BR rates than BP. The results of the present study indicated BP to be more dynamic than BR and the variation in BGE was due to the fluctuation of BP rather than BR. Like BGE, BP values also did not record any seasonal variability in the coastal water. The mean BR rates in the pre- and post-monsoon seasons were 15 and 12.39 $\mu g\ C\ l^{-1}\ h^{-1}$ (Fig. 6.5).

In the Mandovi estuary, significant relationship ($p<0.001$) between BP and BGE was observed where BP explained 89% of the variation in BGE (Fig. 6.6). BR was less seasonally dynamic (CV (SD/Mean%) = 65) than BP (CV= 83). No relationship was observed between BR and BGE. In the Zuari estuary BP showed a significant relationship with BGE ($p<0.001$) and explained 74% of the variation in BGE values (Fig. 6.6). The CV of the BGE in Zuari estuary was 71%

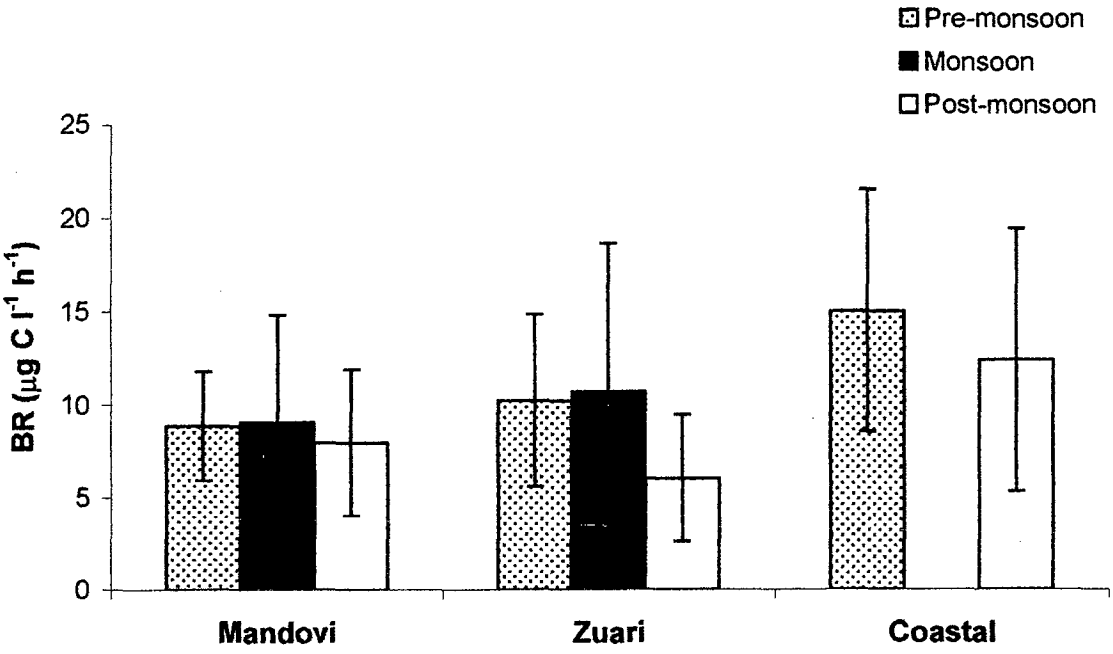

Fig. 6.5. Seasonal variation in bacterial respiration (BR) at the study stations. Standard deviation is indicated as error bars

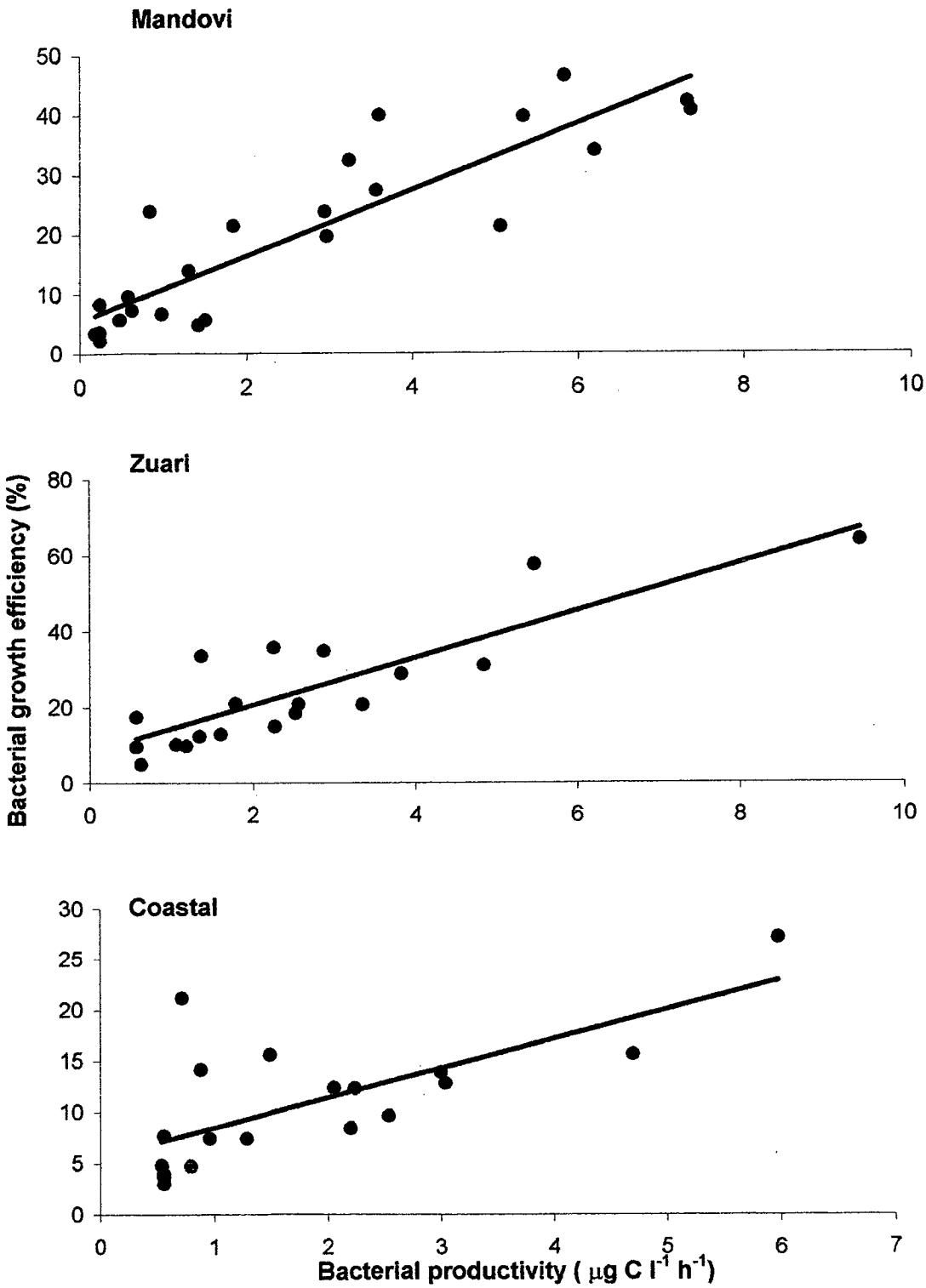

Fig. 6.6. Relationship between bacterial productivity and growth efficiency at Mandovi (n=25, r=0.89,p<0.001), Zuari (n=21, r=0.74, p<0.001) and coastal (n=18, r=0.70, p<0.001) stations

and was higher than that of Mandovi station. At the coastal station, BGE correlated well with BP values (p<0.001) and explained 70% of variation in BGE (Fig. 6.6). Although the relationship was significant the low value of 70% when compared to estuarine stations was due to high BR rates, which led to low BGE indicating that BP was less dynamic than BR.

In the present study, 85% of the values were in the range 4.7 to 46.56%. The CV% of BGE in Mandovi estuary was 68. On an average the median BGE in the Mandovi estuary was 21.43 ±15.01%. In Zuari estuary 80% of the values fell in the range 4.98 to 59.24% with a mean value of 23.53 ±18.16%. The observed value was higher than that of Mandovi estuary. At the coastal station the overall BGE value was 11.24 ±7.17% (Fig. 6.7).

Factors controlling bacterial growth and activity

In order to determine the factors controlling or regulating the BGE, statistical analyses were carried out with physico-chemical and biological parameters to bring out their significance. Further, a series of laboratory experiments were carried out for delineating the factors controlling BGE. Enrichment experiments were carried out in GF/F (0.7 μm) filtered water samples collected at all the three study stations and response of the bacterioplankton to nutrient enrichments (organic and inorganic) was assessed in terms of growth rate, bacterial production and bacterial growth efficiency. Nutrient enrichment bioassays represent the most direct method for assessing the nutrient status of bacterioplankton population (Coveny and Wetzel 1992) in-spite of the limitation due to enclosure of water samples (Torreton et al. 2000). Assessing the nutrient

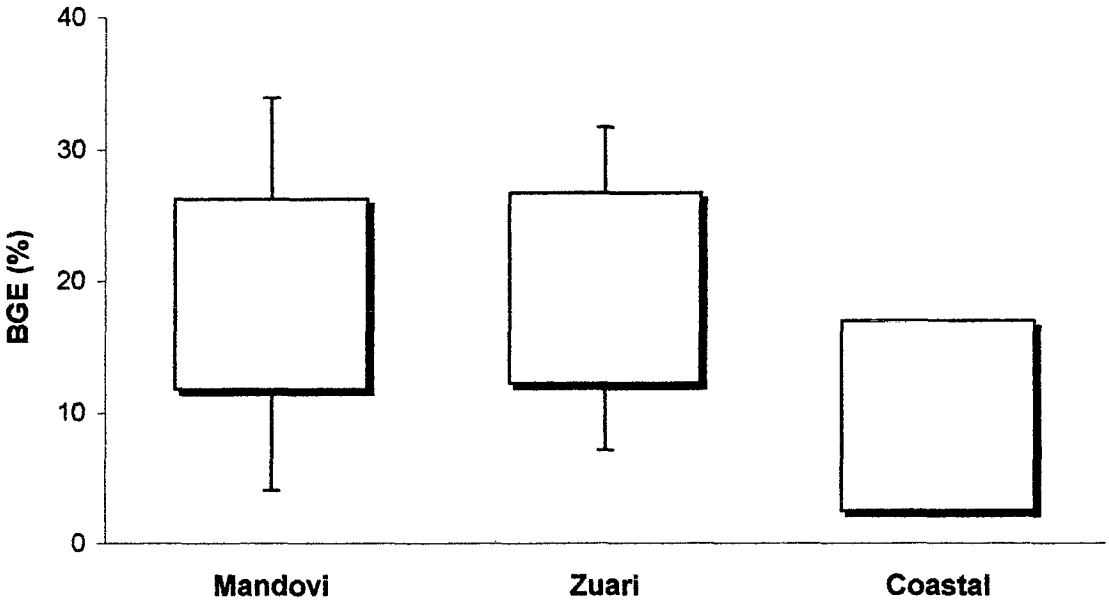

Fig. 6.7. Summary of the data on direct measurements of BGE in the Mandovi-Zuari estuarine system. Box and whisker plot shows the median and range of values (bars)

status of bacterioplankton assemblages in aquatic systems is a pre-requisite in-spite of the fact that no single and direct method is available. This is due to the difficulty in separating the labile pool from the recalcitrant fraction. For nutrient enrichment studies filtration of water samples through GF/F filters served two functions: 1. removal of bacterivores to allow free bacterial growth and 2. removal of most of the algae to ensure that bacteria respond directly to available nutrient in the water. As enrichment microcosms could reflect the nutrient status of bacterioplankton, to a large extent the response of the growth to nutrients in our experimental conditions provides as index of the potentially limiting nutrient.

a) Effect of nutrients

For nutrient enrichments experiments, glucose was used as a source of organic carbon (C) (final concentration of 100 μM C), ammonium as inorganic nitrogen source (N) (20 μM N) and potassium di-hydrogen orthophosphate as inorganic phosphate source (P) (5 μM P). The water samples were individually amended with the above nutrients and also in combination with different molar ratios. The molar ratios of added nutrients included C:N (5:1), N:P (4:1), C:P (20:1) and C:N:P (5:1:0.25). All the stocks were prepared in autoclaved de-ionized water. The amended samples in 300 ml capacity acid washed glass bottles were incubated in the dark at *in situ* temperature (25 ±2 °C).

b). Effect of temperature

Effect of temperature on the bacterial growth rate and activity was carried out by incubating the GF/F filtered water samples at 18°, 28° and 38°C and

calculating the Q_{10} values. The Q_{10} value was calculated by using the formula $Q_{10}= (K_1/K_2)^{10.(t1-t2)}$.

c). Effect of grazing and mortality

Laboratory experiments to determine the grazing and mortality rates were carried out by the disappearance of added ^{3}H-Thymidine from the water sample. In the present study incubation of unfiltered and filtered waters samples with ^{3}H-Thymidine was carried out separately both in to study the effect of grazers on bacterial growth activity. With the above-mentioned approach the unfiltered water samples containing bacteria and grazers were studied for grazing or bacterial removal rates and the filtered water samples, which contains mostly bacterioplankton, were monitored for calculation of natural bacterial mortality rates.

d). Response of bacterioplankton to different organic amendments

Laboratory experiments were conducted to study the response of bacteria to nitrogenous compounds like amino acid, urea and ammonium chloride at 20 μM concentration; organic substrates like cellobiose (20 μM), starch (2 mg l^{-1}), vanillic acid (20 μM), leucine (20 μM), 'Tween 80' (1.5 ml l^{-1}), bovine serum albumin (2 mg l^{-1}), chitin (2 mg l^{-1}), tannic acid (20 μM); phytoplankton extract from *Isochrysis sp.* (44 μM) and *Tetraselmis sp.* (36 μM) and zooplankton extract (2.5 mg C l^{-1}) for a short incubation period (24-36 hours).

Weak relationship between BGE and water temperature ($p<0.05$) was evident only in the surface waters in Mandovi. The Q_{10} values calculated for

temperature 18 to 28° and 28 to 38°C in the present study was 0.9, and 2.9 for bacterial productivity.

On combining both the estuarine stations significant relationship between BGE and inorganic nutrients i.e. ammonia ($p<0.001$) and phosphate ($p<0.05$) was observed (Fig. 6.8). Nutrient assay substantiate that BP and BGE increased significantly in water samples amended with P and N+P source. The BP rates were 2.50 and 2.06 μg C l^{-1} h^{-1} for P and N+P amended samples in Mandovi and 2.4 and 2.64 μg C l^{-1} h^{-1} for Zuari estuary. Correspondingly the BGE rates also increased. These rates were significantly higher ($p<0.05$) than carbon amended samples, which showed little response. High respiration rates of 53.64 and 67.07 μg C l^{-1} h^{-1} observed in carbon amended water samples at both Mandovi and Zuari estuaries led to low BGE. Increase in BP, BR and BGE with addition of inorganic nutrients indicated N and P to be limiting in the estuarine waters. The response of bacterioplankton with the addition of inorganic and organic nutrients at the estuarine stations is shown in Fig. 6.9.

In contrast to the estuaries, the coastal station showed high values in bacterial growth and activity in water samples amended with glucose. The response was significantly ($p<0.001$) higher than inorganic amendments. The BP and BGE values recorded in glucose amended water samples were 9 μg C l^{-1} h^{-1} and 22% (Fig. 6.9). Likewise, SGR was also higher in water samples amended with glucose (0.030 h^{-1}) with a generation time of 23 hours. This high response indicated carbon to be limiting factor in the coastal environments.

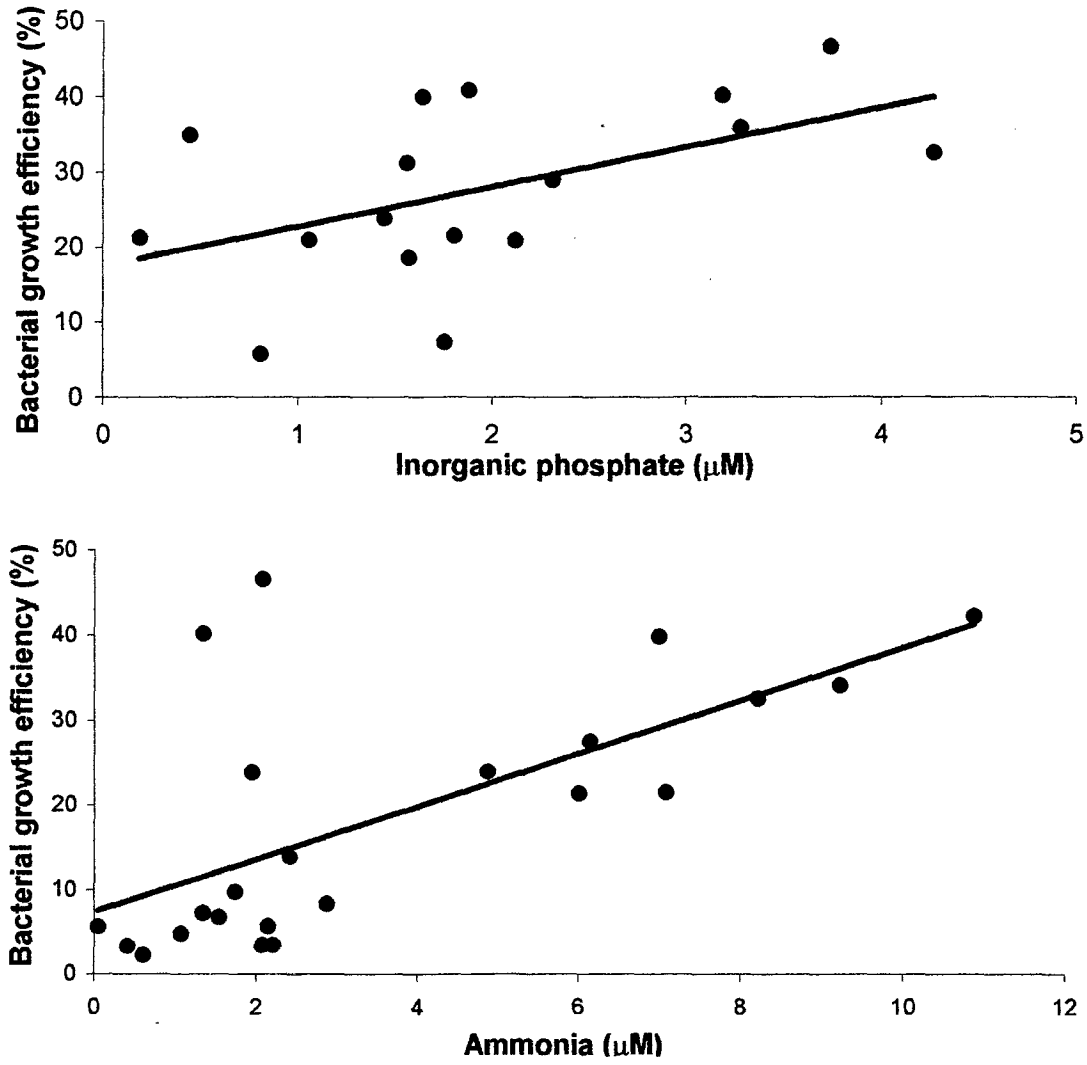

Fig. 6.8. Relationship between inorganic phosphate (n=19, r=0.51, $p<0.05$) and ammonia (n=23, r=0.66, $p<0.001$) with bacterial growth efficiency at the estuarine stations

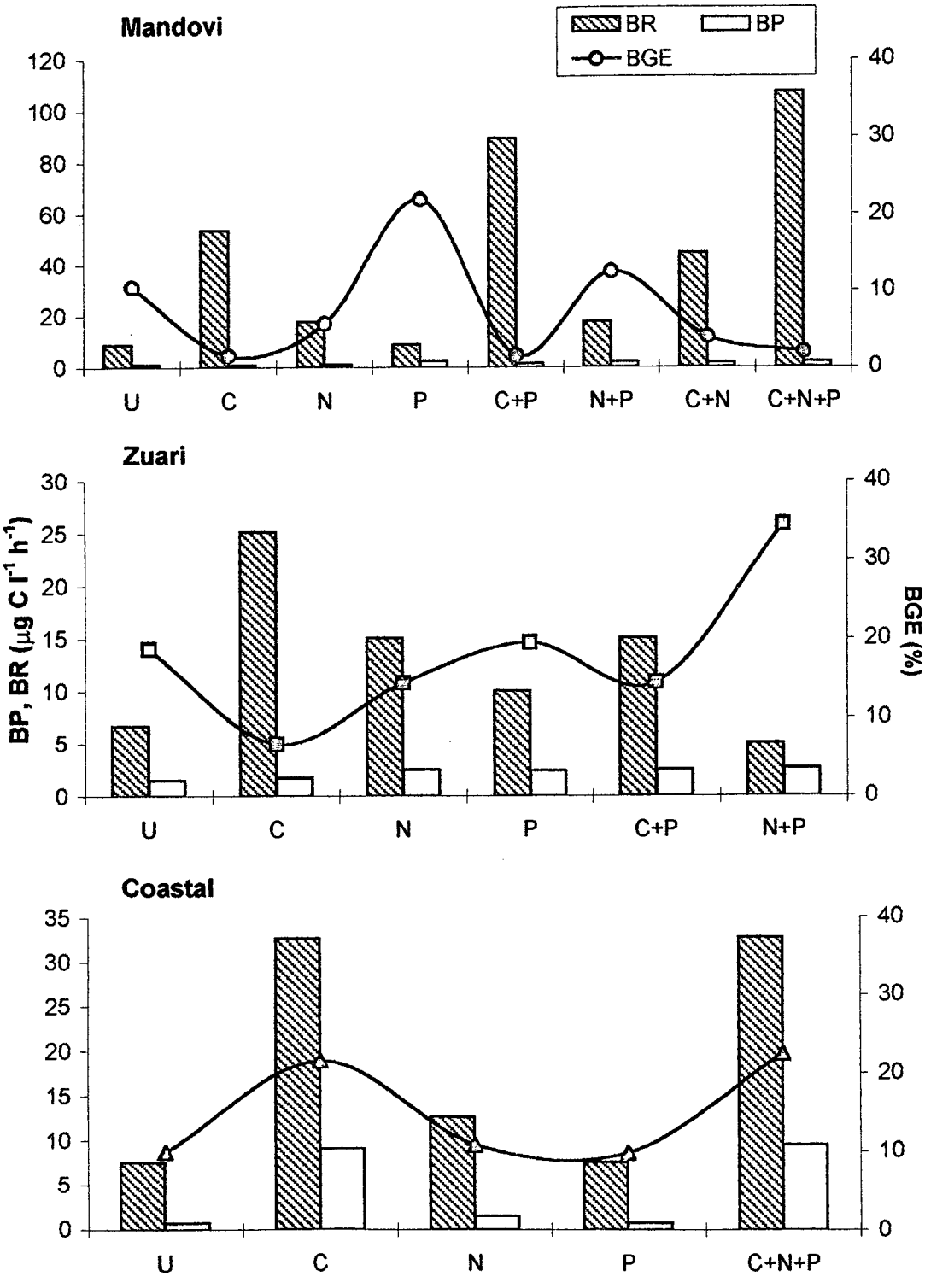

Fig. 6.9. Changes in the bacterial production (BP), respiration (BR) and growth efficiency (BGE) in organic and inorganic amended water samples from the study stations. U=unamended, C=carbon source, N=nitogen source, P=phosphate source

In the filtered samples BP increased steadily up to 24 hours, after which a decrease in the rate was observed (Fig. 6.10). The minimum estimate of bacterial mortality was calculated to be in the range of 0.019 and 0.021 h^{-1}. In the unfiltered fraction thymidine incorporation rates were linear up to 6 hours and afterwards a decrease in BP rates was noted (Fig. 6.10). This decrease in BP rates was due to grazing activity by ciliates, flagellates and microzooplankton present in the unfiltered water samples. The grazing rate was calculated to be 0.012 to 0.013 h^{-1}. However in the present study, the mortality and grazing rates were lower to the bacterial growth rate in filtered and unfiltered water samples. Unlike BP, the bacterial cells in filtered samples increased up to 24 hours and reached a plateau. The SGR was higher in filtered (0.026 h^{-1}) when compared to unfiltered water samples (0.023 h^{-1}). Influence of grazers on BGE was carried out by incubating the unfiltered (with grazers) and filtered (without grazers) water samples for 36-hour period. Decrease in BGE values was observed in the presence of grazers. Although higher BR rates were observed in the presence of grazers, BP showed significantly ($p<0.05$) higher values in the grazer free samples, which led to high BGE (Table 6.1).

Although the BGE did not correlate significantly with primary productivity, the low values coincided with low PP values at the surface waters. BGE related inversely to particulate C:N ratio and showed a strong relationship ($p<0.001$) at the three stations (Fig. 6.11). In the coastal station, BGE was found to correlate significantly with PON ($p<0.05$) than with inorganic nutrients. Water sample amended with bovine serum albumin recorded highest response than other

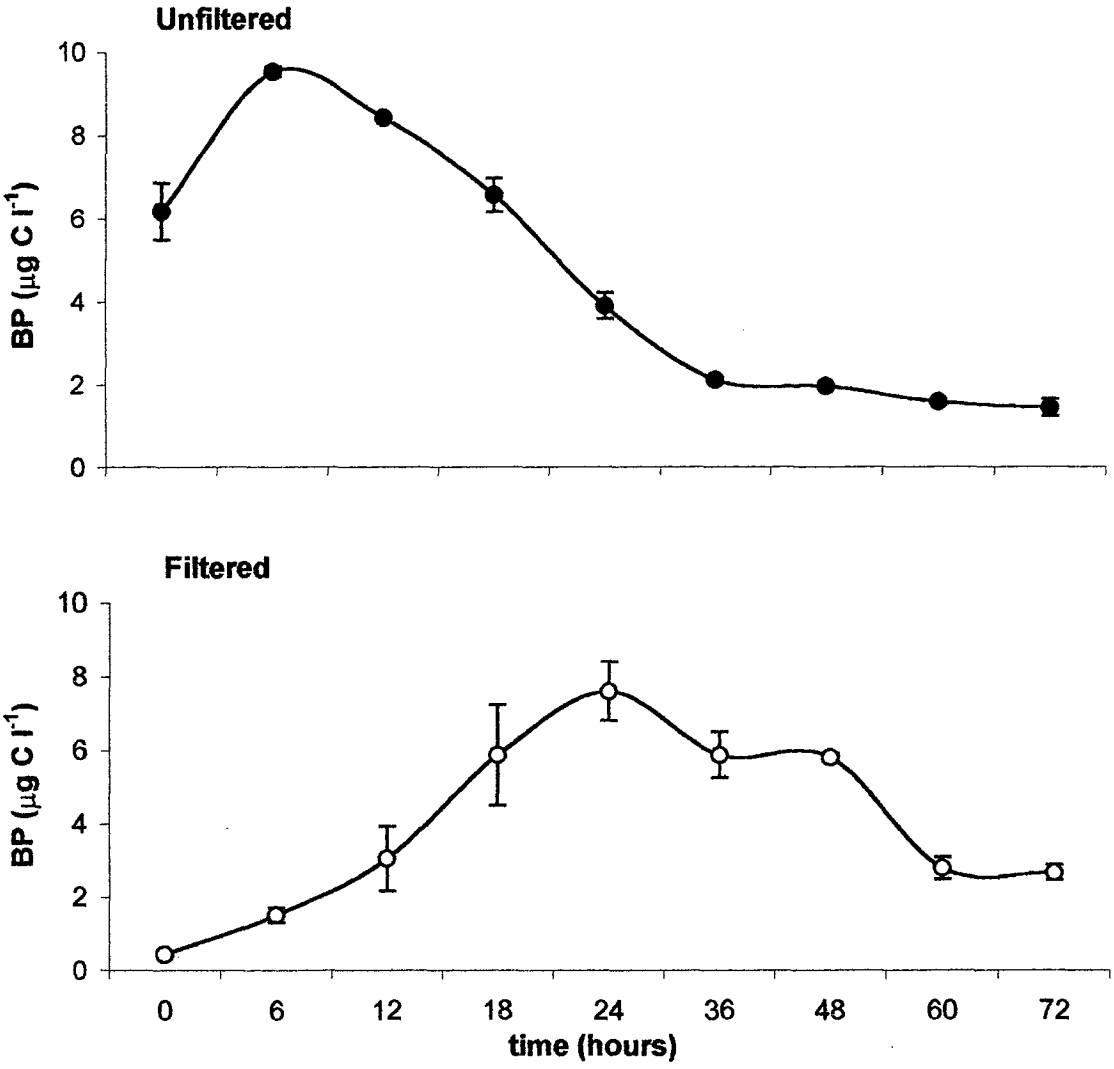

Fig. 6.10. Change in the bacterial productivity (BP) in the unfiltered and filtered estuarine water samples during the course of incubation

Table 6.1. Effect of grazing on BGE. Mean ±SD

Treatment	BR ($\mu M\ C\ h^{-1}$)	BP ($\mu M\ C\ h^{-1}$)	BGE (%)
Unfiltered	0.45 ±0.058	0.09 ±0.009	16.23 ±0.93
Filtered	0.42 ±0.057	0.13 ±0.003	23.49 ±0.35

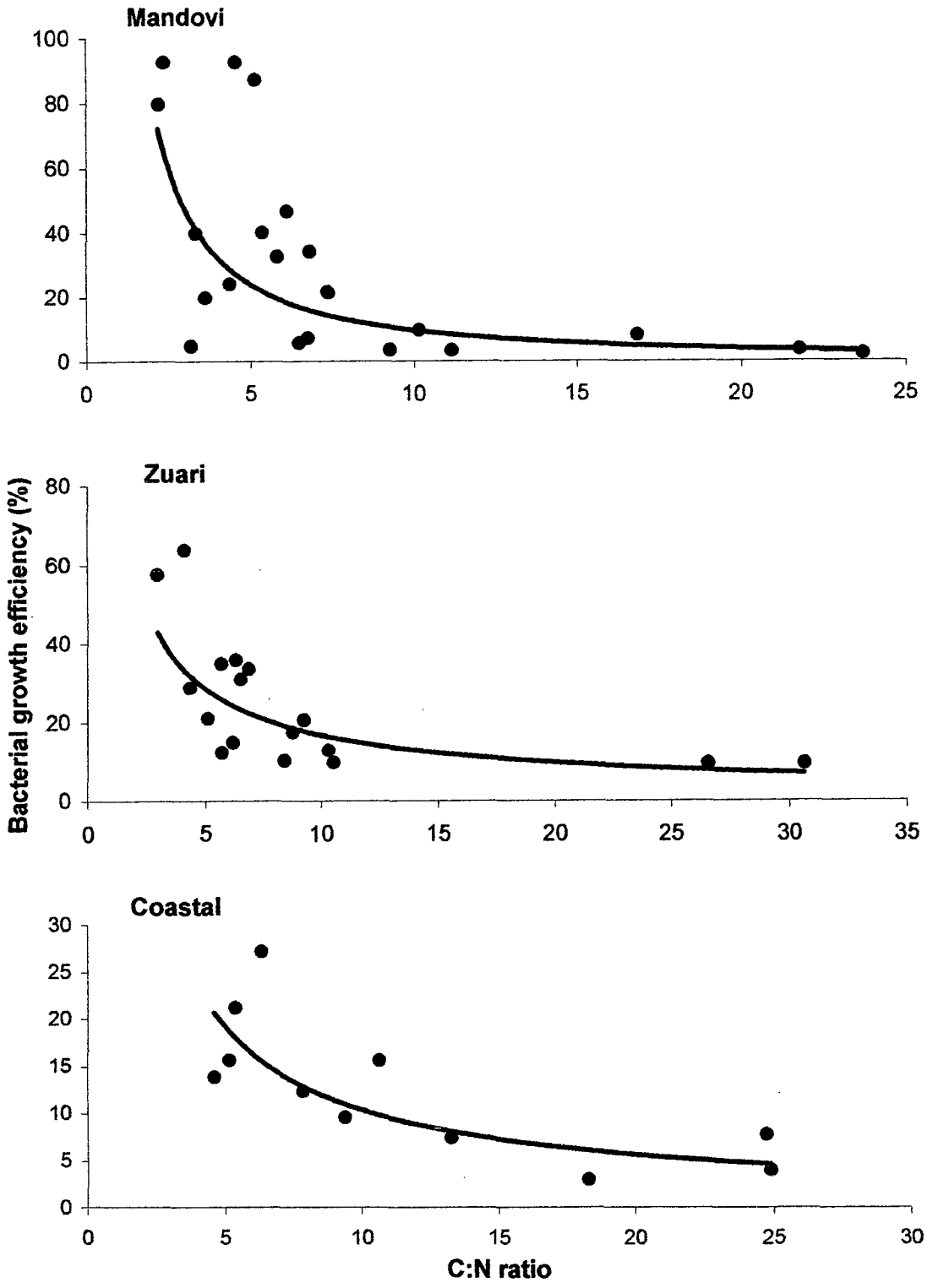

Fig. 6.11. Relationship between C:N ratio and bacterial growth efficiency at Mandovi ($n=21$, $r=0.70$, $p<0.001$), Zuari ($n=18$, $r=0.75$, $p<0.001$) and coastal ($n=11$, $r=0.81$, $p<0.001$) stations

substrates (Fig. 6.12a). The SGR, BP and BGE were calculated to be 0.023 h^{-1}, 2.02 μg C l^{-1} h^{-1} and 28.4% respectively. Water samples amended with amino acid and urea also showed increased BP and BGE than those amended with ammonium chloride (Fig. 6.12b). The BP and BGE values were 5.58 μg C l^{-1} h^{-1} and 21.73% for amino acid, and 5.54 μg C l^{-1} h^{-1} and 20.16% for urea respectively. Similarly high response in BP and BGE was also observed in water samples amended with phytoplankton extract (*Isochrysis sp.*) and vanillic acid. The BP and BGE values were 2.09 μg C l^{-1} h^{-1}, 20.09% for *Isochrysis* and 2.06 μg C l^{-1} h^{-1}, 20% for vanillic acid (Fig. 6.12c). Apart from the above, other substrate amendments showed little or no response.

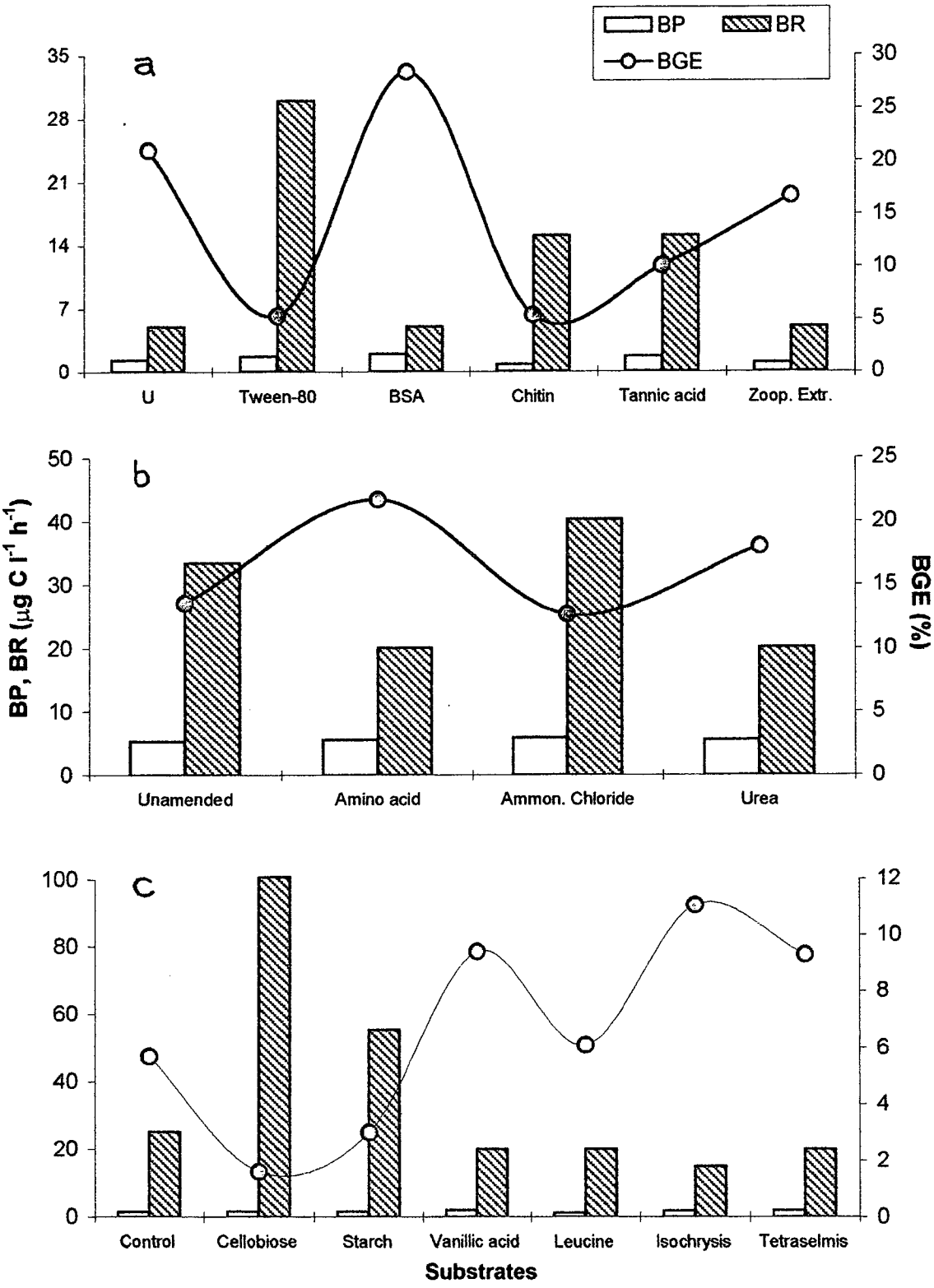

Fig. 6.12. Changes in the bacterial production (BP), respiration (BR) and growth efficiency (BGE) in estuarine water samples amended with different organic subsrates

6.3 Discussion

Knowledge of BGE is a pre-requisite to evaluate the role of bacteria in determining the fate of organic carbon in aquatic systems. These values are assumed to be representative of *in situ* bacterial processes. Although the decrease in DOC utilization has been used in some studies for calculation of BGE (Carlson and Ducklow 1996), in the present study, BP and BR were used for calculation of BGE rather than DOC utilization as it would take longer time (days) to detect a decrease in DOC levels, which can lead to the underestimation of BGE. When considering the metabolism, the accuracy in the estimation of BP and BR is of utmost importance in determining the BGE of natural bacterioplankton. Accuracy in the estimation of BP is largely dependent on the application of conversion factor (CF), which can be responsible for large variation in BP rates. A wide range of conversion factors has been reported and used for the calculation of BP. In order to avoid any bias in choosing the CF, we derived our own CF of 1.96×10^{18} cell $mole^{-1}$. Our values were much closer to those, generally reported and commonly used (1.4 to 2.2×10^{18} cells $mole^{-1}$) (Fuhrman and Azam 1982, Kirchman et al. 1982, Bell et al. 1992). CF for thymidine incorporation has been discussed in detail in the previous chapter. In spite of its limitation associated in measuring BP using labeled compounds (Hollibaugh 1994, Smits and Reimann 1988), this technique is useful to determine the total uptake of organic matter by bacterial assemblage in calculating their average growth rates. This approach has been used to monitor the nutritional status of aquatic bacteria seasonally (Berman et al. 1994).

Earlier studies have calculated BR rates assuming BGE in the range of 40 to 60%, mostly deduced on the basis of the uptake and efficiency conversion of simple ^{14}C-labeled organic compounds (Crawford et al. 1974). There is now indication that growth efficiencies of bacteria using natural substrates are substantially lower than the assumed values (Kirchman et al. 1991, Jahnke and Craven 1995). Oxygen consumption in dark provides simplest and least ambiguous estimate of determining BR that can be directly related to the oxidation of organic matter (Williams 1981, Hopkinson 1989). Although a number of approaches have been served to measure dissolved oxygen, most of the researchers needing high accuracy or precision still rely on the titrimetric method of Winkler (Aminot 1988, Carignan et al. 1998), which was used in the present study. The advantage of this method is that all carbon respired by bacteria is accounted for in the estimate. In the present study, bacterioplankton account for more than 70% of the total community respiration (TR). Similar values of high percentage of BR over total respiration have been reported for Lake Superior and Lake Christmas (USA) where the contribution of BR to TR was 82-98% (Biddanda et al. 2001). Several workers have found that the contribution of BR to TR to be more than 40% (Griffith et al. 1990, Chin-Leo and Benner 1992). Biddanda et al. (1994) showed BR to be half of total respiration in the Gulf of Mexico. In the NE Pacific BR: TR was found to vary from 42 to 83% in spring than other seasons (Sherry et al.1999). Our results are consistent with the general contention that bacterioplankton community is responsible for a large fraction of oxygen consumption in the water column.

The BGE values for Mandovi and Zuari estuaries were 21 and 24% respectively. Overall in the Mandovi-Zuari estuarine system the BGE was low, lower than previously assumed estimate of 50%. BGE has been found to range from <5 to >60% across system (del Giorgio and Cole 1998). The values reported (23%) in the present study in the estuarine waters are very close to the reported median BGE of 24 ± 23% (data derived from 20 published articles and reports) by del Giorgio and Cole (1998). Comparison of our BGE values with those reported elsewhere is shown in Table 6.2. High BGE in the estuarine stations than coastal waters indicated regional variability. Similar spatial variation of high BGE values have been reported in the Florida Bay (43%) when compared to the adjacent coastal waters in the Gulf of Mexico (16%) (Coffin et al. 1993). There is consistent evidence of constant pattern of decreasing BGE with increasing distance across the coastal waters. Griffith et al. (1990) found BGE decreasing with increasing distance from the shore (11% in estuarine, 6% in near shore waters, 2% in shelf areas) in the shelf waters of Georgia.

As seen from the present study, BGE were generally highest in the environments with highest bacterial activity. System dependent variations with lower efficiency in oligotrophic as opposed to more productive marine areas have also been reported elsewhere (Biddanda et al. 1994, Pomeroy 1995, Carlson and Ducklow 1996). The large difference in BGE values between the estuarine and coastal water reported in our study could suggest either physiological changes in the bacterial population or shifts in species composition. The variance in the BGE in our study stations could be attributed to the difference in primary production

Table 6.2. Published sources of *in situ* bacterial growth efficiency in selected coastal and estuarine waters

Location	Bacterial growth efficiency (%)	Reference
Mississippi R. Plume, USA	14-22	Amon and Benner (1998)
Hudson Estuary, USA	7-61	Findlay et al. (1991)
Georgia estuarine waters, USA	11	Griffith et al (1990)
Gulf of Mexico, USA	26-61	Kroer et al. (1993)
Louisiana shelf, USA	18-55	Biddanda et al. (1994)
Sargasso Sea	4-30	Carlson and Ducklow (1996)
Ross Sea	9-38	Carlson et al. (1999)
Mandovi Estuary	4-47	The present study
Zuari Estuary	5-64	The present study
Adjacent coastal waters (Arabian Sea)	3-27	The present study

(system dependent), where the estuarine sites recorded two to three folds higher values than coastal. Relationship between BP, BR and PP could change in time and space due to variable plankton composition and availability of resources (Jensen et al. 1990). In this tropical estuarine system, BGE was found to be higher in places where the PP was high. However no significant relationship could be established between the two. In normal conditions, the natural marine environment has a specific microbiota adapted to particular environmental conditions. Due to interference of many variables simple linear correlations cannot be expected. The observed trend of higher BGE in the estuarine versus lower efficiency in the coastal waters may also reflect substrate quality as well quantity (Pomeroy and Wiebe 1993).

The large difference in BGE values between the estuarine and coastal waters reported in our study could be due to physiological changes in the bacterial population or shifts in species composition. In the present study the average BP estimates in the estuarine and adjacent waters were 1.80 and 2.82 $\mu g\ C\ l^{-1}\ h^{-1}$. These values are much higher than that of the mean values reported in coastal areas and estuaries (0.10 ±0.01, 0.22 ±0.04 $\mu g\ C\ l^{-1}\ h^{-1}$) (del Giorgio and Cole 2000). BR rates were higher in the coastal station and significantly ($p<0.05$) differed from that of estuarine stations. Due to high BR rates, the BGE was low with a mean value of 11%. At all the stations BR exceeded BP by an order which indicated that respiratory loss of carbon is larger than the net assimilation of organic matter into bacterial biomass under almost any condition. Similar observation pertaining to the above has been reported in the Hudson

River estuary (Roland and Cole 1999). As seen from other studies, the BR in the present is less variable than BP and the recorded mean value of 8.79 and 13.70 μg C l^{-1} h^{-1} in the estuarine and coastal waters. This value is much higher than those reported in coastal (0.43± 0.05 μg C l^{-1} h^{-1}) and estuaries (0.36 ±0.06 μg C l^{-1} h^{-1}). Comparison of our productivity and respiration measurements with those reported elsewhere is shown in Table 6.3 & 6.4 respectively.

Scatter in the relationship of BP and BR reflects the physiological flexibility of the bacterial community in aquatic systems (del Giorgio and Cole 1998). In the present study, the relationship between BP and BR varied at all the three study sites. BP and BR showed a low significance ($p<0.05$) at Mandovi and a strong relationship ($p<0.01$) at the coastal station (Fig. 6.4). Although a positive significant relationship was observed at the Mandovi and coastal station, the relationship was not linear, which indicated BGE to be variable and not a constant one. Absence of significant relationship in the Zuari estuary was due to large variation in the BGE, which is shown by a high CV of 71%. In Mandovi estuary the BGE was not as high when compared to Zuari and as a result a weak relationship was observed. At coastal station the variation in BGE was minimum, which led to a strong relationship between BP and BR. Absence of significant relationship in the Zuari estuary indicates uncoupling of catabolism and anabolism processes (del Giorgio and Cole 2000). It has been shown that bacteria can dissociate catabolic from anabolic processes. This means that during periods when the growth is limited by the availability of an anabolic substrate, ATP may be synthesized in excess of the biosynthetic demands

Table 6.3. Published sources of bacterial productivity in selected coastal and estuarine waters

Location	Bacterial productivity ($\mu g\ C\ l^{-1}\ h^{-1}$)	Reference
Northern Gulf of Mexico	0.2 – 3.1	Biddanda et al. (1994)
Northwest Florida Estuaries	0.8 – 3.9	Coffin et al. (1993)
Mississippi River Plume	0.21 – 3.75	Chin Leo and Benner (1992)
Chesapeake Bay	0.17 – 2.08	Ducklow and Carlson (1992)
Mandovi Estuary	0.58 – 7.36	Present study
Zuari estuary	0.57 – 9.46	Present study
Adjacent coastal water (Arabian Sea)	0.54 – 5.98	Present study

Table 6.4. Published sources of bacterial respiration rates in selected coastal and estuarine waters

Location	Bacterial respiration ($\mu g\ C\ l^{-1}\ h^{-1}$)	Reference
Northern Gulf of Mexico	0.84 – 5.37	Biddanda et al. (1994)
Georgia coastal waters	3.70 – 28.65	Hopkinson et al. (1989)
Danish Estuary	3.70 – 22.20	Jensen et al. (1990)
Georgia Shelf waters	11.94 – 53.73	Griffith et al. (1990)
Pensacola Bay	2.87 – 7.34	Coffin et al. (1993)
Mandovi Estuary	1.34 – 18.69	Present study
Zuari Estuary	2.68 – 17.44	Present study
Adjacent coastal waters (Arabian Sea)	5.37 – 25.42	Present study

(Hopkinson et al. 1989). This uncoupling observed in the present indicated that BGE is theoretically variable. Since BR is less dynamic than BP, BGE is basically driven by variation in BP and is well correlated to it (Fig. 6.6). This strong relationship ($p<0.001$) indicated that BGE values could be predicted from BP measurement alone and the factors controlling BP also affected BGE values. BP explained 83, 92 and 71% of the variation in BGE at the Mandovi, Zuari and coastal stations. Similar findings have been reported in the Hudson River estuary where BP explained 70% of the variation in BGE values (Roland and Cole 1999). Our present study comprehensively support the fact that the growth efficiencies in marine systems are clustered from 5 to 30% (del Giorgio and Cole 2000) and any assumption of an average value such as 50% used in ecological models would be inappropriate.

Factors regulating BGE

In the present scenario, factors affecting or regulating BGE in natural aquatic system have vehemently gained importance in understanding the role of bacteria in food web dynamics. In general, in this tropical estuarine system the BGE is low. The low BGE in the present study could be attributed to prime factors like substrate availability, temperature and bacterivory, which have been generally identified to control or regulate bacterial growth in aquatic ecosystems. The relation between bacterial variables could asses, what controls these population, whether substrate limitation (bottom up control) or removal (top down control). A wide range of abiotic and biotic factors has been identified in regulating the BGE (del Giorgio and Cole 2000).

In the present study no significant the effect of temperature on bacterial activity was not observed. The Q_{10} values were lower than those reported for other estuaries, particularly in temperate waters where the value ranged from 3.3 to 4.8 (Shiah and Ducklow 1994, Bussmann 1999, Shiah et al. 1999). Moreover, field investigation also showed no significant relationship of temperature with bacterial parameters indicating its influence on microbial abundance and activity to be of minor concern. Similarly, del Giorgio and Cole (1998) did not find any relationship between BGE and temperature or any significant effect of temperature on the relationship between BP and BR. Shiah et al. (1999) reiterated the fact that in warm waters the bacterial growth and activity are largely regulated by substrate supply rather than temperature which is less likely to occur. In the present study a weak relationship ($p<0.05$) was observed between BGE and temperature only in the surface waters of Mandovi. Although not strong it could be further concluded that temperature is not an overriding factor of BGE in this tropical system.

Previous studies have indicated that nanoflagellates were shown to be capable of actively grazing on bacteria at natural densities in most aquatic environments (0.5 to 5 x 10^6 cells l^{-1}) (Fenchel 1982). The fact that total bacterial population in the present study was more than 1 x 10^6 cells l^{-1} indicates that grazing could play a major role in this tropical estuarine system. Like in most other cases, the present study showed grazing to obey first order kinetics.

The present study indicated the grazing rate to range from 0.012 to 0.013 h^{-1} and natural mortality rates from 0.019 to 0.021 h^{-1}. Mortality and grazing rates

similar to ours have been reported in estuarine and coastal waters of Scheldt River Estuary, Belgian coastal zone, Mediterranean coastal zone, Holland coastal zone where the values ranged from 0.01 to 0.03 h^{-1} for mortality and 0 to 0.02 h^{-1} for grazing respectively (Servais et al. 1985, Servais et al. 1989). These rates were close and almost equal to the bacterial specific growth rates observed in the present study. Large excessive growth rates over grazing are reported from shallow high productive environments like Kaneohe Bay, Chesapeake Bay and San Francisco Bay (USA). The above results indicate that grazing could be one of the factors controlling bacterial growth and activity. Moreover the absence of significant relationship between bacterial abundance and productivity in the field investigations further substantiates the above statement. Pararnhos et al. (2001) attributed the lack of relationship between abundance and production to grazing effect in the inner site of tropical coastal Guanabara Bay (Brazil). In the present study, high BGE was observed in the filtered (without grazers) than unfiltered (with grazers) water samples. This difference in BGE value may be related to physiological characteristics of the bacteria or removal of bacteria by grazers. Hence, the low BGE may be either due to the selective feeding of the larger actively growing cells (Sherr et al. 1992, Gasol et al. 1995, Posch et al. 1997, Hahn and Hofle 1998) or increased recycling of organic matter in these cultures due to grazing, which may increase community respiration and decrease community net production. The decrease in growth yield due to grazers would lead to effects of internal carbon recycling in the bacterial community and rather necessarily change the physiological characteristics of the population.

In the estuarine and coastal waters the availability of nutrients is not only from *in situ* biological process but also from allochthonous input from terrestrial or mangrove litter ecosystem (Wafar et al. 1997). In the present study at the estuarine stations inorganic nutrients (N&P) limited the bacterial growth and activity. Both the estuarine sites showed increase in the BP and BGE in water samples amended with P alone or in combination with other nutrients. The BGE values in the P amended water samples were significantly higher ($p<0.05$) than that of unamended samples. Increasing body of evidence of P limitation has been reported in the marine environments (Zohary and Robarts 1998, Pomeroy et al. 1995, Rivkin and Anderson 1997). Studies have indicated bacteria to be effective competitors with phytoplankton for ammonium and phosphate sources (Kirchman and Rich 1997) and in some systems bacterial uptake of mineral nutrients dominates nutrient fluxes (Cotner et al. 1992). del Giorgio and Cole (1998) suggested that the P limited cells could store organic carbon without dividing and could thus maintain higher growth efficiency. Moreover significant relationship between BGE and P observed in the field investigation indicated P to be the limiting factor in the estuarine environments. High phosphatase activity (as discussed in the earlier chapter) during the field investigation also substantiated the fact of P limitation in the estuarine waters. Studies on diel variation experiment conducted in the Baltic Sea and Mediterranean showed that the BGE was related significantly to the phosphate concentration suggesting that P was preferentially utilized over C and N which was found to be in excess (Zweifel et al. 1993). Further there is increasing evidence of P controlling BGE in marine

systems, especially in the Gulf of Mexico (Pomeroy et al. 1995). Cotner et al. (1997) found both phytoplankton and bacteria to be limited by phosphate during summer and production of alkaline phosphatase to hydrolyze organic phosphorus compounds Sargasso Sea. Similarly high rates of phosphatase activity indicating P limitation have also been reported in Sargasso Sea and Catalan coast, Spain (Cotner et al. 1997, Rose and Axler 1998, Sala et al. 2001).

In contrast to the estuarine samples, nutrient enrichment experiments carried out in the coastal water samples showed increase in BP and BGE in organic carbon amended water samples than those with inorganic nutrients, which indicated that organic carbon was a limiting factor. Moreover the field observations indicating strong relationship between BP and PP substantiated the presumption that organic carbon was the factor limiting bacterial growth in coastal waters. The above finding could further be substantiated by the lack of significant relationship between BGE and organic carbon indicating that organic carbon was not a limiting factor in these estuarine environments. The BP and BGE observed in the organic carbon amended samples were 9 $\mu g\ C\ l^{-1}\ h^{-1}$ and 22% respectively. The concept of organic carbon limitation in coastal waters has been widely reported in different oligotrophic environments of Sargasso Sea and Equatorial Pacific regions (Cole et al. 1988, Carlson and Ducklow 1996, Cherrier et al, 1996) and in the subarctic Pacific (Kirchman 1990). In the present study systematic differences observed in PP in the coastal station when compared to estuarine region could have led to carbon limitation. Such organic carbon

limitation had been reported in Gulf of Mexico and in Conception Bay, New Foundland (Pomeroy et al. 1991, Biddanda et al. 1994)

The natural bacterial assemblage showed high affinity (BGE) towards substrates such as algal lysate, amino acid, bovine serum albumin, urea and vanillic acid indicating its efficiency in utilization for biosynthesis. Batch experiments with individually amended organic substrate in water samples indicated that the response was higher in samples amended vanillic acid, bovine serum albumin (BSA), urea and amino acid. High BGE value of 23% was observed in samples amended with the amino acid. Values as high as 70% are obtained for bacteria grown on amino acids (Connolly et al. 1992). Intermediate growth is seen for cells growing on alcohols and alkanes and for esters and organic acids and the efficiency is typically around 40% (Connolly et al. 1992). Similar finding of increased response in water samples amended with amino acid has been reported in eastern North Pacific surface waters (Cherrier et al. 1996). Perhaps the only common result of most addition experiments is that amino acids tend to enhance both BGE and bacterial growth (Kirchman 1990, Carlson and Ducklow 1996). It has also been suggested that it is energetically advantageous to use preformed compounds, but also the energetic cost of transporting amino acids across the membrane greatly offsets this advantage. It is more likely because amino acids have relatively high energy and carbon contents and are also a source of N; they release bacteria from multiple limitations as carbon, energy and N. High BGE was also observed in water samples amended with vanillic acid, BSA and urea indicating the importance of these organic

compounds in the bacterial assimilation. Similar studies carried out in Maumee River to study the trophic dynamics of riverine bacterioplankton indicated greater positive growth response to bovine serum albumin amended samples during spring and summer and, leucine in the autumn (Foremann et al. 1998). Similar studies pertaining to the response of the bacterioplankton to low carbon substrates along with inorganic nutrients on specific growth rates have been carried out in lake water cultures isolated from Lawrence Lake, USA (Coveney and Wetzel 1992). High respiration rates led to low BGE in water samples amended with organic carbon (glucose). Hence the present study showed that utilization of organic carbon in the tropical system is largely dependent on the availability of mineral nutrients i.e. N and P. Many studies similar to ours have focused on the dependency of BGE on the relative availability of mineral nutrients and organic carbon in natural systems (Billen and Fontigny 1987, Vallino et al. 1996). The result of the present study is in contrast to organic carbon limitation, which was the prevailing theory in the previous decade (Cole 1982, Findlay et al. 1986). The magnitude of bacterial response observed in the present study due to addition of inorganic nutrients indicated, the presence of large pool of labile organic carbon, which could be utilized depending on the availability of mineral nutrients. Hence in the present study the natural bacterioplankton was unable to utilize excess glucose efficiently without inorganic nutrients. Thus the availability of inorganic nutrients could play an important role in regulating accumulation and consumption of the bio-reactive DOC in the water column (Shiah et al. 1998).

Dissolved organic matter (DOM) constitutes a large portion of the organic carbon in aquatic systems and is often a significant resource for heterotrophic microorganisms. DOM is generally a complex mixture of compounds and availability of this DOM for bacterial assimilation is largely dependent on the elemental stoichiometry of the substrate i.e. C:N ratio. Depending on the composition of the DOM pool and the ratio at which the various sources contribute to it, the activity and growth efficiency of bacteria will vary widely (Connolly et al. 1992). Previous studies have indicated that both the quantity and quality of DOM have been identified as the primary factors affecting its bacterial remineralization and trophic assimilation (Cole et al. 1988, Kirchman 1990, Keil and Kirchman 1994). High efficiency in water samples amended with with phytoplankton extract (algal lysate) of *Isochrysis* and *Tetraselmis*, indicated phytoplankton to be an important source of organic matter to bacterial community due to its high lability. Laboratory studies conducted in North Atlantic have indicated that the DOM derived from phytoplankton is incorporated with highest efficiency than other substrates (Biddanda 1988, Cherrier et al. 1996). Enhanced BP and BGE were observed in laboratory experiments with water samples amended with algal lysate in Sargasso Sea (Carlson and Ducklow 1996). Research with cultured bacteria (Russel and Cook 1995) as well models of bacterial energetics and growth in aquatic ecosystem (Anderson 1992, Conolly et al. 1992, Vallino et al. 1996) have suggested that the substrate C:N ratio and inorganic nutrient availability are the most important factors controlling BGE. . In the present study, the particulate C:N ratio of the substrate was used as a

surrogate for substrate quality (Shiah et al. 1999). Increase in the BGE value with the decrease in C:N ratio of the organic matter at the estuarine stations indicated that organic carbon was not a factor limiting bacterial growth. Billen (1984) and Goldman et al. (1987) have unequivocally shown that BGE of natural assemblage of marine bacteria grown on a range of substrates is inversely related to C:N ratio of the substrate. In the present study, the C:N ratio was found to range from 5.42 to 13.35 with a mean value of 7.39. Our results further substantiated the fact that as the C:N ratio of the substrate increases the efficiency in its utilization decreases, which is shown by the significant negative relationship between the BGE and C:N (Fig. 6.11). Thus it could be suggested that the C:N of the available substrate could be a major determinant of BGE in this estuarine system. Kroer (1993) reported the inverse relationship between BGE and C:N ratio in Santa Rosa Sound (USA). Similar relation between substrate C:N ratio and BGE was experimentally verified by Goldman et al. (1987) who observed a large increase in BGE when C:N ratio of the substrate was below 6. Cimbleris and Kalff (1998) also reported that the substrate utilization by the natural bacterioplankton would be maximum when the C:N ratio of the available substrate is close to the intracellular stoichiometry of the natural bacterioplankton. Biddanda et al. (2001) suggested an inverse relationship between C:N and BGE to increased maintenance cost and attributed to the availability of inorganic nutrients in Minnesota lakes. Moreover, significant relationship between BGE and population generation time observed at the study stations suggests increase in the relative importance of maintenance metabolism

and physiological shifts of the bacterial population toward utilization of more refractory substrates (Middelboe and Sondergaard 1993).

In this study it can be inferred that even though excess of labile organic carbon is available for bacterial assimilation, the efficiency of retaining it into the bacterial biomass is dictated by the availability of inorganic nutrients (either N or P) to achieve the stoichiometry.

6.4 CONCLUSION

1. The median BGE value in the estuarine stations ranged from 5.5 to 59.4% with a mean value of 22% and in the coastal site it was 3.64 to 21.19% with a mean value of 11.1%.
2. Overall in this tropical system, BGE are low indicating a greater percentage of substrate is respired to support cellular maintenance energy.
3. BR is less variable than bacterial production and it is clear that BGE is driven by changes in BP rather than BR.
4. Field investigation indicates that BGE is controlled by the availability of inorganic (N&P) nutrients and related inversely to the C:N ratio. Nutrient amendments made in both the estuarine and coastal water samples were successful in inducing changes in both BP and BR and therefore generating range in the BGE, thus indicating that bacteria were substrate limited. The outcome of the nutrient amendments indicated that the bacterioplankton community was phosphate limited in Mandovi and Zuari estuaries and carbon limited in the coastal waters.
5. Low Q_{10} values and absence of significant relationship with the bacteriological parameters suggests temperature to be of minor concern.
6. The grazing rates were not significantly lower than specific growth rates and the absence of significant relationship between bacterial abundance and production indicated that grazing could be one of the factors at times in regulating bacterial growth and activity.

Chapter 7

CARBON FLUX

7.1 INTRODUCTION

Heterotrophic bacterioplankton play an important role in regulating accumulation, export, remineralization and transformation of the largest organic pool (DOC) in marine systems over small (local) and large (global) scales (Cole et al. 1988, Hansell and Carlson 1998, Carlson et al. 1999). The sources of substrate supply for heterotrophic bacteria mainly come from phytoplankton exudation, zooplankton sloppy feeding, excretion from other plankton, release from dead particles and viral induced lysis (Ducklow and Carlson 1992, Fuhrman 1992). However, on longer time (seasonal) or spatial (across ecosystem) scales, it has been suggested that primary production is the 'ultimate' factor controlling bacterial growth (Cole et al. 1988, Conan et al. 1999), primarily due to the theory that phytoplankton exudation has been considered the most important source of organic substrate in supporting bacterial production. Thus, carbon is the currency of choice for examining the fate of primary production in the marine system and the impact on geo-chemical cycles is perennial topics in oceanography. The origin and coupling between sources of phytoplankton-derived material and its consumption by bacteria play a main role in variability of carbon flux. The real magnitude of organic carbon flow through bacterioplankton in most of the aquatic systems especially in the tropics remains largely unknown and there exists paucity in the knowledge for understanding the bacterial mediated carbon flux measurements.

The role of heterotrophic bacteria in the flux of organic matter in the Mandovi-Zuari estuarine system has not yet been understood. The amount of

carbon flowing through bacterioplankton was calculated with BP/PP:BGE. The measurements of these parameters have been described in Materials and methods section. This approach followed in the present study will be useful in assessing the relative contribution of specific system to large-scale carbon budget. It will also greatly enable to comprehend the flow of organic carbon through bacterioplankton and could lead to better understanding of food web interactions in a tropical ecosystem.

7.2 RESULTS

7.2.1 Bacterial productivity: Primary productivity (BP:PP) ratio

Depth integrated primary productivity ranged from 10.10 to 146.6 mmol C m^{-2} d^{-1} in Mandovi and 23.6 to 153.17 mmol C m^{-2} d^{-1} in the Zuari estuary. In the present study, both the estuarine stations showed a high of 121.27 and 119.19 mmol C m^{-2} d^{-1} in the pre-monsoon season and a low of 47.59 and 72.07 mmol C m^{-2} d^{-1} during the monsoon season in the Mandovi and Zuari estuaries (Fig. 7.1). PP varied significantly ($p<0.007$) in all the three seasons in Mandovi than Zuari estuary. No significant variation in PP was observed between the estuarine stations. At the coastal station, high BP values ranging from 13.39 to 104.58 mmol C m^{-2} d^{-1} were observed in the pre-monsoon (CV=71%) than in post-monsoon season. At the estuarine site PP dominated BP during the non-monsoon season and BP over PP in monsoon season (Fig. 7.1). In the coastal station the PP rates were higher during the post-monsoon season and lower in the pre-monsoon season (Table 7.1). However on an annual basis integrated primary productivity did not show any significant variation between coastal and estuarine stations. Bacterial production rates were higher and exceeded primary production rates in the monsoon season at Mandovi and Zuari with a mean value of 71 and 163 mmol C m^{-2} d^{-1} respectively (Table 7.1). In the present study, significant relation between BP and PP was observed at the Zuari ($p<0.05$) and coastal stations ($p<0.001$) (refer chapter 5).

The BP:PP ratio, indicative of trophic status of an ecosystem, ranged from 0.10 to 1.90. Both Mandovi and Zuari estuaries showed high BP:PP ratios in the

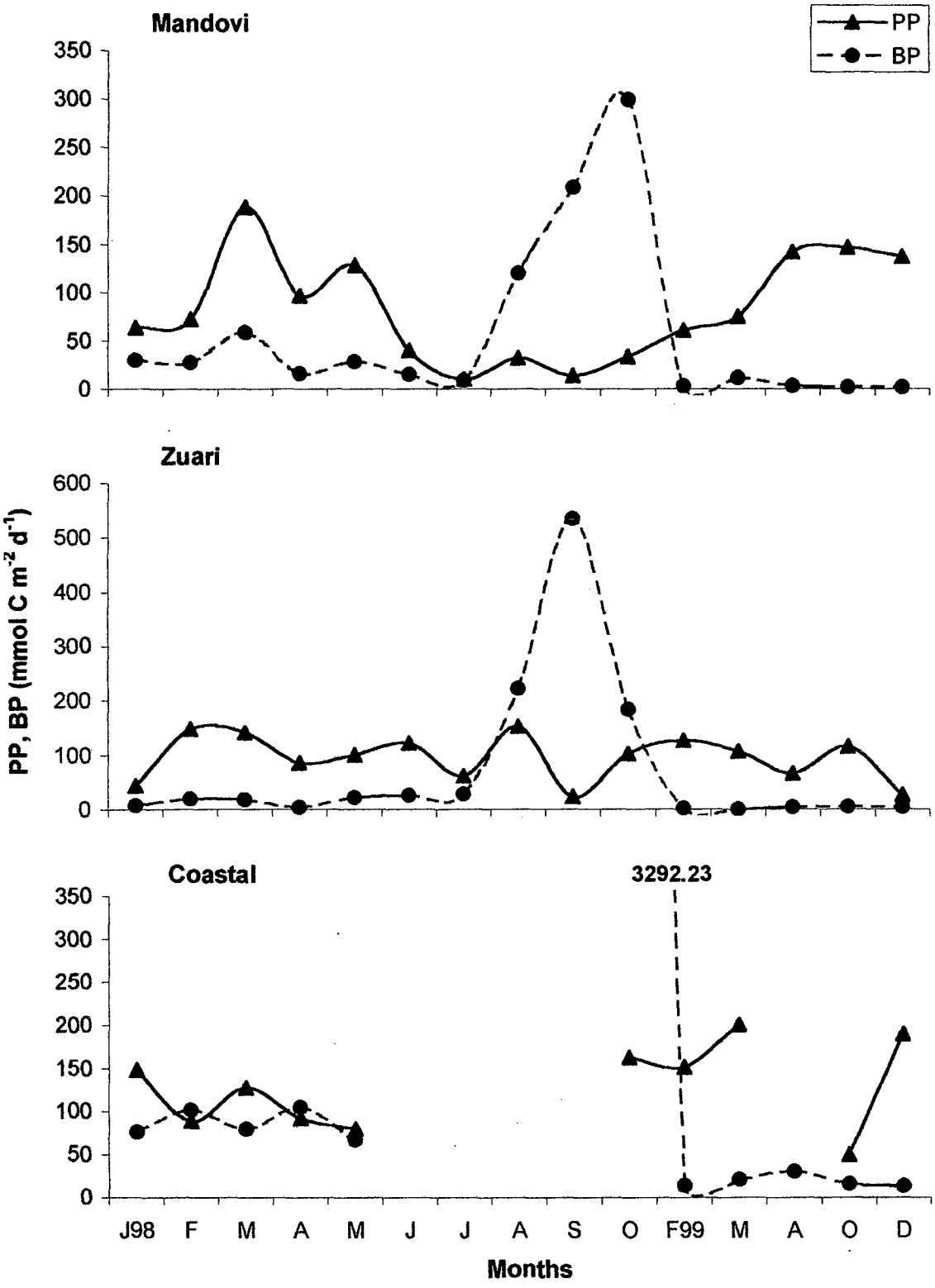

Fig. 7.1. Monthly variation in the depth integrated primary (PP) and bacterial production (BP) rates

Table 7.1. Seasonal variation in the carbon flow in the Mandovi-Zuari estuarine system.

BP and PP are expressed in mmol C m^{-2} d^{-1}

Parameter	Mandovi			Zuari			Coastal		
	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
BP	32.64	71.07	83.07	1582	163.07	49.90	88.03	-	35.01
PP	121.27	47.59	95.16	119.19	85.54	72.07	96.86	-	136.95
BP:PP	26.91	149.00	87.29	13.27	190.64	69.24	90.87	-	25.56
BGE (%)	32.48	22.85	17.28	19.33	33.11	12.89	15.89	-	8.26
BP:PP/BGE(%)	83.12	652.07	57.87	67.25	573.84	69.82	572.68	-	326.87

monsoon season with a mean value of 1.49 and 1.90 respectively (Table 7.1), when compared to non-monsoon months, which ranged from 0.13 to 0.87 (Table 7.1). The coastal station showed a mean value of 0.91 and 0.26 in the pre- and post-monsoon seasons.

Significant correlation was observed between BP:PP ratio and phytoplankton efficiency (PE) in both Mandovi and Zuari estuaries (Fig. 7.2). PE was relatively high in the non-monsoon which lead to low BP:PP ratio. Thus the high PP was sufficient to support bacterial carbon demand. The reverse trend was observed in these two stations during the monsoon season.

The amount of phytoplankton carbon passing through bacterioplankton was calculated by dividing the BP:PP ratio with corresponding BGE values determined seasonally at the study stations. In Mandovi estuary, the percentage of PP passing through bacterioplankton was calculated to be 83 and 58% in the pre- and post-monsoon seasons respectively (Table 7.1). In the monsoon season high value of 652% was observed, indicating the utilization of exogenous carbon by the natural bacterial assemblage. In Zuari estuary the bacterioplankton utilized 67 and 70% of the PP in the pre- and post-monsoon seasons. In the monsoon season the bacterioplankton utilized 574% of PP (Table 7.1). At the coastal station bacterioplankton utilized 573 and 327% carbon of PP in the pre- and post-monsoon seasons respectively.

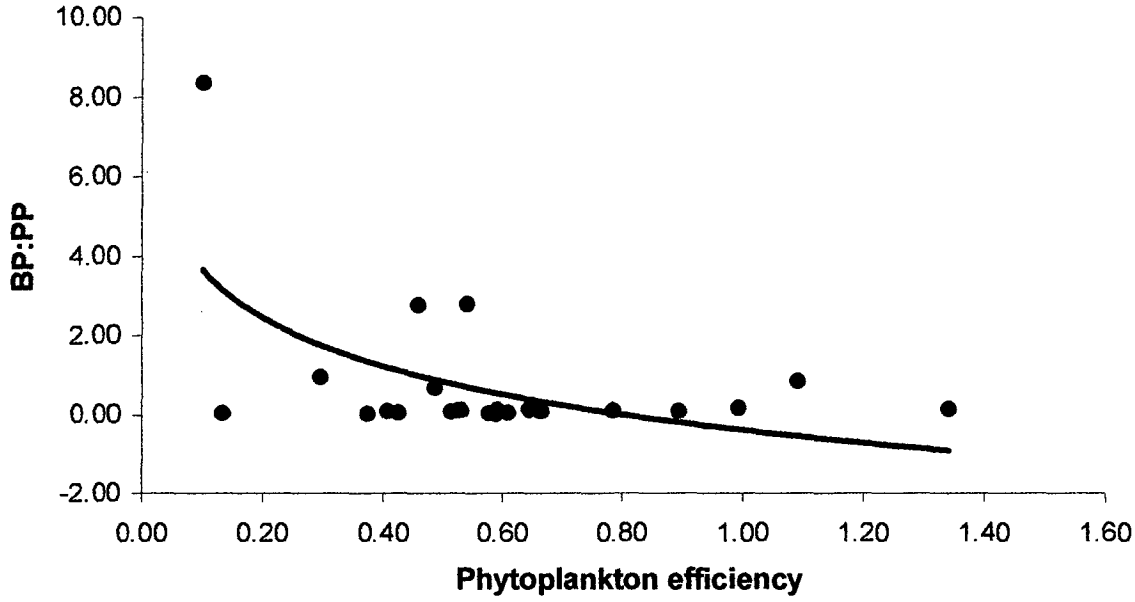

Fig. 7.2. Scatter plot between BP:PP ratio and phytoplankton efficiency ($n=23$, $r=0.57$, $p<0.01$) at the estuarine stations

7.3 DISCUSSION

In marine environments the autotrophs and heterotrophs constitute two of the most fundamental and complementary functional units. Hence, understanding the nature of auto-heterotrophic processes is necessary to define their contribution to the organic metabolism and trophic status in an ecosystem. Bacteria are the main consumers of dissolved organic matter generated from phytoplankton and carbon flow from organic matter into bacteria is one of the major pathways of material and energy flow in pelagic food webs. The origin and coupling between sources of phytoplankton derived material and its consumption by bacteria play a main role in the variability of BP:PP ratio. Comparison of our BP:PP ratios with other regions are shown in Table 7.2.

The ratio between BP and PP (BP:PP) in the estuary showed a high variability depending on the season. At both the estuarine stations bacterial production exceeded PP in the monsoon season with a mean ratio of 1.7 than non-monsoon seasons which showed a mean ratio of 0.15. The BP:PP ratio(expressed in percentage) in the tropical estuarine system was low during the non-monsoon seasons and ranged from 10 to 27%. In the present study, the values of BP:PP recorded in the non-monsoon seasons compared well with Chesapeake Bay, where the BP:PP ratio was found to range from 15 to 22%. Other authors have reported mean values, which range from 20 to 30% when reviewing data from a wide variety of aquatic systems (Williams 1981, Cole et al. 1988). Significant relationship was observed between BP:PP and PE (phytoplankton efficiency, calculated from primary productivity and chlorophyll

Table 7.2. Published sources of Bacterial productivity: Primary productivity (%) in selected coastal and estuarine waters

Location	Bacterial productivity: Primary productivity (%)	Reference
St. Lawrence estuary	28-81	Vincent et al. (1996)
Indus R. Delta	120-490	Bano et al. (1990)
Delaware estuary	5-3410	Hoch and Kirchman (1993)
Schelde estuary	37-452	Goosen et al. (1997)
Urdaibai estuary	1-469	Revilla et al. (2000)
East China Sea	4-57	Shiah et al. (2001)
Sargasso Sea	18-130	Carlson et al. (1996)
Mediterranean Sea (East)	18-54	Turley et al. (2000)
Mandovi estuary	8-278	Present study
Zuari estuary	1-68	Present study
Adjacent coastal waters	2-52	Present study

concentration) ($p<0.01$) at the surface waters of the estuarine stations. This relationship indicates that when phytoplankton are efficient in carbon production (i.e. PE>1.5 mg C mg^{-1} chl h^{-1}), the amount of organic matter produced is sufficient to support the BP directly through phytoplankton. PE is low indicating that the phytoplankton are less efficient in production of organic carbon, which may result in bacteria utilizing increased proportions of PP. As a result, the bacterial carbon demand (BCD) could be met either by the low PE or indirectly through a range of other routes such as sloppy feeding and release zooplankton (Jumars et al. 1989), dissolution of fecal pellets, egestion of digestion vacuoles and cell lysis including lysis by viruses (Baines and Pace 1991). From our study it is clear that during the non-monsoon period large amount of material is available for higher trophic level of marine food web and/or for export. However, in the monsoon season this carbon demand could not be met by the phytoplankton. In coastal waters an inverse relationship between BP:PP to PE was observed. This inverse relationship has also been observed in Mediterranean Sea, which ranged from 10 to 30% (Conan et al. 1999). Shiah et al. (2001) also showed negative trend in the relationship between BP:PP to PE where the ratio of BP:PP ranged from 4 to 57% with a mean of 15 $\pm$8% in the East China Sea. Lochte et al. (1997) reported similar observation in the cold Southern Ocean and attributed it to low exudation rate of DOC by phytoplankton, low bacterial uptake or conversion of organic material and expression of bacterial metabolism due it low temperature or high grazing pressure. Significant inverse relationship was observed between BP:PP to PP at all the study stations during the monsoon season (Fig. 7.3),

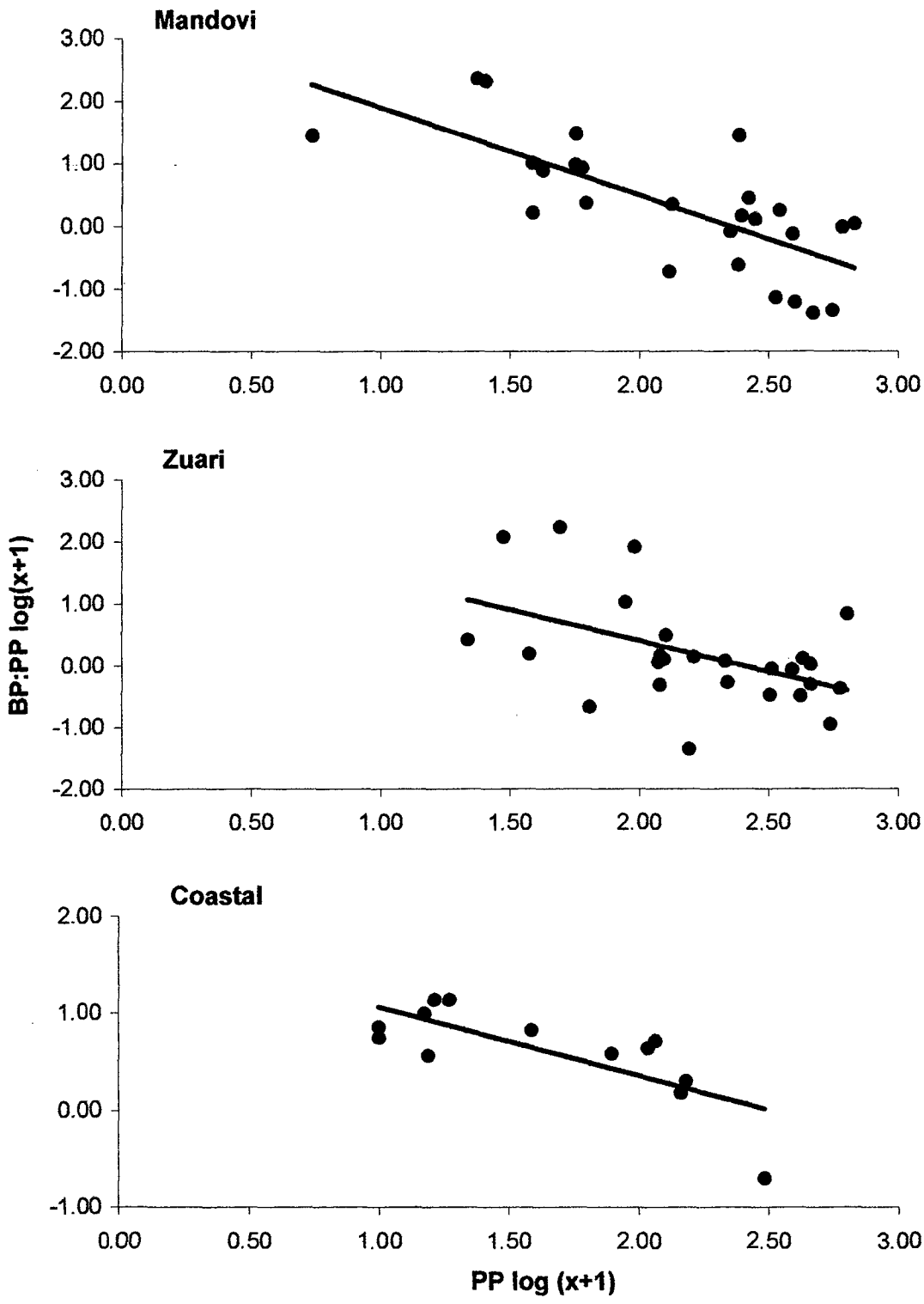

Fig. 7.3. Scatter plot between BP:PP and PP (Log transformed) at Mandovi (n=26, r=0.73, p<0.001), Zuari (n=26, r=0.53, p<0.001) and coastal (n=15, r=0.75, p<0.01) stations

indicating uncoupling or time lag between phytoplankton and bacterial development. The average values being 54 and 55% in the estuarine and coastal waters respectively (Table 7.3). Though the relationship has been reported in several ecosystems, its ecological implication is not very clear and must be further explored.

To model the total carbon flux through bacterioplankton a thorough knowledge of bacterial growth efficiency (BGE) is a pre-requisite. Utilization of PP by bacterioplankton is largely dependent upon BGE. In the present study the BGE values were low, suggesting that the bacterioplankton in this ecosystem would be responsible for a greater overall flux of carbon and would require a larger fraction of PP to maintain measured BP. The amount of PP channeled through bacteria in marine environments is reported to vary from 10% to over 100% with a mean of 30-40% (Cole et al. 1988, Ducklow and Carlson 1992). Goosen et al. (1997) reported very high values ranging from 138 to 1508% in the Schelde estuary. Simon and Tilzer (1987) calculated 40% of the PP to pass through bacterioplankton using a growth efficiency of 50% in Lake Constance. In the present study, during the non-monsoon season the percentage of PP passing through bacterioplankton was calculated to be 70 and 69% in the Mandovi and Zuari estuaries. High percentage values of 652 and 574% were recorded in the Mandovi and Zuari estuaries in the monsoon season. From the present study, it is evident that during the monsoon season the bacterial carbon demand is supported by the utilization of exogenous organic carbon or DOC reservoir source in addition to those derived from local contemporaneous phytoplankton.

Table 7.3. Comparison of carbon flow between estuarine and coastal sites

Parameters	Estuarine	Coastal
Integrated bacterial productivity (BP) (mmol C m^{-2} d^{-1})	49.84	61.52
Integrated primary productivity (PP) (mmol C m^{-2} d^{-1})	91.87	112.77
BP:PP (%)	54	55
Bacterial growth efficiency (BGE) (%)	23	12
BP:PP/BGE (%)	234	458

In both the estuaries during the non-monsoon seasons 53 to 83% of PP is sufficient to support BP with a low BGE of 21%. Thus the general feature of this system is that it is productive with loose auto-heterotrophic coupling. The high values observed could be due to other processes such as cell lysis, POM hydrolysis, external inputs of substrate by turbulent vertical diffusion and /or by advection presumably become increasingly important compared to direct phytoplankton release and grazing alone. The link between BP and physiological state of the phytoplankton could explain the wide range of BP:PP ratios as found previously in other studies (Ducklow and Carlson 1992). High BP:PP ratio and weak or non existent correlation between bacteria and phytoplankton has also been found in other heterotrophic systems like the Delaware estuary (Hoch and Kirchman 1993), the upper Lawrence estuary (Painchaud and Therriault 1989), the Hudson estuary (Findlay et al. 1991) in USA and Rhone River plume (Germany) (Kirchman et al. 1995). Cole et al. (1988) when reviewing data from a large number of marine and freshwater systems suggested that 60% of PP would be fluxed through bacterioplankton.

The coastal station showed higher percentage of bacterial utilization of PP than estuarine stations and did not vary with seasons. BGE values are lower indicating greater amount of carbon passing through bacterioplankton. In the euphotic zone, the proportion of primary production required to support bacterial production varies from 2 to 190% (Ducklow and Carlson 1992) and is around 40% in most environments (Cole et al. 1988). Biddanda et al. (1994) indicated that carbon flow through bacterioplankton was 25% of PP at the highly productive

shelf region and 69% of PP at the less productive slope in Gulf of Mexico. In the western and eastern Mediterranean Sea 110 and 170% of the PP was routed through DOC reservoir to support the bacterial carbon demand when BGE was assumed to have a value of 20% (Turley et al. 2000).

The annual integrated data (Table 7.3) clearly showed that the carbon flow through bacterioplankton in estuarine and coastal stations could be as high as 234 and 458% of the PP in the productive estuarine and coastal waters. The percentage of PP utilized in our ecosystem may be highly variable with season depending on the supply and nature of allochthonous carbon inputs. Low carbon conversion efficiency in this system especially in the coastal waters implies high BR rates, which explains the use of large amounts of dissolved substrate and increased participation of bacteria in the carbon fluxes.

7.4 CONCLUSION

1. There is seasonal variability in the carbon flux in the estuarine stations. i.e. high BP:PP ratios during the monsoon and low in the non-monsoon seasons. In the coastal waters the ratio was high through out the year.
2. Low BP:PP in the non-monsoon season indicated the availability of sufficient material available for higher trophic level.
3. PP supported 53 to 82% of the bacterial carbon demand in the non-monsoon seasons, while high percentages of >100% in the monsoon season was linked to exogenous supply of organic matter.

Chapter 8

NET PRODUCTION

8.1 INTRODUCTION

The contribution of any biological system to the global carbon budget relies on a balance between carbon production and consumption. Metabolism within the system represents the sum of two fundamental and complementary processes viz. primary production and community respiration. They are the major pathways by which organic matter is produced and utilized within the ecosystem and the balance between them is termed as net ecosystem production (NEP). NEP is calculated as the difference between gross primary production (GPP) and dark community respiration (R) (Smith and Hollibaugh 1997). Measurements of these variables are pre-requisite to assess the trophic status and to bring out the relative importance of external organic matter inputs versus internal sources in aquatic ecosystems (Caffrey et al. 1998). NEP may be equated to 'new' production, which represents the maximum exportable biomass to preserve the long-term integrity of the system (Quinones and Platt 1991, Eppley 1992), and allows calculation of the availability of surplus of primary production, and hence the determination of the potential fate of organic carbon produced in the euphotic zone. NEP could be positive indicating net autotrophy (GPP>R) or negative indicating net heterotrophy (R>GPP), thereby representing the overall balance of the dynamics of whole ecosystem better than the activity of primary producers and heterotrophs (Howarth et al. 1996). This balance between net autotrophy and net heterotrophy within aquatic systems is strongly affected by the nature of exogenous inputs. The organic substrate supply rate from the non-algal sources mentioned above also co-vary with primary production, which indicate that

bacteria-phytoplankton coupling might not be a 'direct' casual relationship and lead to imbalances in the carbon budgets (del Giorgio et al. 1997, Duarte and Agusti 1998). At present there is still difficulty in quantifying the relative importance of non-biogenic and biogenic components in contributing organic substrate in different ecosystems. The significant contribution of the marine biota to the global carbon cycle (Sarmiento and Siegenthaler 1992) ultimately results from the balance between gross primary production (P) (all photosynthesis independent of its fate) and community respiration (R) (the oxidative consumption of organic matter by autotrophic and heterotrophic organisms).

In recent years, there has been a debate on the role of the plankton community as sources (del Giorgio and Cole 1998, Duarte and Agusti 1998, Duarte et al. 1999, Duarte et al. 2001) or sinks (Williams 1998, Williams and Bower 1999) of carbon in sub tropical and temperate waters. Although data sets on GPP are available substantially over the past four decades, knowledge on the rates of respiration in the marine systems is disturbingly poor, resulting in unclear quantification of such a balance. Specifically the paucity of respiration data has been suggested to be the reason why most marine carbon flux models fail to balance (Biddanda et al. 1994, Jahnke and Craven 1995).

As the present knowledge is insufficient to understand the quantitative carbon cycling in this tropical region and since this natural environment is vulnerable to human influence and vice versa, it is of immediate interest to carry out a detailed study of the factors controlling carbon dioxide in this region The balance between net autotrophy and net heterotrophy within a system is strongly

affected by the nature of exogenous inputs. High biological diversity, primary and secondary productivity and high turnover rates of organic materials are typical of pollution-free tropical estuaries. Tropical Mandovi-Zuari estuarine system, receives intermittent input of large amount of organic matter from riverine sources- especially during the monsoon months, which will significantly affect the net carbon balance of the coastal waters. In addition, substrates from allochthonous inputs such as riverine runoff, sediment re-suspension also support substantial bacterial growth, particularly in the near shore systems

The main objective of this chapter is to examine whether there is a seasonal change in the trophic status in a tropical estuary and the adjacent coastal waters of the Arabian Sea. Subsequently the quantification of the net trophic balance over biogeochemically significant scales study will help in assessing the relative contribution of specific system to large-scale carbon budgets leading to better understanding of the tropical ecosystem. In order to resolve major aspects of global ocean organic budget, it is ultimately necessary to define the steady state rates of transfer of materials to or from particular carbon reservoirs. For the calculation of NEP, depth integrated approach was followed to overcome bias in the data sets (Williams 1998, Duarte and Agusti 2001), thereby giving a more realistic picture of the distribution of the balance of autotrophy and heterotrophy in these waters.

8.2 RESULTS

8.2.1 Integrated primary productivity (P)

Depth integrated primary productivity (P) did not show any significant variation between estuarine and adjacent coastal waters. Productivity ranged from 10.10 to 188.18 mmol C m^{-2} d^{-1} in the Mandovi and 23.60 to 148.16 mmol C m^{-2} d^{-1} in Zuari stations (Fig. 8.1). Seasonal variation in the primary productivity was more prominent in Mandovi between non-monsoon and monsoon seasons than in Zuari estuary. The mean productivity in the Mandovi and Zuari estuaries during the monsoon season was 24.04 and 85.54 mmol C m^{-2} d^{-1} respectively (Table 8.1). Primary productivity did not differ significantly between the estuarine stations. Productivity at the coastal station ranged from 49.56 to 188.94 mmol C m^{-2} d^{-1} with post-monsoon season recording a mean value of 136.95 mmol C m^{-2} d^{-1}, higher than that of the pre-monsoon season (96.86 mmol C m^{-2} d^{-1}) (Table 8.1).

8.2.2 Integrated dark community respiration (R)

Dark community respiration rates (R) ranged from 36.17 to 159.36 mmol C m^{-2} d^{-1} in the Mandovi and 21.43 to 148.23 mmol C m^{-2} d^{-1} in the Zuari estuaries (Fig. 8.1). High respiration was observed in both Mandovi and Zuari stations during the monsoon. The mean R rates in the Mandovi and Zuari was 81.81 and 80.02 mmol C m^{-2} d^{-1} respectively (Table 8.1). Although no significant variation in R rates was observed between the estuaries, significant seasonal variation ($p<0.03$) in R rates were observed between monsoon and non-monsoon seasons at both the estuaries. At the coastal station R rates were higher and ranged from

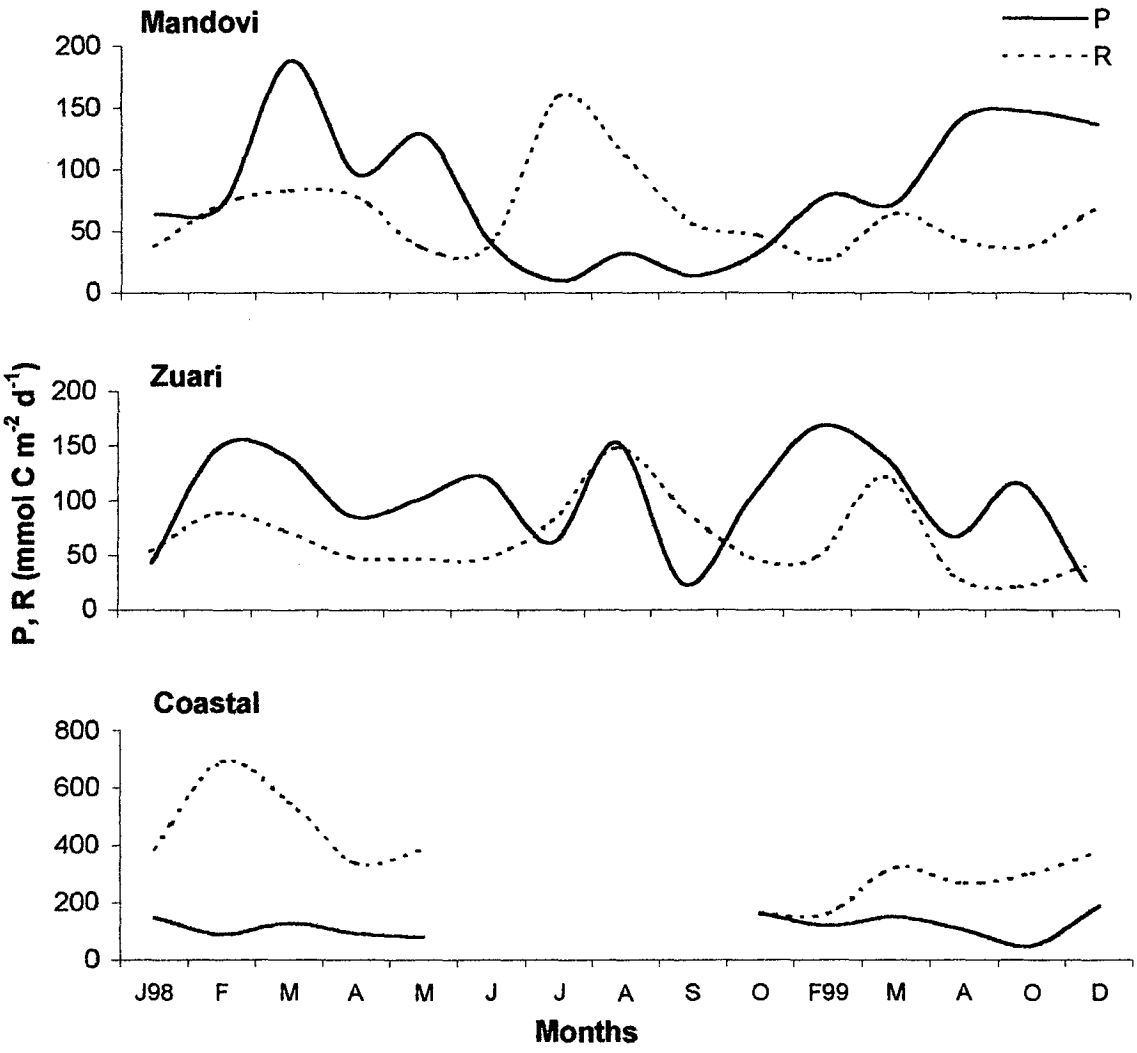

Fig. 8.1. Monthly variation in the integrated primary production (P) and respiration (R)

Table 8.1. Seasonal variation in the primary production (P), respiration (R) and P:R ratio

Station	Season	P (mmol C m^{-2} d^{-1})	R (mmol C m^{-2} d^{-1})	P:R
Mandovi	Pre-monsoon	121.27 (±50.13)	67.43 (±21.30)	1.80
	Monsoon	24.40 (±14.33)	81.81 (±51.74)	0.29
	Post-monsoon	95.16 (±55.39)	47.89 (±15.09)	1.99
Zuari	Pre-monsoon	119.19 (±30.41)	64.28 (±20.40)	1.85
	Monsoon	85.54 (±51.65)	80.02 (±45.02)	1.07
	Post-monsoon	72.07 (±43.91)	40.90 (±14.12)	1.76
Coastal	Pre-monsoon	96.86 (±20.93)	491.67 (±162.41)	0.20
	Monsoon	-	-	-
	Post-monsoon	136.95 (±60.74)	306.68 (±104.61)	0.45

161 to 692.50 mmol C m^{-2} d^{-1}. In contrast to P, R rates were higher in the pre-monsoon (491.67 mmol C m^{-2} d^{-1}) than post-monsoon season (306.68 mmol C m^{-2} d^{-1}) (Table 8.1).

8.2.3 Primary productivity: Dark community respiration (P: R)

In the estuarine stations the dominance of P over R and vice versa was observed in certain months of the year (Fig. 8.1). In the Mandovi the dominance of R over P was observed in the monsoon months indicating net heterotrophy with P: R ratio of 0.29. In the Zuari estuary certain months of monsoon and post-monsoon season indicated the dominance of R over P, whereas in the other months particularly in the pre-monsoon season net autotrophy was observed (P:R= 1.85) (Fig. 8.1). The change in the trophic status in both Mandovi and Zuari estuaries coincided with either high riverine discharge or terrestrial runoff in the monsoon. During this period the secchi disc reading measured was 6% of the water column. In contrast to the estuarine stations, the coastal station recorded higher R than P with an average ratio of 0.33, indicating net heterotrophy throughout (Table 8.1).

8.2.4 Net ecosystem production (NEP)

Seasonal fluctuation in NEP was more prominent at the estuarine than in the coastal station (Fig. 8.2). NEP ranged from –149.26 to 109.14 mmol C m^{-2} d^{-1} in the Mandovi estuary and from –67.97 to 119.71 mmol C m^{-2} d^{-1} in the Zuari estuary (Fig. 8.2). At Mandovi station, the mean NEP values were 53.84, -34.22 and 47.27 mmol C m^{-2} d^{-1} in the pre-monsoon, monsoon and post-monsoon seasons respectively (Fig. 8.3). In Zuari estuary, although some months

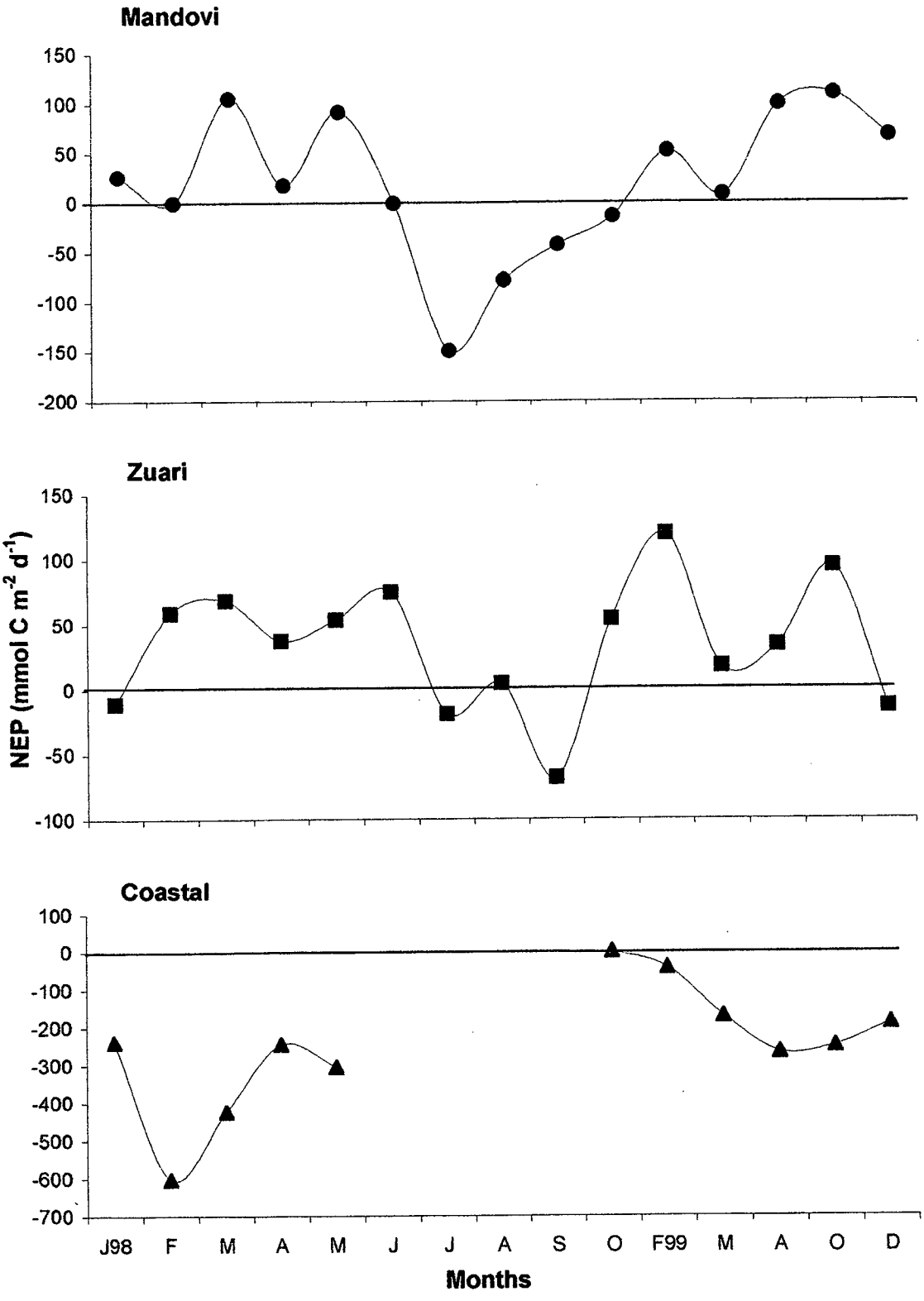

Fig. 8.2. Monthly variation in the net ecosystem production (NEP) measurements.

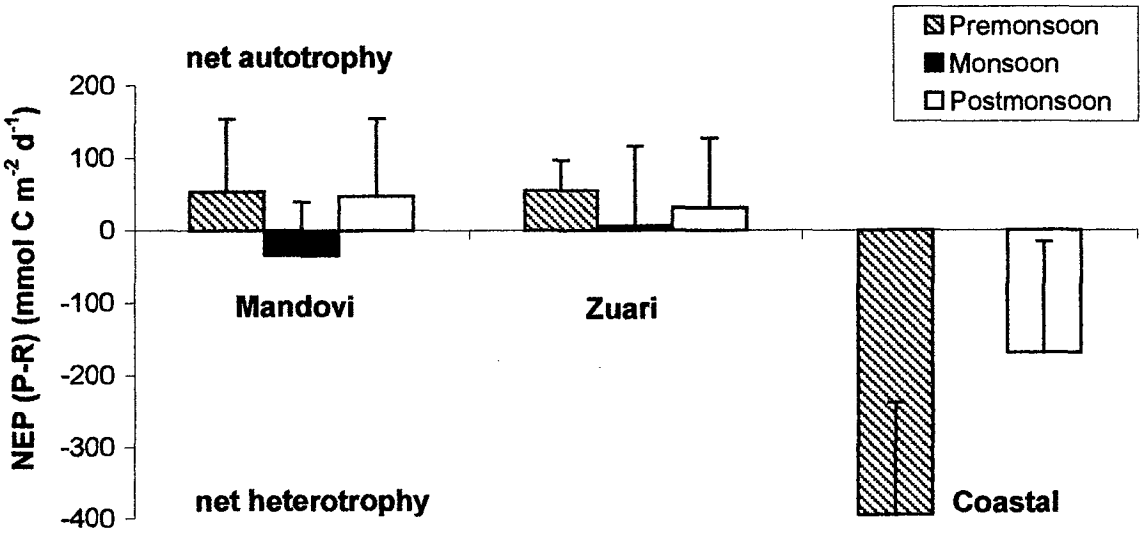

Fig. 8.3. Seasonal variation in the net ecosystem production at the study stations

indicated NEP values to be negative, the overall mean value showed NEM to be positive indicating net autotrophy. The mean NEP values recorded in the Zuari were 54.91, 5.52 and 31.17 mmol C m^{-2} d^{-1} in the pre-monsoon, monsoon and post-monsoon seasons (Fig. 8.3). In the coastal station NEP values were negative throughout, except in the month of October (0.78 mmol C m^{-2} d^{-1}). The NEP values ranged from –603.67 to 0.78 mmol C m^{-2} d^{-1} (Fig. 8.2). Mean NEP value of –394.64 and –169.74 mmol C m^{-2} d^{-1} was observed in the pre- and post-monsoon seasons respectively (Fig. 8.3).

Scatter plot between P and NEP showed the trend to be different for the estuarine and coastal stations. The pattern that emerged for estuaries was more towards autotrophy and with increased P (Fig. 8.4). These are represented by the regression equation NEP= 69.95 ln (P) – 275.21. On an annual basis at the estuarine stations 65% of the values showed autotrophy and the rest showed heterotrophy. In the coastal station the scatter was large and did not relate significantly to P and as a result net heterotrophy decreased with increase in P with prominent outliers (Fig. 8.4). At coastal station negative values of NEP were observed at low production rates. Net heterotrophy increased with R and related significantly ($p<0.001$) when compared to estuarine system where a weak relationship was observed (Fig. 8.5).

Annual integrated data on P, R and NEP has been presented in Table 8.2. The annual NEP in both the estuaries showed the dominance of P over R indicating the trophic status to be net autotrophic (sink). The P: R-values of Mandovi and Zuari estuaries were 1.36 and 1.56 respectively. The coastal water

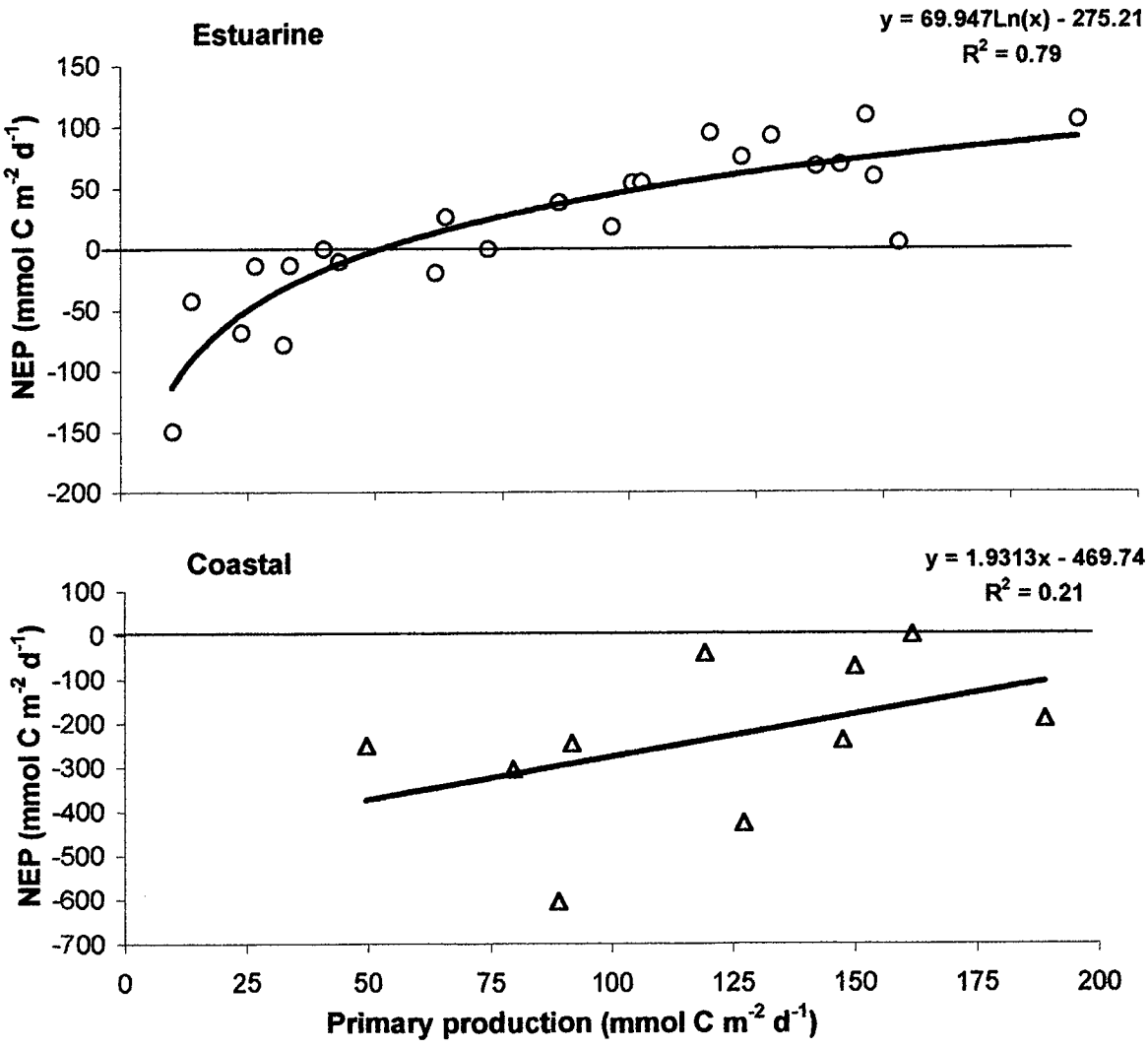

Fig. 8.4. Scatter plot between net ecosystem production (NEP) and primary production (P) at the estuarine and coastal stations

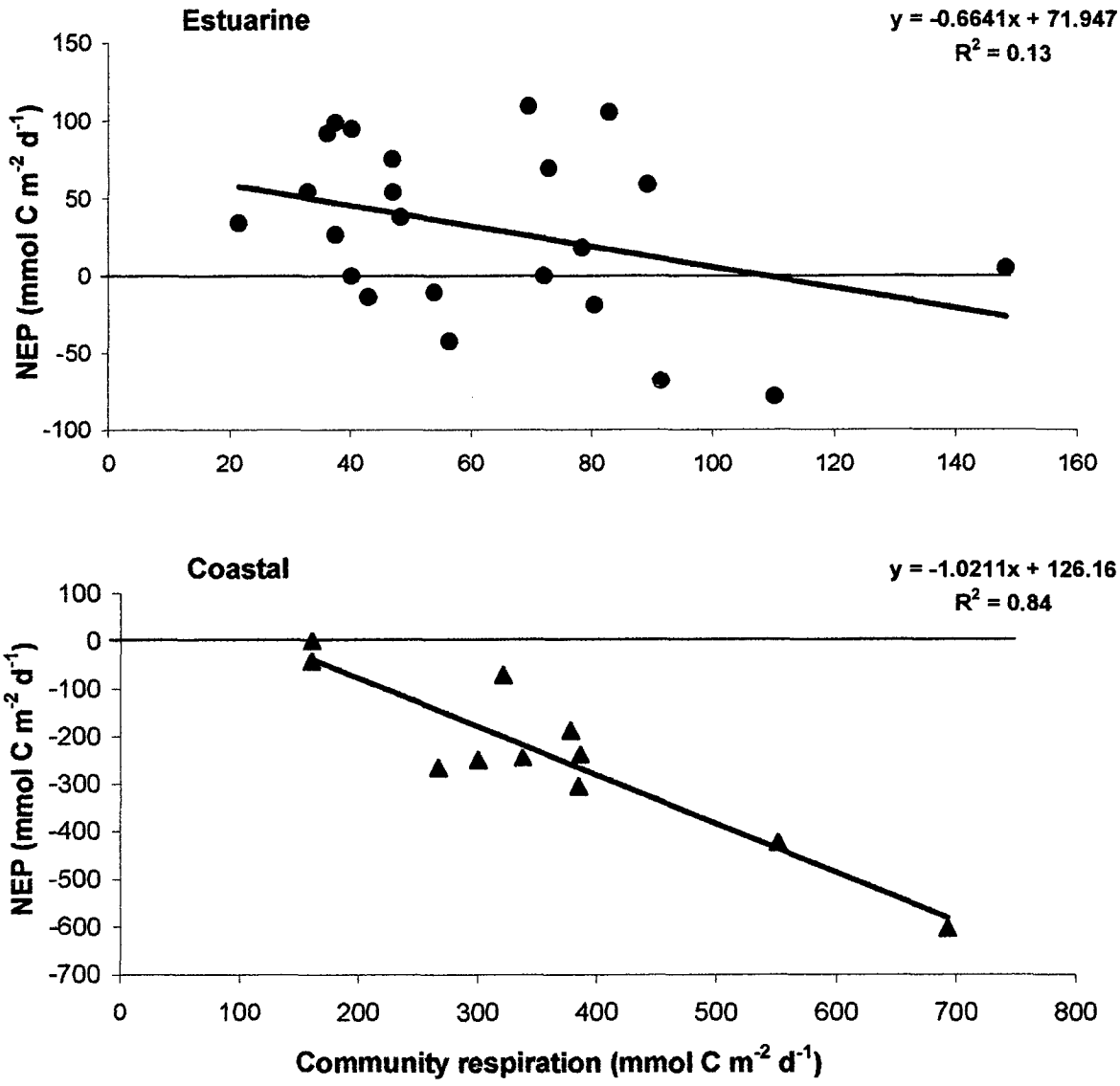

Fig. 8.5. Scatter plot between net ecosystem production (NEP) and respiration (R) at estuarine and coastal stations

Table 8.2. Annual trend in the net trophic balance at the study stations

Station	Area (km^2)	P ($mol\ C\ m^{-2}\ y^{-1}$)	R ($mol\ C\ m^{-2}\ y^{-1}$)	NEP (P-R) ($mol\ C\ m^{-2}\ y^{-1}$)	P:R	Trophic status
Mandovi	1527	30.54 (± 19.82)	23.40 (± 12.645)	7.14 (± 17.30)	1.31	Net autotrophic
Zuari	973	36.52 (± 17.20)	24.12 (± 12.69)	12.40 (± 14.65)	1.51	Net autotrophic
Coastal	3630	43.47 (± 14.95)	130.67 (± 56.84)	-87.20 (± 53.28)	0.33	Net heterotrophic

showed the reverse trend wherein the respiration was more than production with a P: R value of 0.33 indicating the persistence of net heterotrophy (source). Rates of metabolism measured in the present study at the estuarine and coastal stations are tabulated per unit (map) area (Table 8.3).

Table 8.3. Estimates (areawise) of net trophic balance at the study stations

Parameters	Mandovi (x 10^5 mole C y^{-1})	Zuari (x 10^5 mole C y^{-1})	Coastal (x 10^5 mole C y^{-1})
Primary production (P)	0.47	0.36	1.57
Community respiration (R)	0.36	0.24	4.74
P:R	1.31	1.51	0.33
Trophic status	Net autotrophic	Net autotrophic	Net heterotrophic

8.3 Discussion

The NEP in the estuarine stations was more dynamic and varied seasonally when compared to the coastal station. The average NEP values for estuarine stations in the monsoon and non-monsoon seasons were –34.22 and 50.56 mmol C m^{-2} d^{-1} respectively, whereas, the coastal station showed a dominance of heterotrophy. Such annual or seasonal scales of variability of NEP in the temperate waters (Kemp et al. 1992, Nixon and Pilson 1984, Smith and Hollibaugh 1997) have been reported. Apart from seasonal variation, in Roskilde Fjord (Denmark) and Waquoit Bay (Massachusetts) daily and weekly variability in NEP was observed (Jensen et al. 1990, D'Avanzo et al. 1996). Kemp et al. (1997) reported variability along the trophic gradient of Chesapeake Bay where the upper Bay exhibited net heterotrophy, middle balanced and lower bay net autotrophic. Conspicuous pattern of heterotrophy in the Mandovi during the monsoon season could be attributed to large amount of riverine discharge than Zuari as Mandovi encompasses a larger catchment area (1527 km^2) than Zuari estuary (973 km^2). Depth factor and persistence of stratification in the monsoon months in Zuari estuary could also play a role in explaining the above. These factors may have influenced the primary production (P) and community respiration (R). The low P: R ratio of 0.29 observed in the monsoon season could be due to combined effects of allochthonous organic carbon source and high turbidity, thereby enhancing respiration and inhibiting phytoplankton photosynthesis (Smith and Kemp 1995). Primary productivity during the monsoon season was low and ranged from 10.10 to 40.05 mmol C m^{-2} d^{-1}. In the present

study the estuaries showed low P rates in the monsoon season especially in the Mandovi. This low P rates particularly in the Mandovi was due to high turbidity caused by re-suspension of sediment particles in water column. Due to turbidity, the amount of light penetration into the water column got reduced to 6% in the monsoon when compared to pre monsoon (13%) and post-monsoon (25%) seasons. In such situation the community would have utilized the labile fraction of organic matter. Such utilization of labile organic matter has been observed in the upper region of Chesapeake Bay (Howarth et al. 1996). Although, the $^{14}CO_2$ method measures neither net nor gross primary productivity our rates were closer to gross productivity. However experimental studies conducted by Davis and Williams (1984) showed that rate of $^{14}CO_2$ determined photosynthetic production and gross photosynthetic oxygen production agreed well, within the precision of each technique. No evidence of marked errors in the ^{14}C technique for measuring P was found. Caution was exercised in interpreting net primary productivity to gross primary productivity (GPP). We restrained in applying correction factor to calculate GPP from net primary productivity, although previous workers have assumed values ranging from 5 to 50% of the P_{max} (Raven and Beardall 1981). Cole et al. (1991, 1992) and Howarth et al. (1996) have used a correction factor of 5% of P_{max} for the calculation of GPP in the turbid Hudson River. However, in the present study in spite of using 5% of P, neither the trend nor the values varied significantly. Thus our estimate is at the lower end of GPP. P and R measurements made in the present study are compared with those values reported elsewhere (Table 8.4). From the present

Table 8.4. Published sources of production (P), respiration (R) and P:R values in selected marine systems. P and R are expressed in mol C m^{-2} y^{-1}

System	P	R	P:R	Reference
Georgia coast	44.92	36.25	0.71	Hopkinson (1985)*
Tomales Bay, California	27.42	31.00	0.88	Smith et al. (1991)
Narragansett Bay	25.83	19.17	1.35	Nixon and Pilson (1984)*
North Atlantic Bight	19.17	23.33	0.82	Rowe et al. (1986)
New Port River Estuary	17.58	20.17	0.87	Kenny et al. (1988)
Spencer Gulf, Australia	7.67	7.00	1.10	Smith and Veeh (1989)
Columbia River Estuary	5.92	2.33	2.54	Simenstad et al. (1984)
Mandovi Estuary	30.54	23.40	1.31	Present study
Zuari Estuary	36.52	24.12	1.51	Present study
Adjacent coastal water (Arabian Sea)	43.47	130.67	0.33	Present study

* - see reference chapter

Smith SV, Hollibaugh JT, Dollar SJ, Vink S (1991) Tomales Bay metabolism: C-N-P stoichiometry and ecosystem heterotrophy at the land-sea interface. Estuar Coast Shelf Sci 33: 223-257

Rowe GT, Smith S, Falkowski P, Whitledge R, Theroux, R, Phoel W, Ducklow H (1986) Do continental shelves export organic matter? Nature 324: 559-561

Smith SV, Veeh HH (1989) Mass balance of biogeochemically active materials (C, N, P) in a hypersaline gulf. Estuar Coast Shelf Sci 26: 195-215

Kenny BE, Litaker W, Duke CS, Ramus J (1988) Community oxygen metabolism in a shallow tidal estuary. Estuar Coast Shelf Sci 27: 33-43

Simenstead C, Jay D, McIntire CD, Nehlsen W, Sherwood C, Small L (1984) The dynamics of the Columbia River Estuarine Ecosystem. Vol. 1, Columbia River Estuarine Development Program, University of Washington, Seattle

study it is clear that there exists seasonality in NEP and that in this tropical estuarine ecosystem auto-heterotrophic coupling could be transient phenomenon rather than a permanent one.

The balance between net autotrophy and net heterotrophy within aquatic systems is strongly affected by the nature of exogenous inputs. Estuarine system such as Narragansett Bay (Nixon and Pilson 1984) and Chesapeake Bay (Kemp et al. 1997) (USA) which receive large inorganic nutrients inputs appears to be net autotrophic while coastal system with organic inputs such as Georgia Bight (Hopkinson 1985) and Tomales Bay (USA) (Smith and Hollibaugh 1997) appear to be net heterotrophic. However a seasonal study in San Francisco Bay (USA) indicated net autotrophy in shallow areas in the spring months and net heterotrophy in deeper regions during other seasons (Caffery et al. 1988). In the coastal station R exceeded P throughout the seasons (except October '98) indicating the trophic status of the ecosystem to be net heterotrophic i.e. NEP is negative. In the case of coastal waters physical factors operating at this station could also play a role in regulating NEP as it has been shown in Tomales Bay where coastal upwelling led to net heterotrophy (Smith and Hollibaugh 1997).

The data on NEP in the present study were corroborated with apparent oxygen utilization (AOU) and partial pressure carbon dioxide (pCO_2) measurements. AOU values indicated undersaturation of oxygen in the estuarine system, indicating the supersaturation of carbon dioxide. Sarma et al. (1998) reported high pCO_2 value of 1153 μatm (at salinity <10), which is three times higher to that in atmosphere during the southwest monsoon season in the

Mandovi-Zuari estuarine system. High pCO_2 and low pH values during the monsoon coincided with high respiration rates indicating the system to be net heterotrophic. This net heterotrophy was evident from increased flow of organic matter by riverine discharge fuelling bacterial activity as seen from previous chapters (Chapter 5 and 6). This high pCO_2 build up could be due to the bacterial degradation of allochthonous carbon (del Giorgio and Cole 1998). Significant relationship ($p<0.01$) between bacterial productivity and R was recorded in the coastal and Zuari estuary indicating that bacterioplankton may be responsible for a significant fraction of total respiration. Moreover laboratory studies as discussed earlier suggest that bacterioplankton contributed more than 70% of the total community respiration.

As no relationship between P and R could be seen, the observed R might have been fuelled by other sources in addition to phytoplankton photosynthesis. The observed uncoupling between P and R could potentially be due to the flow of organic carbon from autochthonous and allochthonous sources (Howarth et al. 1996). Similar observations during spring bloom in South San Francisco Bay (Caffrey et al. 1998) and in the Bay of Biscay (Serret et al. 1999) have also been reported. Pomeroy and Wiebe (1993) stated that a net heterotrophic water column is possible not only because of temporary imbalance of photosynthesis and consumption but also because of organic inputs from rivers and atmospheric fallouts. Uncoupling between P and R could also be due to potential time lag, usually less than a month in length and they should not substantially complicate the interpretation of heterotrophic periods in this study (Hopkinson 1985).

Heterotrophy in the coastal waters as observed from our study could indicate slow degradation of organic matter accumulated over decades from either residual autochthonous or allochthonous matter. Azam et al. (1994) observed net heterotrophy during the inter-monsoon season in highly productive Arabian Sea and attributed it to the slow degradation of dissolved organic carbon pool.

A significant relationship was observed between NEP and P at the estuarine stations (Fig. 8.4). The trend showed that NEP become more positive with increasing P, indicating autotrophy i.e. organic matter production is more than consumption. These data are reasonably well represented by the geometric regression equation

NEP = 69.95 ln(P) - 275.21 (Fig. 8.4).

Comparing NEP estimated in Mandovi-Zuari system with values reported for other estuarine ecosystems reveals several interesting relations. Smith and Hollibaugh (1993) suggested an inverse relationship between NEP and P to heterotrophic metabolism and that they are the most productive ecosystem. They also suggested that nutrient delivery to the estuaries was derived from terrestrial organic matter inputs. However in the present study positive relationship between P and NEP indicated that organic inputs from terrestrial sources were negligible in the non-monsoon season. Previous studies in this estuarine system indicate that the riverine discharge during the non-monsoon months is insignificant (Qasim and Sengupta 1981). The coastal station did not show any significant relationship between NEP and P, indicating that this region is supplied with more organic inputs than inorganic. This station showed a general trend of increasing

net heterotrophy (i.e. P-R becoming more negative) indicating that more organic matter is oxidized than what is produced or available. This confirms that the excess organic matter in the coastal station came from the estuarine region, which showed net autotrophy. On an annual basis, in the estuarine systems 65% of the values indicated autotrophy while the remaining indicated net heterotrophy (Fig. 8.4). Like in most of the aquatic systems, the present study indicates that the nutrient supply, which supports P, is essentially derived from recycling. This led to the finding that respiration is primarily supported by the phytoplankton productivity (Garside and Malone 1978, Ittekot and Laane 1991).

NEP of any aquatic ecosystem depends on the ratio of inorganic to organic inputs (I_{inorg}:I_{org}) with high ratios favoring autotrophy and lower ratios favoring heterotrophy. Cole et al. (2000) demonstrated the shift in the trophic status from heterotrophy to autotrophy in mesocosm experiments with addition of inorganic nutrients conducted in lakes of Wisconsin (USA) indicating the significance of I_{inorg}:I_{org}. Increased inputs of inorganic nutrients to estuarine ecosystem tend to stimulate P and NEM. This has been demonstrated clearly in inorganic enrichments studies with experimental coastal marine ecosystems, where NEP increased from balanced metabolism under low nutrient river to 100 g C m^{-2} y^{-1} (Oviatt et al. 1986). Chesapeake Bay and Narragansett Bays (USA) represent examples of temperate estuaries generating substantial positive NEP from the large input of inorganic nutrients received via agricultural land runoff and wastewater discharges. The inorganic nutrient concentrations were higher in the

estuarine than in coastal waters, which could have led to NEP being positive with increase in P.

Annual integrated data indicated the persistence of net autotrophy (P:R=1.46) in the estuaries and net heterotrophy (P:R=0.33) in the adjacent coastal waters. This suggests that the organic matter have ultimately exported or transported hydrologically to adjacent coastal region thereby proving the 'outwelling' hypothesis or 'coastal heterotrophy' proposed by Odum (1968). The outwelling hypothesis presumes that NPP of estuaries greatly exceeds local degradation and storage of carbon, and that the excess organic matter is exported to the adjacent ocean where it is finally degraded and incorporated into the offshore food web. Similar observation pertaining to ours has been reported by Hopkinson (1985) in the Georgia Bight, where evidence supports the estuarine outwelling hypothesis proposed by Odum (1968).

It is evident that the coastal waters are net consumers of organic matter rather than producers and release inorganic nutrients. It has been reported that net heterotrophy leads to release of dissolved inorganic phosphorous to the ocean and dissolved inorganic carbon to the atmosphere (Smith and Hollibaugh 1993, Sarma et al. 2001). R>P in the coastal region implies that the system was not in a steady state during the year as suggested by Vezina and Savenkof (1999) and that the heterotrophic bacteria which have been shown to be the dominant component in this tropical ocean were consuming dissolved organic matter either accumulated at a previous time or advected into the area.

8.4 CONCLUSION

1. Pronounced seasonality in the trophic status was observed in the estuarine than at the adjacent coastal station with a shift from autotrophy in non-monsoon seasons to hetrotrophy in monsoon season.
2. Persistence of net autotrophy at the estuarine stations was linked to high inorganic nutrients and net heterotrophy at the coastal station was due to high organic matter.
3. Seasonal changes in the NEP at the estuarine stations reflect changes in the pathways of energy flow in these shallow coastal ecosystems.

Chapter 9

ACTION PLAN

Understanding and predicting how human activities affect ecosystem structure and function is major challenge for the science in ecology. Of all ecosystems 'coastal ocean' are considered to be important because they are the most vulnerable to human activities (Holligan and Reiners 1992), as a result managing their resources becomes very critical in today's text. Hetrotrophic bacteria are the most abundant and biological component involved in transformation and mineralization of organic matter in aquatic system. They exhibit tremendous versatility not only in surviving in extreme environments but also in their contribution to biotechnology.

My study was able to demonstrate that bacterial growth was limited by inorganic nutrients in the estuaries. This implies that any physical perturbations such as massive release of inorganic nutrients trapped in sediments during storm processes or due to human perturbations would lead to a shift in the trophic structure of the system. The above could thus lead to eutrophication, a feature, which should be kept in mind in the future management of economic development plans of Mandovi-Zuari estuarine system. Studies regarding inorganic nutrients in this tropical coastal ocean are patchy and data regarding inorganic inputs over a long period of time (several decades) has not been recorded. As a result, the trend in the loading pattern over a period cannot be ascertained.

Bioremediation is particularly dependent on factors, which influence the metabolic capability of the appropriate microorganisms e.g. the prevailing environmental conditions. Moreover as the total viable bacterial population (CFU)

is greater than 10^6 g^{-1} dry weight sediment this estuarine system offers favorable environmental conditions for bioremediation studies. It is generally accepted that the addition of N and P offers a great promise as a counter measure against oil spills (Prince 1993) and the ratios of C, N and P to support and optimal oil degradation have been defined (Bragg et al. 1994, Venosa et al. 1996). As these estuarine waters are limited by inorganic nutrients i.e. N and P, bioremediation measures could be possible particularly in case of oil spills.

Study on the response of these organisms to know labile organic compounds validate the fact that they are able to utilize a wide variety of compounds. They also exhibited an array of enzyme activities. Survival strategies of bacteria in aquatic environment will have great implication in water quality assessment. More knowledge is necessary to understand the relation between the waste load and bacterial growth. An important problem is in what way activity and development of heterotrophic microorganisms are influenced by wastewater of different quantity and quality. Thus a better understanding of the responses of coastal ecosystem to anthropogenic nutrient loadings would be useful in understanding and managing a very practical problem.

The high BGE in the estuarine than coastal region as observed in the present study suggest bacteria to be a better vector in the export of organic carbon to higher trophic levels. del Giorgio and Cole (2000) suggested that although the BGE could be low, the amount of carbon transported to higher level would still be sufficient to support higher organisms.

Knowledge on the trophic status of the system i.e. net autotrophy or heterotrophy is very essential. NEP in the photic zone of pelagic estuarine region also produces particulate organic matter, which is available to sink to the bottom waters and support oxygen consumption. As net production of an estuarine ecosystem (NEP) increases, so does it biomass, which represents increased availability of food to support fisheries harvest from the ecosystem. As indicated earlier (Chapter 8), the NEP of estuarine systems depends largely on the ratios of inorganic to organic (I_{inorg}:I_{org}) inputs with high ratios favoring autotrophic and lower ratios favoring heterotrophy. The present and previous studies carried out so far in this estuarine system indicated the persistence of comparatively high inorganic nutrients at the estuarine stations when compared to adjacent coastal region. These general trends of increased inorganic nutrient loading with higher ratio of I_{inorg}:I_{org} inputs would likely to lead to general increase in NEP in this tropical coastal ocean. As seen from the previous chapters, bacteria were responsible for significant consumption of dissolved oxygen in degrading organic matter, especially in the pre-monsoon summer months. These observations on carbon balance for this tropical Mandovi–Zuari estuarine system have implications regarding potential strategies for managing coastal resources. While waste management efforts in industrial regions focused initially on reducing the inputs of organic carbon to coastal waters, present concerns are aimed primarily at removal of inorganic nutrients; however, both can contribute to oxygen depletion (Officer and Ryther 1977). It appears that resource managers are faced with the inevitable trend of increasing NEP in coastal ecosystems worldwide. By

determining the input rates of nutrients and through appropriate management practices, unfavorable conditions leading to events like pollution can be effectively controlled. It is necessary to develop strategies for fostering the associated increased production of fisheries population, while attempting to mitigate the potential detrimental effects of anoxia and resulting lost habitat for demersal species (Kemp et al. 1997).

Chapter 10

SUMMARY

In the framework of the present concern for future global environmental changes, the primary interest is to evaluate the relative strength of the various causes for high production in the coastal zone. Although, it is impossible to eliminate the influence of anthropogenic activities on the modification of natural fluxes and processes, at least its possible effects could be visualized. The primary goal of the present study was to have a sound basic knowledge of the microbial processes in the tropical estuarine system and to form a forum for predictive advice. Bacteria being the prime mediators of all biogeochemical cycles, information on their activity is a pre-requisite for predicting changes in systems. This study will highlight the response of bacterioplankton to natural and anthropogenic environmental changes and also for the management of marine resources.

The present study was carried out for a period of two years in the tropical Mandovi-Zuari estuarine system (Arabian Sea), which is one of the largest riverine networks in the southwest coast of India. This system is characterized by extreme wind forcing from seasonal reversal of monsoon winds, which makes it an interesting region to study and evolve strategies required for managing marine resources.

Hydrological study of the three study stations showed that the estuary is well mixed throughout the year except in the monsoon season wherein the stratification is caused by salinity gradient. Inorganic nutrient concentration was higher at the estuarine stations when compared to the adjacent coastal station. The organic matter showed low particulate C:N ratio (7-8) in the pre- and post-

monsoon season and high ratio (>10) in the monsoon season at the estuarine stations, which were indicative of biogenic and terrestrial sources of matter. Coastal station was less dynamic than the estuarine stations, as it did not show any marked variability in hydrological parameters. In this station, the dissolved organic carbon was significantly higher (two fold) than particulate organic carbon. Like hydrological parameters, biological variables also indicated that the estuary was more dynamic than the adjacent coastal regions.

The estuarine system showed the dominance of bacteria over phytoplankton biomass suggesting that they could contribute significantly to the nutrient turnover. Lack of strong relationship and uncoupling between bacterial (BP) and primary productivity (PP) in the Zuari and Mandovi estuaries indicate the importance of exogenous input in fuelling bacterial activity. Significant positive relationship between BP and PP in the coastal station indicated PP to be a prime source of organic matter to support bacterial carbon demand. Among all exo-enzymes, phosphatase showed the highest activity. In the present study bacteria belonging to the division C of Proteobacteria, subdivision C3: Gamma class dominated. 68% of isolates belonged to genus *Pseudomonas* that is known to take part in nutrient recycling and biodegradation.

Bacterial growth efficiency (BGE) was low in this tropical ecosystem indicating that a greater percentage of substrate is respired for cellular maintenance. The BGE values for estuarine and coastal waters were 18($\pm$7.84%) and 11($\pm$4.19%), respectively. The low BGE was due to substrate limitation as both field and laboratory investigation showed inverse relationship with C:N ratio

of organic matter. The observed variation in BGE was attributed to bacterial productivity. Absence of significant relationship between bacterial productivity and abundance in the field investigation and difference in BGE between water samples with and without grazers in the laboratory experiments indicated that grazing could be one of the factors regulating BGE. Nutrient limitation studies indicated inorganic nutrients limited bacterial growth in the estuarine water and organic carbon in the coastal water.

Seasonal variability in the carbon flux was more at estuarine stations than in coastal waters. Low BP:PP ratio in the non-monsoon season indicated that sufficient material was available for higher trophic levels. PP supported 53 to 86% of the bacterial carbon demand in the non-monsoon. However, >100% in the monsoon season was linked to exogenous supply of organic matter.

In the estuarine stations, net ecosystem production showed monthly variation and a transition from net autotrophy of 49 mmol C m^{-2} d^{-1} during the non-monsoon seasons (pre and post monsoon) to net hetrotrophy of –46 mmol C m^{-2} d^{-1} in the monsoon season. Seasonal monsoon driven changes such as increased allochthonous inputs resulted in enhanced heterotrophic respiration and reduced primary production in the estuaries. In the coastal station, the monthly variation in net ecosystem production was not significant and net heterotrophy was prevalent whenever measurements were made, thereby serving as net source of carbon dioxide to atmosphere. These results provide evidence to suggest that the excess organic matter from these tropical estuaries support heterotrophy in the adjacent coastal ecosystem.

Comparatively higher inorganic nutrients persisted at the estuarine stations when compared to adjacent coastal region. Based on the approaches adopted in the present study the general trends of increased inorganic nutrient loading with higher ratio of I_{inorg}:I_{org} would lead to better understanding of the impact of eutrophication in marine systems. The present study links the biological investigations to physical processes and bears significance to tropical estuaries, which are prone to eutrophication.

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LIST OF PUBLICATIONS

Published/Accepted

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2. **Pradeep Ram AS**, Loka Bharathi PA, Nair S, Chandramohan D (2001) Bacteria blamed for high nitrate level. Nature News India, October, pp. 11
3. **Pradeep Ram AS**, Loka Bharathi PA, Nair S, Chandramohan D (2001) A deep-sea bacterium with unique nitrifying property. Current Science 80: 1222-1224
4. **Pradeep Ram AS**, Balasubramanian T (2000) Bacterial flora associated with a commercial shrimp feed. Fishing Chimes 20: 26-28

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1. **Pradeep Ram AS**, Nair S, Chandramohan D. Seasonal shift in net ecosystem production in a tropical estuary -Limnology and Oceanography (under revision)