

**SOME ASPECTS OF THE NITROGEN CYCLE IN  
MANGROVE AND ESTUARINE WATERS**

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by

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**May 2000**

## **STATEMENT**

As required under the University Ordinance 0.19.8 (iv), I hereby state that the present thesis entitled '**SOME ASPECTS OF THE NITROGEN CYCLE IN MANGROVE AND ESTUARINE 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.



**ANJALI MENEZES HEREDIA**

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This is to certify that the thesis entitled '**SOME ASPECTS OF THE NITROGEN CYCLE IN MANGROVE AND ESTUARINE WATERS**' submitted by Anjali Menezes Heredia for the award of the degree of Doctor of Philosophy in Marine Science is based on original studies carried out by her under my supervision.

The thesis or any part thereof has not been previously submitted for any other degree or diploma in any Universities or Institutions.

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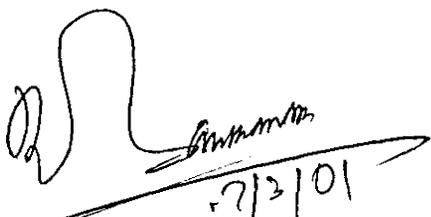


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## ABSTRACT

This study focused on nutrient dynamics in mangrove and estuarine waters. The seasonal changes of N nutrients ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and urea), regeneration rates (ammonium and nitrite) and uptake rates of N nutrients ( $p\text{NO}_3^-$ ,  $p\text{NO}_2^-$ ,  $p\text{NH}_4^+$  and  $p\text{urea}$ ) were investigated in a mangrove ecosystem on the west coast of India during 1997-1998. Nitrate was the major form of N in the dissolved pool (72%), followed by ammonium (16%), and nitrite and urea (6% each). The changes of nutrient concentrations followed clear seasonal cycles. In the case of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  advection with freshwater in monsoon and *in situ* biological processes during the rest of the year controlled concentration changes. In the case of reduced forms ( $\text{NH}_4^+$  and urea), they were solely regulated by biological *in situ* production rates. The gradual increase in concentrations of the reduced forms ( $\text{NH}_4^+$  and urea) in the dry months (from post to pre-monsoon) was mainly due to intense microbial decomposition of organic matter.

Particulate organic nitrogen (PON) concentrations showed clear seasonal variations and ranged from 12.1 to 207.9  $\mu\text{g at N l}^{-1}$ . Litterfall appeared to be the major source of PON to the water column. Phytoplankton contribution to the total PON was comparatively less significant than litterfall. Unlike that of PON, the chlorophyll *a* followed a different seasonal trend and concentrations ranged from 0.1 to 21.6  $\mu\text{g chl a l}^{-1}$ . The phytoplankton biomass was high in the dry season (pre-monsoon: 9.9 and post-monsoon: 10.2  $\mu\text{g chl a l}^{-1}$ ) compared to the monsoon season. The cumulative effect of low salinity, low irradiance, turbidity and high water currents reduced phytoplankton biomass in the monsoon season. However, in the dry season, the increased insolation, water clarity and water stability along with adequate supply of dissolved N nutrients enhanced phytoplankton biomass. The phytoplankton community structure and composition changed significantly with the change in salinity and nutrient availability. The microphytoplankton were abundant at low salinity (10-25 PSU) and high  $\text{NO}_3^-$  availability whereas nanophytoplankton were abundant at high salinity (>30 PSU) and when  $\text{NH}_4^+$  concentrations were adequate (>0.3  $\mu\text{g at NH}_4^+\text{-N l}^{-1}$ ).

*Trichodesmium erythraeum* occurred in bloom formations in the month of May during two consecutive years.

Ammonium was the major nutrient utilized by phytoplankton (60%), by  $\text{NO}_3^-$  (29%), urea (7%) and nitrite (4%). Ranges in absolute uptake rates were 4 to 1194  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$  with  $\text{NH}_4^+$ , 2 to 368  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$  with  $\text{NO}_3^-$ , 1 to 94  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$  with  $\text{NO}_2^-$  and 0 to 63  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$  with urea.

The seasonal patterns of uptake rates were distinct, with a dominance of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  uptake in the post-monsoon, followed by dominance of  $\text{NH}_4^+$  and urea uptake in pre-monsoon. The high and prolonged utilization of  $\text{NO}_3^-$  at the beginning of the productive season was due to strong allochthonous supply of  $\text{NO}_3^-$ , dominance of microphytoplankton and low rates of  $\text{NH}_4^+$  regeneration. The relation of N uptake with chl *a* showed that heterotrophs may take up all 4 nutrients and that they could account for half of the total N uptake in a year.

Ammonium (10-1500  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$ ) and nitrite production (0.1-96.7  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$ ) rates were among the highest known for nearshore waters and showed clear seasonal patterns. Production and utilization of  $\text{NH}_4^+$  were closely coupled throughout the year. Nitrite production rates related with  $\text{NH}_4^+$  production rates in a rectangular hyperbolic fit. The *in situ* production ( $\text{NH}_4^+$  and  $\text{NO}_2^-$ ) provides on an average 20% more N than assimilated by plankton.

Efflux from the sediments to the overlying waters also regulates aquatic primary production and therefore, benthic fluxes of N should be even more important than water column fluxes.

**Key words:** nutrient, phytoplankton, community, uptake, regeneration and nitrification

## CONTENTS

	<b>Page No.</b>
<b>Chapter – I: INTRODUCTION</b>	<b>1</b>
<b>Chapter – II: MATERIAL AND METHODS</b>	
<b>2.1 Description of the study area</b>	<b>21</b>
2.1.1 Distribution of mangrove species	21
2.1.2 Climatological features	22
2.1.3 Choice and description of stations	23
<b>2.2 Sampling programme and processing</b>	<b>24</b>
<b>2.3 Analytical techniques</b>	<b>27</b>
2.3.1 Hydrological parameters	
2.3.1.1 Temperature	27
2.3.1.2 Salinity	27
2.3.1.3 Dissolved oxygen	28
2.3.2 Biological parameters	28
2.3.2.1 Chlorophyll -a	28
2.3.2.2 Phytoplankton cell counts and species composition	29
2.3.3 Ambient nitrogen concentrations	30
2.3.3.1 Nitrate	30
2.3.3.2 Nitrite	30
2.3.3.3 Ammonium	30
2.3.3.4 Urea	31
2.3.3.5 Particulate organic nitrogen (PON)	32
<b>2.4 Tracer techniques</b>	<b>34</b>
2.4.1 Nitrogen uptake studies	34
2.4.1.1 Unfractionated uptake	34
2.4.1.2 Size-fractionated uptake	35
2.4.2 Measurement of ammonium regeneration rates	36
2.4.2.1 Principle	36
2.4.2.2. Extraction of ammonium by direct diffusion	37
2.4.3 Measurement of nitrification rates	39
2.4.3.1 Experimental protocol of nitrite extraction	39

2.4.4	Isotopic analyses of emission spectrometry	41
2.4.4.1	Principle of detection	42
2.4.4.2	Preparation of samples for emission spectrometry	42
2.4.4.3	Calculations	44
2.5	Litterfall studies	47
 <b>Chapter -III: RESULTS</b>		
3.1	Hydrological parameters	48
3.1.1	Temperature	48
3.1.2	Salinity	48
3.1.3	Dissolved oxygen	49
3.2	Ambient nitrogen concentrations	49
3.2.1	Nitrate	49
3.2.2	Nitrite	51
3.2.3	Ammonium	52
3.2.4	Urea	54
3.2.5	Particulate organic nitrogen	55
3.3	Biological parameters	57
3.3.1	Chlorophyll-a	57
3.3.2	Chlorophyll -a of size fractions	58
3.3.3	Phytoplankton cell counts and species distribution	59
3.4	Regeneration of nitrogen	63
3.4.1	Ammonification rates	63
3.4.2	Nitrification rates	65
3.5	Nitrogen uptake studies	66
3.5.1	Unfractionated uptake	66
3.5.1.1	Nitrate	66
3.5.1.2	Nitrite	70
3.5.1.3	Ammonium	73
3.5.1.4	Urea	76
3.5.2	Size-fractionates uptake	80
3.5.2.1	Nitrate	80
3.5.2.2	Nitrite	83
3.5.2.3	Ammonium	87

3.5.2.4 Urea	90
<b>3.6 Salient features of results</b>	<b>94</b>
3.6.1 Ambient nitrogen concentrations	94
3.6.2 Biological parameters	96
3.6.3 Regeneration of nitrogen	97
3.6.3.1 Ammonification rates	97
3.6.3.2 Nitrification rates	98
3.6.4 Uptake studies	
3.6.4.1 Unfractionated uptake	98
3.6.4.2 Size-fractionated uptake	99
<b>Chapter – IV: DISCUSSION</b>	
<b>4.1 Ambient nitrogen</b>	<b>101</b>
4.1.1 Nitrate	101
4.1.2 Nitrite	104
4.1.3 Ammonium	108
4.1.4 Urea	113
4.1.5 Particulate organic nitrogen (PON)	118
<b>4.2 Biological parameters</b>	<b>123</b>
4.2.1 Chlorophyll –a and phytoplankton taxonomic composition	123
<b>4.3 Regeneration of nitrogen</b>	<b>131</b>
4.3.1 Ammonification rates	131
4.3.2 Nitrification rates	137
<b>4.4 Nitrogen uptake studies</b>	<b>142</b>
4.4.1 Introduction	142
4.4.2 Nitrate uptake	156
4.4.3 Nitrite uptake	164
4.4.4 Ammonium uptake	170
4.4.5 Urea uptake	176
<b>Chapter -V: INTEGRATED DISCUSSION</b>	<b>181</b>

**BIBLIOGRAPHY**

## CHAPTER I

### INTRODUCTION

The diversity of ecosystems of the global world ocean is as much varied, if not greater than, as in the terrestrial domain. In a latitudinal axis, the oceanic realm can be broadly divided into boreal, temperate, sub-tropical and tropical waters. On the depth scale, the euphotic waters which occupy a thin layer of a few dozens of meters thickness but nevertheless support all of the autotrophic production of organic matter, yield progressively to the larger pelagic ecosystem, followed by mesopelagic, bathypelagic and abyssal environments.

Within these broad classes, the diversity is still large and its extent is a function of the temperature cycle. While the boreal waters are practically monotonous, the temperate waters sustain a relatively larger suite of ecosystems. These include seasonally stratified coastal and oceanic waters, permanently well-mixed waters, temperate rocky and sandy beaches, sea grass meadows and estuaries. However, the degree of diversity is the largest in the tropical waters - productive coastal waters, oligotrophic oceans, upwellings - both coastal and equatorial, coral reefs, monsoonal estuaries, mangroves, high saline lakes, lagoons, rocky and sandy beaches, and sea grass ecosystems.

Notwithstanding the diversity, tropical marine ecosystems differ from temperate or boreal ecosystems in two important characteristics. Not only do they sustain high levels of biological productivity throughout the year in chronically nutrient-limited situations, but are also structurally and functionally different, enabling them to harbour a highly varied biodiversity. In the tropics, a substantial proportion of the coastlines is occupied by highly productive mangroves (Boto *et al.*, 1984; Por, 1984). These ecosystems, besides supporting a unique assembly of animals and plants, act as an important linkage and buffer zone between the land and ocean (Morell and Corredor, 1993). They are also sites of high production of organic matter that not only sustain a large secondary food chain within, but also influence, by export, the biological productivity of adjacent waters including their fisheries (Robertson, 1987; Robertson and Alongi, 1992; Wafar *et al.* 1997). The extent to which mangroves exchange dissolved and particulate nutrients with adjacent ecosystems depends upon several factors, including geomorphology, tidal regime, climate and freshwater advection.

Studies on primary production in mangroves have generally been restricted to leaf photosynthesis and litter production rates. These range from 1 to 2 g C m<sup>-2</sup> d<sup>-1</sup>, as a function of the age of the stand and its geographical location, leading to the generally accepted conclusion that the terrestrial component is important for the primary productivity of mangroves (Wafar *et al.*, 1997). In contrast, studies on productivity of aquatic vegetation in mangroves are rather

few. The mangrove waters being normally very turbid, are generally considered to contain low populations of phytoplankton (Gong, 1984). Nevertheless, assessments on aquatic primary production are subject to speculation - there are reports of fairly high values for such production (Krishnamurthy *et al.*, 1975). Phytoplankton production has been found to vary from 11 to 91 mg C m<sup>-3</sup> h<sup>-1</sup> (Tundusi, 1969; Teixeira *et al.*; 1969; Untawale *et al.*, 1977; Pant *et al.*, 1980). However, Sundararaj and Krishnamurthy (1973) reported net phytoplankton production of about 525 mg C m<sup>-3</sup> h<sup>-1</sup> from the Pichavaram mangroves of India.

In tropical coastal marine ecosystems, nutrient levels are generally low (Qasim and Wafar, 1990) and productivity is almost solely regulated by the supply of nitrogen (Caperon and Meyer, 1972; Eppley *et al.*, 1979a; McCarthy and Goldman, 1979). The importance of nitrogen (N) in the marine environment and the role it plays in the biological productivity cycles have been the subject of 50 years of research. Though continuous, the evolution of research in this field has been marked by several turning points. To cite some of them: N is assimilated in the same ratio to phosphorus in which it occurs in the sea and in the phytoplankton cells (Harvey, 1957); the concept of Redfield ratios (Redfield *et al.*, 1963); ammonium and urea, besides nitrate, are major N nutrients (Vaccaro, 1963; McCarthy, 1972); development of the <sup>15</sup>N tracer method (Neess *et al.*, 1962) and the concept of 'new' and 'regenerated' production (Dugdale and Goering, 1967); concept of f-ratio and export

production (Eppley and Peterson, 1979); N recycling through the microbial loop (Harrison, 1991) and finally, the usefulness of the f-ratio in global biogeochemical models and as a diagnostic tool of the state of the ecosystem (Platt *et al.*, 1991).

Progress in understanding the processes by which nitrogen is imported into the marine environment and assimilated/regenerated has also been substantial (Carpenter, Capone, 1983). The major processes involved are autotrophic assimilation, nitrogen fixation, ammonification, nitrification and denitrification besides the physical ones such as advection and diffusion. In almost all of the marine ecosystems, more than one process operates at any level and at anytime. Quantification of these is relatively easier in ecosystems such as the open sea where the food chain operates along the phytoplankton  $\Rightarrow$  zooplankton  $\Rightarrow$  herbivore  $\Rightarrow$  carnivore hierarchy and where most of the processes are spatially decoupled. For example, assimilation of nitrogen occurs in the euphotic zone whereas, ammonification of the particulate organic nitrogen and nitrification occur below. Similarly, diffusion of N occurs across the thermocline into the euphotic zone but advection is generally unimportant.

In nearshore ecosystems, the pattern of nitrogen cycles becomes more complex. Advection from terrestrial sources can add nitrogen at varying intervals and quantities thus perturbing the *in situ* nitrogen fluxes. The

multiplicity of the producer components – phytoplankton, microphytobenthos, macroalgae, autotrophic bacteria and their preference for one or the other form of nitrogen can influence N assimilation patterns. In a similar way, the shallow depths can cause an overlap of processes of assimilation and remineralization, and the export flux, instead of the sinking pathway as in the open ocean, will be controlled by coastal currents and tidal oscillations.

### **Sources of Nitrogen in mangrove and estuarine waters**

Sources of nitrogen in mangrove ecosystems include atmospheric, riverine, marine, sedimentary influx, inputs through biological processes and through human activities.

The bacterial degradation of organic matter leads to the production of ammonia which, when volatile, escapes into the atmosphere and its short residence time (Holland, 1978) makes it easily returnable to the system through rainfall. Nitrate from the atmosphere is also removed by rainfall. Input through fresh water runoff is an important source of nitrogen (Qasim and Wafar, 1990), transporting nutrients in dissolved and particulate phases.

Nitrogen from the marine sources is regulated by hydrodynamic processes, mainly driven by tides. Hydrodynamic processes influence the productivity of marine ecological systems through nutrient and material exchanges between coastal and marine ecosystems (Kjerfve *et al.*, 1981; Wolanski *et al.*, 1980).

Studies carried out by Johnson (1996) in a tropical mangrove-dominated bay showed that hydrodynamic patterns are driven by tides that generate strong reversing tidal currents, winds and alongshore currents generated by wave breaking. These processes facilitate nutrient exchanges and promote the coastal trapping of turbid brackish water and its inherent nutrient content.

Estuarine and coastal sediments add nitrogen to the overlying waters and are a significant source of inorganic nitrogen made available for pelagic primary production (Boynton *et al.*, 1980; Billen and Lancelot, 1988). These fluxes are largely driven by diffusion (Jorgensen and Revsbech, 1985), advection (Riedle *et al.*, 1972) or sediment resuspension (Sondergaard *et al.*, 1992). Earlier reports on nutrient exchanges between mangrove sediments and overlying waters indicate low rates of nutrient influx from mangrove sediments (Kristensen *et al.*, 1988) and from sub-tidal sediments near mangroves (Alongi, 1990).

Biological processes through which nitrogen can enter into the dissolved pool of the system include bacterial remineralization of organic matter, metabolic activity of heterotrophs and nitrogen fixation.

The degradation of particulate organic matter (detrital material) plays an important role in the recycling of nitrogen in the marine environment as it contributes to the dissolved organic nitrogen pool. Although decomposition studies on mangrove litter have shown that percentage nitrogen increases and carbon:nitrogen ratio

decreases (e.g. Tam *et al.*, 1990), the metabolism of the microorganisms involved (bacteria and fungi) is poorly understood and further research is required.

Ammonium is the primary excretory product of nitrogen metabolism of microheterotrophs and can be a significant source of regenerated N in estuarine and coastal ecosystems. Direct measurements of ammonium excretion have not been carried out in mangroves.

Nitrogen fixation by bacteria and blue-green algae is yet another source of nitrogen in mangrove ecosystems where free nitrogen is fixed by these organisms to form ammonia. Although a relatively well studied process in mangroves (e.g. Iizumi, 1986; Mann and Steinke, 1989; Boto and Robertson, 1990), the rates are low compared to other estuarine and marine habitats (Capone, 1983).

Nitrogen supplied through human activity includes those derived from the use of nitrogen fertilizers and use of mangrove areas as sewage dumps. The burning of oil and coal in industrial areas close to mangrove ecosystems is a source of ammonia in the atmosphere.

### **Nitrogen in mangroves**

In general, the dynamics of nitrogen in tropical waters and sediments have been poorly studied compared with their temperate counterparts (Furnas,

1983) and most of the available information for the tropics are only from coral reef systems (D'Elia and Wiebe, 1990).

Dissolved nitrogenous nutrient fluxes are mainly influenced by physical and biological processes (Rendell *et al.*, 1997). This aspect has not been comprehensively studied in mangrove ecosystems, although a few measurements have been made. The variations in concentrations are associated with extent of fresh water and ground water input, degree of solar insolation, oxygen availability and standing stocks and productivity of phytoplankton and bacterioplankton (Alongi *et al.*, 1992). Dissolved nitrogen concentrations are high at the freshwater end of the mangroves during the intense monsoon season as in the mangroves of Southeast Asia, but they decrease with increasing salinity at the seaward end (Nixon *et al.*, 1984; Wong, 1984). Lowest concentrations are generally recorded in the pre-monsoon season, coincident with high rates of primary productivity (Sarala Devi *et al.*, 1983). Studies conducted by Boto and Wellington (1988) in the northern Australian mangroves showed that dissolved nitrogen was mainly influenced by tidal action. Few investigations have been carried out on dissolved organic nitrogen (DON) in mangrove and estuarine waters. In the Malaysian Creek waters, DON concentrations decreased with increasing salinity (Nixon *et al.*, 1984), while in the Pichavaram mangroves, South India, Balasubramanian and Venugopalan (1984) attributed the high DON

concentrations to chlorophyll-*a*, suggesting that decomposing plant matter may be responsible.

The fact that mangrove ecosystems influence coastal food webs is evident from the exchange of particulate and dissolved materials with adjacent marine ecosystems (Boto and Wellington, 1988; Morell and Corredor, 1993; Rivera-Monroy *et al.*, 1995a, b; Alongi, 1996). Most forests appear to exchange substantial amounts of nutrients (e.g. northern Australia: Alongi *et al.* 1992; Kenya: Hemminga *et al.*, 1994; Papua New Guinea; Robertson and Alongi, 1996; Mexico: Rivera-Monroy *et al.*, 1995a), but some do not (e.g. southwest Florida: Twilley, 1985). Moreover, as suggested by Wiebe (1989), mangroves are a sink for nutrients. In Terminos Lagoon, Mexico, Rivera-Monroy *et al.* (1995a) found that a fringe mangrove forest acts as a sink for  $\text{NH}_4^+$  and  $\text{NO}_2^- + \text{NO}_3^-$  and as a source of particulate N and DON, with rainfall and river discharge controlling the extent and direction of the flux.

#### **Utilization / loss of nitrogen in mangrove ecosystems**

Phytoplankton utilize dissolved inorganic (nitrate, nitrite and ammonium) and dissolved organic (urea and amino acids) nitrogen for their growth and hence production of new biomass. Besides the inorganic and organic nitrogen sources, dissolved gaseous nitrogen is also a possible source for autotrophic uptake. Studies by Dugdale *et al.* (1964), in the Arabian and Sargasso Seas

showed that *Trichodesmium* sp. fixed gaseous nitrogen besides utilizing ammonium and/or nitrate.

Phytoplankton abundance in estuaries is known to depend on physical and chemical factors such as light (Cloern, 1987), nutrients (Gallegos and Jordan, 1997), stability of the water column (Paerl *et al.*, 1998), turbidity (Cloern, 1987), river flow (Fisher *et al.*, 1992) and temperature (Pennock and Sharp, 1994). Of all these factors, nutrient availability has frequently outweighed others (Hendzel *et al.*, 1994). There are few studies concerning the interaction between supply and utilization of nutrients in estuarine systems. It is generally believed that the ambient nutrient concentrations are a balance between uptake and supply and uptake rates are mostly dependent on the availability of nutrients (Mengesha *et al.*, 1998).

Several methods have been used to identify the nutrient most limiting to photosynthetic production of organic carbon in estuaries (Paerl *et al.*, 1995; Pinckney *et al.*, 1997) and the most recognized growth-limiting nutrients are species of nitrogen and phosphorus, although silica is also important for growth limitation of diatoms (Roelke *et al.*, 1999).

Specific nutrient uptake rates vary as a function of the size structure and taxonomic composition of the community (Malone, 1980; Probyn, 1985). Numerous studies have shown that the development of phytoplankton

communities are mainly based on nitrate (Wafar *et al.*, 1983; Glibert and Garside, 1992; Dauchez *et al.*, 1996), while other investigations emphasize a major contribution of ammonium (Probyn and Painting, 1985; L'Helguen *et al.*, 1996). This apparent contradiction is partly related to variations in the supply of nutrients, size structure of the phytoplankton community and their nutrient preferences (Gallegos *et al.*, 1997; Levine *et al.*, 1997)

In mangrove ecosystems, studies on nutrient uptake are restricted to mangrove plants (*e.g.* Boto, 1992; Clough, 1992), and no reports exist to date on nutrient uptake by phytoplankton, which are also important primary producers.

### **Regeneration of nitrogen**

The importance of nitrogen recycling through the microbial community was first recognized by Azam *et al.* (1983) and later on in a number of studies (Harrison, 1991). The biologically mediated processes of ammonium regeneration (ammonification) and nitrification are the major transformations of the nitrogen cycle.

The nitrogen present in organic matter can be utilized by the autotrophs only after being remineralized. The regeneration of nitrogen can take place in two ways, either directly by excretion or indirectly by bacterial decomposition of organic matter. It is believed that microheterotrophs (organisms less than 200

$\mu\text{m}$ , mainly represented by protozoans, ciliates, naupliar and post-naupliar stages of copepods etc. and bacteria) contribute to much of this activity (McCarthy and Carpenter, 1983; Glibert, 1988a; Harrison, 1992).

Several studies showed that ammonium formed bulk of the total nitrogenous excretion products. For example, Jawed (1969) found that ammonium comprised as much as 82-85% of the total nitrogen excreted by the euphausiid, *Euphausia pacifica*. Similarly, Corner *et al.* (1976) found that ammonium accounted for 60 to 80% of the nitrogen excreted by the temperate copepod, *Calanus helgolandicus*. It is generally accepted that bacteria less than 1  $\mu\text{m}$  in size are associated with heterotrophic activity (Harrison *et al.*, 1977). The bacteria regenerate nitrogen from dissolved organic matter or from organic detritus. The microbial decomposition of detritus and DOM is an important pathway of release of ammonium in coastal and estuarine ecosystems. In mangroves, a major portion of the detritus being terrestrially derived, is unavailable to consumers, but serves as the substrate for the growth of microorganisms (bacteria and fungi).

Measurements of ammonium regeneration in mangrove ecosystems is restricted to mangrove sediments and the present study is the first of its kind in mangrove and estuarine waters. Direct measurements of ammonification rates have however been made in other habitats (Bidigare, 1983; Meyer and Schultz, 1985; Ip *et al.*, 1990). High rates of ammonification in mangrove

sediments were reported by Iizumi (1986) at Hinchinbrook Island (420 to 1820  $\text{ng N g}^{-1} \text{d}^{-1}$ ).

The ammonium produced as a result of mineralization of organic matter undergoes oxidation to nitrite and nitrate (a process called nitrification). All marine nitrifying bacteria are gram-negative, obligate chemolithotrophs (Kelly, 1971). The genera *Nitrosomonas* and *Nitrosococcus* mediate the first step of nitrification, while the *Nitrobacter*, *Nitrospira* and *Nitrococcus* are involved in the second step of the nitrification process. The process of nitrification is an important source of nitrite (the intermediary product) and results in the formation of a nitrite maximum beneath the photic zone in most tropical and temperate waters (Rakestraw, 1936). Later, and with the use of  $^{15}\text{N}$  techniques, it was concluded that this process was responsible for much of the nitrite in sub-surface waters (Wada and Hattori, 1972; Miyazaki *et al.*, 1973; 1975). Nitrification rates have not yet been measured in mangrove waters, although these have been estimated in mangrove sediments (Iizumi, 1986; Shaiful *et al.*, 1986).

Since ammonium is the substrate for nitrification, its availability is an important factor influencing nitrification rates. Several studies report that nitrification in deep ocean and coastal systems is faster at the interfaces due to the adequate supply of ammonium from the sediment (Hansen *et al.*, 1981; Ward *et al.*, 1984). However, nitrifiers appear to have a high affinity for the

substrate, as they are able to nitrify and grow at sub-micromolar substrate concentrations (Ward, 1986). Berounsky and Nixon (1993) observed a strong relationship between ammonium concentrations and nitrification rates along an estuarine gradient in Narragansett Bay.

In pelagic systems, light is an important factor influencing the distribution and magnitude of nitrification (Kaplan, 1983) and the activity of nitrifiers appears to be inhibited by light (Bock, 1965). Temperature also influences the growth of nitrifiers. It was reported that optimal activity of ammonium oxidizers took place at temperatures between 25 and 35 °C (Focht and Verstraete, 1977) and at low temperatures, nitrifiers do not grow (Horrigan, 1981). Oxygen serves as the terminal electron acceptor in the oxidation of ammonium and is incorporated directly into the substrate (Rees and Nason, 1966). However, marine nitrifiers can grow and oxidize their substrates at very low oxygen levels (Kaplan, 1983).

The oxidized forms of nitrogen produced *via* nitrification can be reduced to either ammonium (dissimilatory nitrate reduction to ammonium) or gaseous nitrogen, N<sub>2</sub> (denitrification). Dissimilatory nitrate reduction is the process in which nitrate is used by facultative and some obligate anaerobic bacteria as the terminal electron acceptor, substituting for oxygen under low oxygen conditions. This process has not yet been addressed to in mangrove ecosystems. On the other hand, reports on denitrification (a process mediated

by facultative anaerobes) in mangroves are many, though mainly concerned with sediments (e.g. Rivera-Monroy and Twilley, 1996; Corredor *et al.*, 1999). The processes of dissimilatory nitrate reduction and denitrification may not be significant in the present study since these waters are oxic and unpolluted.

### **Nitrogen cycle in mangroves**

The mangrove ecosystem can be distinguished into terrestrial, aquatic and benthic compartments. In mangrove ecosystems, the mangrove vegetation contributes to a major part of the primary production. Hence, terrestrial production forms a major source of chemical energy made available to the aquatic and benthic biota of the ecosystem and also regulates the processes involved in the cycling of nitrogen within. A diagrammatic representation of nitrogen cycling in mangrove and estuarine waters is given in Fig.1.1.

- Particulate organic matter is mainly contributed by the fall of litter products, the autotrophs (phytoplankton) and heterotrophs (macrozooplankton, microzooplankton and necton).
- A part of this organic matter is buried into the sediments or is consumed by the heterotrophs.
- The particulate organic matter undergoes microbially-mediated decomposition to form a highly nutritive biomass (detritus).
- This decaying particulate matter is an important source of food to the detritivores (microzooplankton:flagellates, ciliates) and a major source of



dissolved organic nitrogen that is finally remineralized to ammonium (ammonification).

- The heterotrophs are responsible for the direct regeneration of ammonium and urea.
- The ammonium regenerated (by decomposition of organic matter and metabolic activity of heterotrophs) undergoes oxidation to nitrite and nitrate (nitrification).
- The oxidized forms produced as a result of nitrification may get reduced under conditions of low oxygen to either ammonium (dissimilatory nitrate reduction) or nitrous oxide/gaseous nitrogen (denitrification).
- Ammonium produced is reassimilated and gaseous nitrogen either diffuses into the atmosphere or is utilized by nitrogen fixers (bacteria and blue-green algae) to produce organic matter.
- The nitrogenous nutrients (nitrate, nitrite, ammonium and urea) produced are utilized by phytoplankton and mangroves for their growth and produce new biomass.
- There is also exchange of dissolved nutrients between the sediment and the overlying waters.
- Since a mangrove ecosystem is an "open" system, there is a continuous exchange of materials and energy with the open sea/adjacent systems.

## **Aims and objectives**

The output of research on nitrogen during the last twenty years is copious, but when one looks at it objectively, the imbalance between temperate and tropical waters is strikingly evident. Most of the results, hypotheses and models are from temperate waters, whereas, the greatest diversity and biological productivity are from tropical ecosystems. There is clearly a need to enhance our understanding of the nitrogen economy of tropical ecosystems and this is especially important with respect to mangroves.

In mangrove ecosystems, the N flux is partitioned between terrestrial, aquatic and benthic compartments. The flux processes in each of these compartments and their interrelationships contribute to the functioning of the mangrove ecosystem in its entirety. Thus, quantifying the flux processes becomes essential for an assessment of the productivity of these highly specialized and productive ecosystems. Hence, an understanding of N cycling will be of immense application in rational use and management of the biological resources from mangroves.

It may be noted that earlier studies on productivity and energy flows mainly utilized carbon as the tracer. However, as carbon is already abundant in the environment, it can hardly provide an understanding of the nutrient regulation of biological processes. Neither would phosphorus be of use, since it is regenerated rapidly. On the other hand, nitrogen recycling is a step-wise

process, enabling one to gain a good understanding of the influence exerted by nitrogen on biological productivity at all spatio-temporal scales.

The N budget has been studied for the Hinchinbrook mangroves in northern Australia (Alongi *et al.*, 1992). However, this budget does not consider the role played by the phytoplankton and its impact on the dissolved nutrient pool. Also, the processes of ammonification and nitrification in the aquatic compartment of mangroves have not been quantified. Besides, such a budget lacks precision - the flux rates are deduced from concentration changes over time, whereas, the appropriate method would be the use of tracers. The use of the stable isotope tracer,  $^{15}\text{N}$  has not been applied to the mangrove ecosystem so far.

The present study focuses on the use of  $^{15}\text{N}$  as the tracer to quantify nitrogen flux rates in mangrove and estuarine waters and aims at understanding the seasonal and spatial variations in nitrogen fluxes with respect to physical and biological processes.

This study has been conducted with the following objectives:

- ◆ Description of seasonal and spatial variations of ambient nitrogen concentrations in the water column.
- ◆ Evaluation of the abundance, distribution, biomass and diversity of phytoplankton in surface waters.

- ◆ Quantification of N uptake rates by phytoplankton in relation to variations of nutrients, seasonal dynamics of the phytoplankton community and environmental parameters.
- ◆ Quantification of size-fractionated uptake rates of nitrogen by two fractions of phytoplankton : microphytoplankton (200-20  $\mu\text{m}$ ) and nanophytoplankton (20-0.8  $\mu\text{m}$ ).
- ◆ Quantification of ammonium regeneration rates and their seasonal variations.
- ◆ Quantification of nitrification rates and their seasonal variations.

Since the detrital organic nitrogen pool forms an important reservoir of nitrogenous nutrients that support various processes, knowledge on the elemental flux from decaying organic matter (specifically mangrove litter) is obligatory. To achieve this, a simultaneous study on the seasonal variations of litter fall production, elemental composition of litter and its decomposition was conducted. It is important to note that studies on the transfer of energy through mangrove litter have already been carried out (Wafar, 1988; Wafar *et al.*, 1997). However, a simultaneous study on the contribution of mangrove litter to organic matter, its subsequent decomposition, followed by N cycling (together with what is known on benthic nitrogen cycling), would give us a better understanding of the structure and function of the mangrove ecosystem as a whole.

## **Significance**

During the last few decades, there has been widespread exploitation of mangrove resources resulting in impacts in the form of loss of habitat and/or alteration to the habitat. All impacts, natural and man-made, cause modifications in the composition of biodiversity, structural and functional organization at various trophic levels and overall biological productivity. Hence, any one, or all of these, can be an index of the health and sustainability of the ecosystem. Earlier studies have mostly used biodiversity, and at times the biological productivity, as such indices, but not energy flow or nutrient dynamics, though the latter are equally important, both as indices and as key components in modelling the processes of an ecosystem.

## CHAPTER II

### MATERIAL AND METHODS

#### 2.1 DESCRIPTION OF THE STUDY AREA

The study site is an undisturbed mangrove forest of about 300 ha on the central west coast of India ( $16^{\circ} 12' - 16^{\circ} 14' \text{ N}$  and  $73^{\circ} 25' - 73^{\circ} 30' \text{ E}$ ), fed by a small river and connected with the Arabian Sea by a single channel (Fig. 2.1). The estuary is about 20 km in length, with a maximum width of 1.2 km at its midstream and a minimum width of 0.012 km at its mouth. The midstream estuarine region has abundant mangroves. This region extends to a distance of approximately 5 km from the mouth of the estuary and several channelled waterways divide this expanse, creating scattered patches of mangroves on interspersed islets. The north, south and west of the estuary are relatively hilly and the eastern side has flat plains with extensive paddy fields. The estuary lies in a north-south direction before meeting the Arabian Sea.

##### 2.1.1 DISTRIBUTION OF MANGROVE SPECIES

The mangroves along the estuary are distributed in five zones (Table 2.1), demarcated on the basis of species dominance. *Rhizophora mucronata*, *Sonneratia alba*, *S. caseolaris*, *Avicennia marina*, *A. officinalis* and *Excoecaria agallocha* are dominant, while species such as *Bruguiera gymnorhiza*, *Acanthus ilicifolius*, *Rhizophora apiculata*, *Aegiceras corniculatum*, *Derris heterophylla*, and *Clerodendrum inerme* are less abundant.

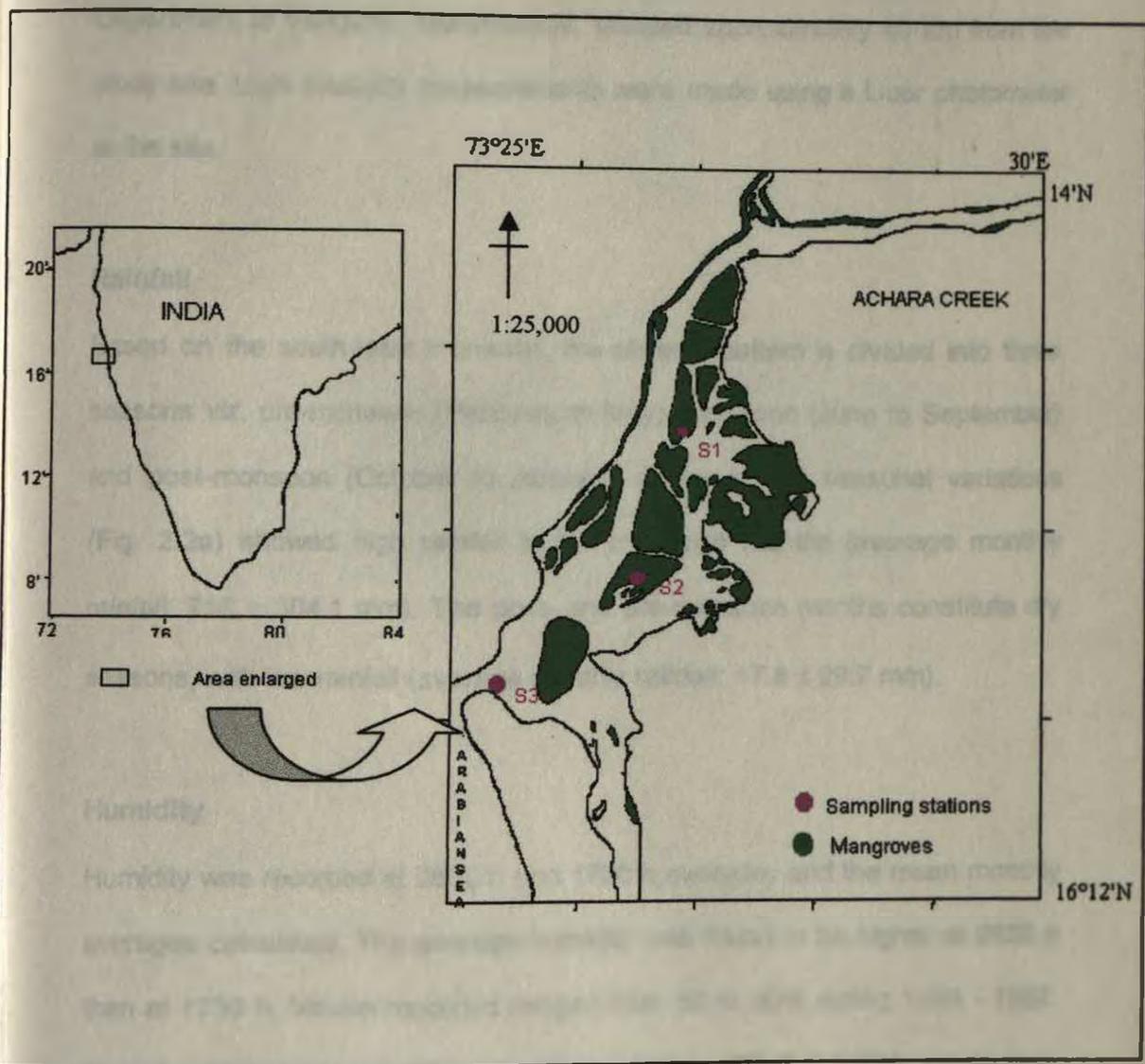


Fig. 2.1 Map of Achara estuary showing location of sampling stations

## 2.1.2 CLIMATOLOGICAL FEATURES

Data on climatological parameters such as rainfall, humidity and air temperature were obtained from the field station of the India Meteorological Department at Vengurla, Maharashtra, situated approximately 40 km from the study site. Light intensity measurements were made using a Licor photometer at the site.

### Rainfall

Based on the south-west monsoon, the climatic pattern is divided into three seasons viz. pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January) seasons. The seasonal variations (Fig. 2.2a) showed high rainfall in the monsoon months (average monthly rainfall:  $716 \pm 384.1$  mm). The post- and pre-monsoon months constitute dry seasons, with low rainfall (average monthly rainfall:  $17.8 \pm 29.7$  mm).

### Humidity

Humidity was recorded at 0830 h and 1730 h everyday and the mean monthly averages calculated. The average humidity was found to be higher at 0830 h than at 1730 h. Values recorded ranged from 50 to 90% during 1996 - 1997. Humidity was maximum in the monsoon season ( $86.0 \pm 2.9\%$ ), moderately high in the post-monsoon season ( $71.9 \pm 5.6\%$ ) and lower during the pre-monsoon season ( $67.8 \pm 4.6\%$ ) (Fig. 2.2b).

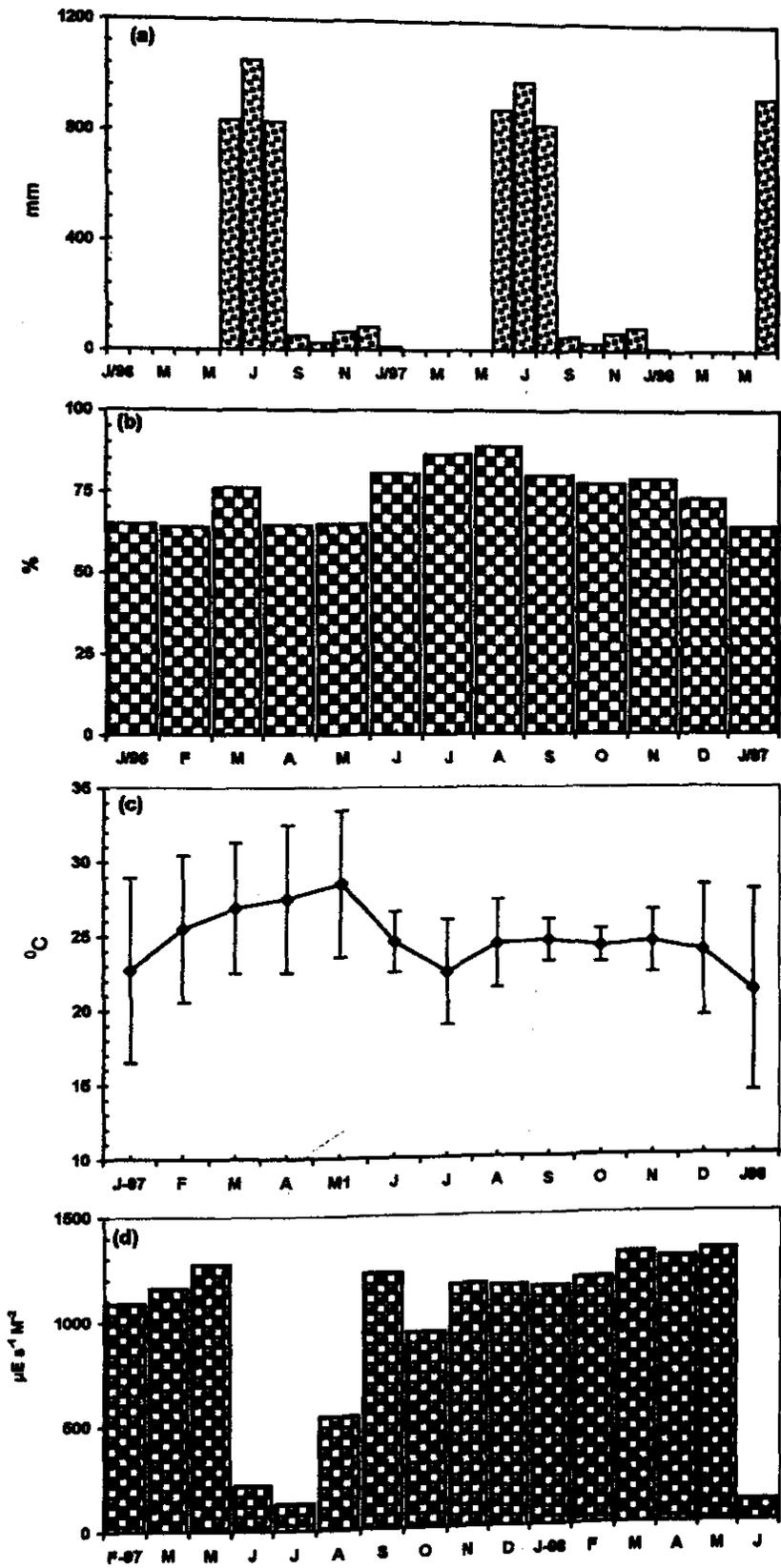


Fig. 2.2. Seasonal variations of monthly averages of (a) rainfall, (b) humidity, (c) air temperature and (d) light intensity

### **Air temperature**

The mean monthly maximum and minimum air temperatures recorded from 1996 to 1997 are shown in Fig. 2.2c. Average temperatures varied from 16.3°C to 32.0°C and followed seasonal changes. Lower temperatures were recorded in the post-monsoon season ( $23.4 \pm 3.6^{\circ}\text{C}$ ) and maximum in the pre-monsoon season ( $27.2 \pm 4.8^{\circ}\text{C}$ ).

### **Light intensity**

The surface incident light per unit area was recorded at hourly intervals for 5 hours during the day when incubations of samples were carried out. The light intensity ranged widely from 98 to 1318.5  $\mu\text{E s}^{-1} \text{M}^2$  (Fig. 2.2d). The light intensity was maximum in the pre-monsoon season (mean: 1231.2  $\mu\text{E s}^{-1} \text{M}^2$ ), moderate in the post-monsoon season (mean: 1103.2  $\mu\text{E s}^{-1} \text{M}^2$ ) and lowest in the monsoon season (mean: 443.3  $\mu\text{E s}^{-1} \text{M}^2$ ).

### **2.1.3 CHOICE AND DESCRIPTION OF STATIONS**

To carry out the investigations in the present study, three sampling stations along the estuary were selected. These represented a gradient in mangrove vegetation, from dense (reference station) through partial (middle station) to sparse (mouth station). The positions of these sampling sites are shown on Fig. 2.1. The physical characteristics of the stations are as follows:

Reference station (S 1): This was located approximately 3 km upstream from the mouth of the estuary. The mangrove species abundantly found include *R. mucronata*, *A. officinalis*, *E. agallocha*, *A. corniculatum*, *C. inermis* and *D.*

*heterophylla* and *R. apiculata*. The mean depth of the water column was 1.8 m and salinity ranged from 0.20 to 36.24 PSU. The sediment at this station was compact, mainly composed of dark brown silt with 63.8% of the particles between 2 and 65  $\mu\text{m}$  and 36.2% of the particles  $<2 \mu\text{m}$ , composed of clay.

**Middle station (S 2):** This was located about 1.5 km downstream from station 1. The mangrove species found here were *R. mucronata*, *A. officinalis*, *S. alba*, *S. caseolaris*, *B. gymnorrhiza* and *A. marina*. The mean depth of the water column was 2.1 m and salinity ranged from 0.21 to 36.83 PSU. Here also, the sediment was compact, composed of brown silty-clay, with 72.3% silt and 27.7% clay.

**Mouth station (S 3):** This was located at approximately 1.5 km from station 2 at the lower reaches of the estuary, where the latter opens into the Arabian Sea. The mangrove distribution at this station was rather patchy and only *R. mucronata*, *A. officinalis* and *A. marina* were found. The mean depth of the water column was 2.7 m and salinity ranged from 0.11 to 36.89 PSU. The sediment consisted of silty-sand with 94.2% of the particles in the size range of 2  $\mu\text{m}$  - 65  $\mu\text{m}$  and a minor contribution of sand.

## **2.2 SAMPLING PROGRAMME AND PROCESSING**

Owing to the relatively shallow depth of the estuary and tidal mixing, the water column at all three stations remained vertically homogeneous at anytime of the year and hence sampling was restricted to only surface waters. The stations were accessible without the need to use a boat. From each sampling station,

surface water samples were collected at monthly intervals beginning from February 1997 until June 1998. Nitrification experiments were carried out from July 1997 to June 1998. Sampling at each station was carried out on three consecutive days during mid-tide. All samples were immediately transferred to an ice box before being analyzed. Sampling normally required one and a half to two hours depending on the prevailing weather, especially during the monsoon months when heavy rain hampered collections. A small laboratory was set up in close proximity to the study area so that processing of the samples could be carried out without delay.

Surface water samples were collected in 10 l plastic carboys. In addition, two samples were collected and immediately fixed for measurements of dissolved oxygen concentrations and were stored away from light. Processing of samples on reaching the laboratory began with chlorophyll-*a* extraction. A known volume of water sample was filtered onto a Whatman GF/C filter pad of 47 mm diameter and chlorophyll-*a* was extracted in 90% acetone from the particulate matter retained on the filter. The filtered water sample was then analyzed for nutrients (ammonium, nitrate, nitrite and urea). Samples for ammonium measurement were fixed with reagents without delay so as to avoid contamination. This was followed by urea, nitrite and nitrate measurements. All nutrient analyses were done manually using a UV-VIS double beam spectrophotometer. Samples for estimating phytoplankton cell counts and species composition were fixed with Lugol's iodine and stored in the dark in polythene bottles of 125 ml capacity. A sample for determining salinity was stored in a 125 ml glass bottle pending measurements with a Guildline Autosol

model 8400A Salinometer. Samples for determination of particulate organic nitrogen (250 or 500 ml) were filtered onto Whatman GF/F filter pads and stored until analyses by Kjeldahl method.

Samples for measuring nitrogen uptake rates were incubated with  $N^{15}$  tracers for four hours under *in situ* conditions. Simultaneously, size fractionated uptake experiments were conducted for two groups of phytoplankton viz. microplankton (200-20  $\mu\text{m}$ ) and nanoplankton (20-0.8  $\mu\text{m}$ ). The latter measurements (fractionated uptake) were made once per season. The light intensity during the period of incubation was recorded at hourly intervals using a LICOR photometer. At the end of the incubation, samples for nitrogen uptake were filtered onto Whatman filter pads and were then dried and stored until analyses of  $^{15}\text{N}:^{14}\text{N}$  isotope ratios. The filtrate recovered by filtering the sample for measurement of ammonium uptake was used for measuring ammonium regeneration rates ( $^{15}\text{N}:^{14}\text{N}$  ratio of ammonium in the filtrate). Samples for measurement of nitrification rates were incubated with  $N^{15}\text{-NH}_4^+\text{Cl}$  and kept in the dark for 24 hours. After the incubation period, the filtrate collected was used for extraction of nitrite as a dye which was adsorbed onto Whatman filter pads of 25 mm diameter. These were stored until analyses of  $^{15}\text{N}:^{14}\text{N}$  isotope ratios.

All Whatman GF/F filters used were of 47 mm diameter (except for nitrification experiments, where they were of 25 mm diameter) and were pre-ignited in a muffle furnace at  $400^\circ\text{C}$  for 4 hours. These were stored in clean, closed petri-dishes before use. All filtrations were carried out under vacuum not exceeding

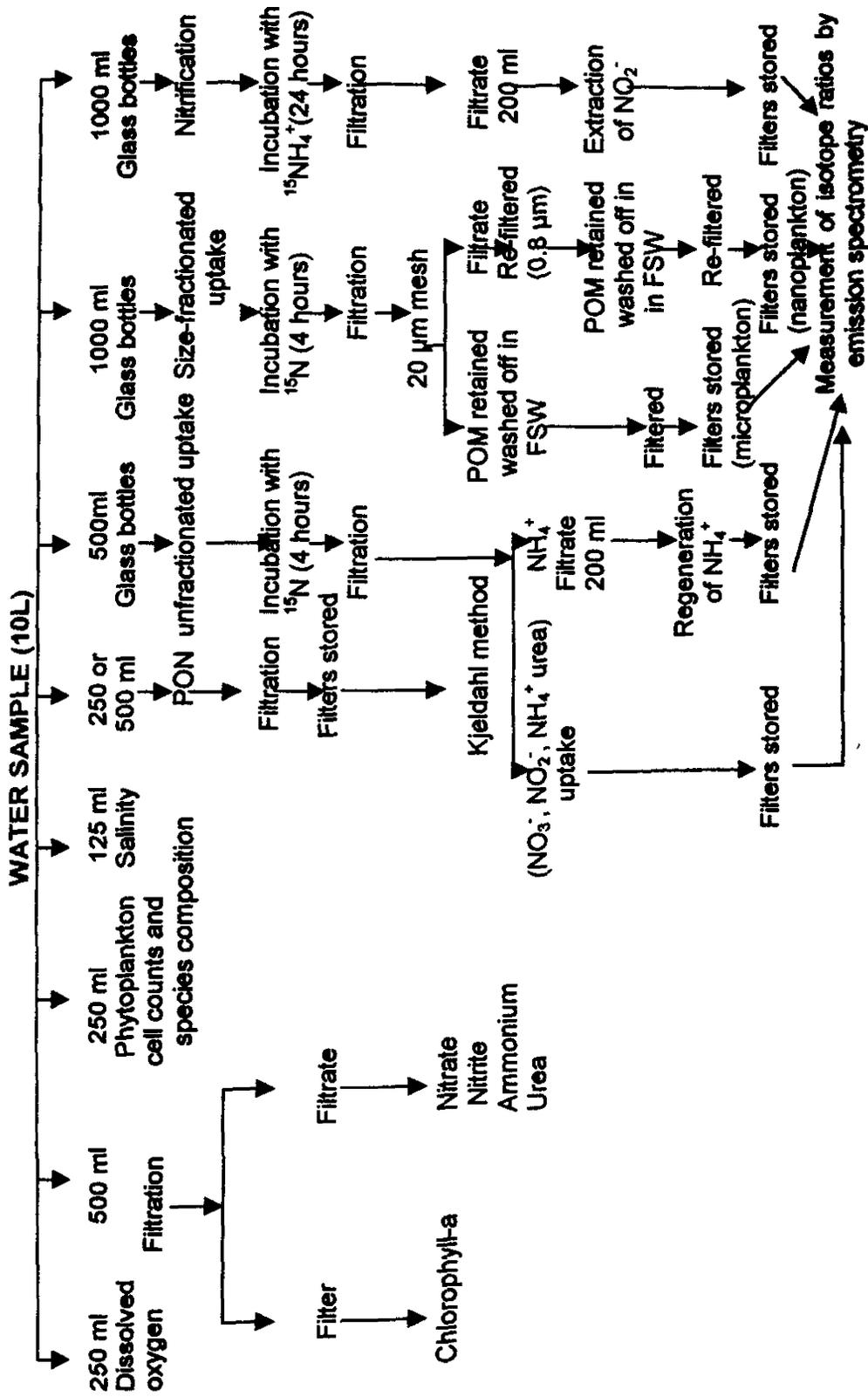


Fig. 2.3 Flow diagram of sample processing

200 mm of Hg. The filters for measurement of uptake, regeneration and nitrification rates were stored in separately labeled sachets and the isotope ratios were measured by emission spectrometry using a Jasco N-150 Nitrogen analyzer. Tracers used were  $\text{Na}^{15}\text{NO}_3$ ,  $\text{Na}^{15}\text{NO}_2$ ,  $^{15}\text{NH}_4\text{Cl}$  and  $\text{CO}(^{15}\text{NH}_2)_2$  (97.4 atom % enrichment for nitrate and 95 atom % for nitrite, ammonium and urea). These isotopes were obtained from KOR-Isotopes, U.S.A. The sampling programme is summarized in Fig. 2.3.

## **2.3 ANALYTICAL TECHNIQUES**

### **2.3.1 HYDROLOGICAL PARAMETERS**

#### **2.3.1.1 Temperature**

Surface water temperatures were measured with a laboratory thermometer (precision:  $0.1^\circ\text{C}$ ).

#### **2.3.1.2 Salinity**

The electrical conductivity ratio of seawater samples was measured in a Guildline "Autosal" model 8400A Salinometer (measurement range: 0.005 - 42 PSU). The salinity of the sample was calculated using the equation for conversion of conductivity ratio to salinity (UNESCO Technical papers in Marine Science 44, 1983).

#### **2.3.1.3 Dissolved oxygen**

The classical Winkler's method described in detail by Strickland and Parsons (1972) was used for measurement of dissolved oxygen concentration.

## 2.3.2 BIOLOGICAL PARAMETERS

### 2.3.2.1 Chlorophyll-a

Pigment analysis is a measure of living plant matter in the particulate organic matter of the sea. The pigments contained in unicellular algae *i.e.* phytoplankton, include chlorophylls and carotenoids. The chlorophyll pigments include chlorophyll -a, -b and -c of which chlorophyll-a is the most important. The carotenoids (carotenes and xanthophylls) are contained in four groups of phytoplankton: Chlorophyta, Cyanophyta, Chrysophyta and Pyrrophyta.

In the present work, chlorophyll-a was determined spectrophotometrically based on the technique described by Richards and Thompson (1952), which was later modified (Strickland and Parsons, 1972). The water sample (0.5 -1 liter) collected for extraction of chlorophyll-a was first passed through 200  $\mu\text{m}$  mesh to discard the larger zooplankton and subsequently filtered onto a Whatman GF/C filter pad of 47 mm diameter. The chlorophyll-a was then extracted from the particulate matter retained on the filter by grinding in 10 ml of 90% acetone. Magnesium carbonate was not added after filtration since extraction was carried out soon after filtration (Moed and Hallegraeff, 1978). The tubes containing the extract were then stored in the refrigerator for 6 hours for complete extraction of the pigment and later centrifuged at 4000-5000 rpm for 5 minutes. The supernatant was transferred into a spectrophotometer cell of 1 cm path length and the absorbance read at 663, 645 and 630 nm. The optical density of 90% acetone at all wavelengths represented the reagent blank, while the sample O.D. taken at 750 nm served as the turbidity blank.

Extraction of pigments was carried out within 1½ hours after collecting the samples. The chlorophyll-*a* content was calculated using the trichromatic equations of Strickland and Parsons (1972).

A second water sample (0.5 - 1 liter) for the estimation of chlorophyll-*a* of size fractions was passed through a 200 µm mesh net to discard the larger zooplankton. This was then filtered through a 20 µm mesh net to obtain the 200-20 µm fraction (microphytoplankton). The particulate matter retained was washed off into a beaker with filtered seawater and re-filtered onto a GF/F filter. For the 20-0.8 µm fraction (nanophytoplankton), the filtrate recovered after passing the sample through the 20 µm mesh net was filtered onto a 0.8 µm cellulose acetate filter pad and the particulate matter retained was washed off into a beaker with filtered seawater and once again filtered onto a GF/F filter. The chlorophyll-*a* from both these fractions was extracted in the same manner as done for the unfractionated sample.

#### 2.3.2.2 Phytoplankton cell counts and species composition

Water samples (250 ml) were fixed with few drops of Lugol's iodine and stored in the dark until enumeration of phytoplankton (microphytoplankton and nanophytoplankton) cell counts in a Sedgewick - Rafter Plankton Counting Chamber. Kite diagrams were drawn to demonstrate the seasonal succession of phytoplankton species at each station. The abundance was expressed as percentage of the total number of cells identified. Shannon-Weaver (1949) diversity indices were calculated for all the identified species.

## 2.3.3 AMBIENT NITROGEN CONCENTRATIONS

### 2.3.3.1 Nitrate

Nitrate was measured by the method of Wood *et al.* (1967). In this method, nitrate is reduced almost quantitatively to nitrite on passing the sample through a cadmium - copper column. The nitrite produced is measured as described below (precision:  $\pm 0.1 \mu\text{g}$  at  $\text{NO}_3^- \text{-N } \Gamma^1$ ).

An earlier method for nitrate measurement was that of Morris and Riley (1963), where reduction was achieved by passing the sample through cadmium amalgamated with mercuric chloride. In a later method (Jones, 1984), the reduction of nitrate to nitrite was achieved by shaking of samples with spongy cadmium. It was demonstrated that shaking of samples for 60-90 minutes resulted in complete reduction of nitrate at concentrations between 1 - 100  $\mu\text{g}$  at  $\text{NO}_3^- \text{-N } \Gamma^1$ .

### 2.3.3.2 Nitrite

The method of Bendschneider and Robinson (1952) was used for measuring nitrite. In this method, dissolved nitrite reacts with sulphanilamide in an acid solution. The resulting diazo-compound then reacts with N-(1-naphthyl)-ethylenediamine to form a highly coloured azo-dye, the optical density of which is measured at 543 nm (precision:  $\pm 0.01 \mu\text{g}$  at  $\text{NO}_2 \text{-N } \Gamma^1$ ).

### 2.3.3.3 Ammonium

The method for measurement of ammonium was first described by Berthelot in 1859. Several improvements to this method have since been made, of which

those by Solorzano (1969) and Koroleff (1969, 1970) have gained most importance. The method used in the present study was that described by Koroleff (1969, 1970) in which dissolved ammonia reacts with hypochlorite at basic pH to form a monochloramine which, in the presence of phenol and sodium nitroprusside (a catalyst), results in the formation of indophenol blue colour. The colour development is complete in six hours at room temperature. The optical density of the blue indophenol obtained at the end of the reaction period was measured at 630 nm (precision:  $\pm 0.05 \mu\text{g at NH}_4^+ - \text{N l}^{-1}$ ).

#### 2.3.3.4 Urea

The method first used to estimate urea directly involved a chromatographic technique (Degens and Reuter, 1964). An indirect method for measuring urea was described by McCarthy (1970). This method measures urea by measuring ammonium produced as the end product of enzymatic hydrolysis by urease. The method used in the present study is based on the direct colorimetric determination of urea described originally by Newell *et al.* (1967) and later improved by (Aminot and Kerouel, 1982). In this method, urea reacts with diacetylmonoxime reagent in a strong acid solution of ferric chloride in the presence of thiosemicarbazide. Colour development is achieved after incubation of samples at  $85^\circ\text{C}$  for 2 hours in a water bath. The reaction mixture is then cooled rapidly and the optical density measured without delay at 520 nm (precision:  $\pm 0.01 \mu\text{g at Urea-N l}^{-1}$ ).

The reagents used for nutrient analyses were prepared freshly before each field trip. All glassware were washed with chromic acid, rinsed well with distilled

water and dried before use. Reagent blanks were run for each set of nutrients to be analyzed. The optical density of all the solutions were measured in a Jasco UV/VIS double beam spectrophotometer (2 nm band width) using a 1 cm cell.

#### **2.3.3.5 Particulate organic nitrogen (PON)**

The water sample (250 or 500 ml) was filtered onto a Whatman GF/F filter pad (pre-ignited for 4 hours at 400<sup>0</sup>C to remove all traces of nitrogen). The filter pad was then allowed to dry at low temperatures and stored until determination by Kjeldahl digestion method.

Although the CHN analyzer has been widely used and accepted for its high precision and repeatability, the Kjeldahl digestion method was used for the estimation of PON in the present study and was preferred for two reasons:

1. The precision of the Kjeldahl method was also close to that of the CHN analyzer (0.01  $\mu\text{g}$  at N) especially when microprocessor controlled titration units were employed as in the present study.
2. The estimation of PON by the Kjeldahl digestion method was less expensive.

The Kjeldahl digestion method involves the conversion of organic nitrogen in the sample to ammonia by digesting with concentrated sulphuric acid, followed by recovery of ammonia by steam distillation of the acid mixture.

### ***Experimental protocol***

a) ***Digestion:*** The dried filters of each sample were finely cut and transferred into a Kjeldahl flask (100 ml capacity). To this, 1.5 g of a catalyst mixture (consisting of selenium granules,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and  $\text{K}_2\text{SO}_4$  in the ratio of 1:10:100) was added, followed by 5 ml of concentrated sulphuric acid. The Kjeldahl flasks were then placed in a Kjeldahl digestion unit and heated until a clear solution was obtained. Total digestion time required was about 6 hours. The Kjeldahl flasks were then removed from the digestion unit and allowed to cool. Deionised water (25 ml) was added carefully to each flask and the contents in the flasks were mixed.

b) ***Steam distillation:*** The digestion mixture and washings of each sample were transferred to a round bottom distillation flask which was connected to a steam distillation unit. To the flask, 25-30 ml of 40% NaOH was added slowly and the sample was steam distilled. The distillate was collected in 10 ml of standard HCl solution (0.01N) contained in a conical flask of 250 ml capacity. Distillation was continued until about 30 ml of distillate was collected.

c) ***Titration:*** Titration of the steam-distilled ammonium was immediately carried out with a microprocessor controlled Metrohm Dosimat-665 unit with 0.01N standard NaOH solution using methyred-methylene blue as the indicator. The PON in the sample was calculated using the formula:

$$\text{PON (mg)} = \frac{\text{Volume of NaOH Unconsumed in the Titration}}{\text{Normality of NaOH}} \times 14$$

## 2.4 TRACER TECHNIQUES

The study of uptake of nitrogenous nutrients by phytoplankton using  $^{15}\text{N}$  as the isotopic tracer was introduced by Neess *et al.* (1962) and applied to marine systems by Dugdale and Goering (1967). Since then, there has been a renewed interest in quantifying nitrogen fluxes in the marine environment. In the last three decades, the uptake of nitrate and ammonium, representing the major fractions of new and regenerated N, has been investigated using  $^{15}\text{N}$  in a number of instances (see Dortch, 1990). Tracer techniques have also been used to assess the nutritional status of phytoplankton and their preferences to different nitrogen forms (Glibert and McCarthy, 1984).

In general, a nitrogen tracer experiment involves the following:

- collection of the sample
- inoculation of the sample with a known quantity of a  $^{15}\text{N}$  labeled compound (nitrate, nitrite, ammonium and urea).
- incubation of the sample for a pre-determined length of time
- filtration (collection of particulate organic matter)
- measurement of change in the isotopic enrichment

### 2.4.1 NITROGEN UPTAKE STUDIES

#### 2.4.1.1 Unfractionated uptake

Surface water samples were pre-screened through 200 $\mu\text{m}$  mesh net to remove the larger zooplankton and transferred into glass bottles (500 ml capacity). Samples were then inoculated in duplicate with labeled nitrogen tracers (in the form of  $\text{Na}^{15}\text{NO}_3$ ,  $\text{Na}^{15}\text{NO}_2$ ,  $\text{NH}_4^{15}\text{Cl}$  and  $\text{CO}(^{15}\text{NH}_2)_2$ ) in quantities not

exceeding 10% of the ambient levels. Amount of tracer added was calculated from the nutrient concentrations measured the previous day at the same site. Later calculations showed that tracer addition rarely exceeded 10% of the ambient. Stock solutions of tracers with 1 cc = 10  $\mu\text{g}$  at N were prepared and stored in the refrigerator. Working solutions were freshly prepared every day before commencement of sampling. The bottles were placed in large troughs filled with seawater and incubated for four hours under *in situ* conditions. The temperature was maintained at ambient with frequent changes of seawater. Incident light on the incubation bottles was measured using a LICOR photometer at hourly intervals during the incubation period that began before noon.

On completion of the incubation, the particulate matter was recovered on pre-ignited (400<sup>o</sup>C for 4 hours) Whatman GF/F filter pads of 47 mm diameter. All filtrations were done under vacuum not exceeding 200 mm Hg. The filters were then dried at low temperatures (40<sup>o</sup>C) and stored in labeled sachets pending processing for measurement of <sup>15</sup>N:<sup>14</sup>N isotope ratios by emission spectrometry. Filtration of the samples for ammonium uptake were done first and the filtrate collected was used for immediate extraction of ammonium (ammonium regeneration).

#### 2.4.1.2 Size-fractionated uptake

The incubation of samples for size-fractionated uptake experiments (1 liter) was carried out in the same manner as done for unfractionated uptake. However, at the end of the incubation period, the micro- and nano-

phytoplankton fractions were recovered by post-fractionated filtration. The sample was first filtered through 20  $\mu\text{m}$  mesh net to remove the microphytoplankton. This was washed off into a beaker with filtered seawater and re-filtered onto a GF/F filter. The filtrate recovered after passing the sample through 20  $\mu\text{m}$  mesh net was filtered onto a 0.8  $\mu\text{m}$  cellulose-acetate filter pad and the particulate matter retained was washed off into a beaker with filtered seawater and re-filtered onto a GF/F filter pad. This retained the 20-0.8  $\mu\text{m}$  fraction constituting the nanophytoplankton. The filters were then dried at 40°C in an oven and stored in labeled sachets and thereafter processed for the measurement of  $^{15}\text{N}:^{14}\text{N}$  isotope ratios by emission spectrometry.

## 2.4.2 MEASUREMENT OF AMMONIUM REGENERATION RATES

Early efforts to study ammonium regeneration rates began with research carried out by Harris (1959) on macrozooplankton in the waters of Long Island Sound. Later, with the development of isotope dilution methods (Harrison, 1978; Caperon *et al.*, 1979), a greater understanding of ammonium recycling in different ecosystems was achieved. With this technique, it became possible to measure rates of ammonium regeneration by microheterotrophs.

### 2.4.2.1 Principle

The isotope dilution method involves the addition of  $^{15}\text{NH}_4^+$  to a sample and measuring the change in the  $^{15}\text{N}:^{14}\text{N}$  isotopic ratio after the period of incubation. Regeneration of unlabeled nitrogen by microheterotrophs will tend to dilute the percent abundance of  $^{15}\text{N}$  in the incubation medium. This dilution is proportional to the regeneration rate. Unlike in uptake and assimilation

experiments where the final sample for isotopic analysis is in the particulate form, regeneration experiments involve the extraction of the dissolved nitrogen species (ammonium, in the present study) before isotopic analysis.

#### 2.4.2.2 Extraction of ammonium by direct diffusion

Several methods for extracting ammonium for isotopic analysis have been described. Dissolved ammonium is distilled out by vacuum distillation (Harrison, 1978; Glibert *et al.*, 1982c) or by micro-diffusion techniques described by Paasche and Kristiansen (1982). Other methods involve precipitation of ammonium in an alkaline solution of mercuric chloride (Fisher and Morrissey, 1985) and extraction in an organic phase as indophenol blue followed by evaporation (Dudek *et al.*, 1986; Selmer and Sorenson, 1986).

The methods described above are time consuming, require the use of hazardous chemicals, are prone to contamination and result in low recovery of ammonium. The direct diffusion method introduced by Kristiansen and Paasche (1989) was used in the present study. In this method, dissolved ammonium is liberated as ammonia in the presence of magnesium oxide (MgO) which raises the pH to > 9; the ammonia liberated is trapped on a filter pad impregnated with 0.5N H<sub>2</sub>SO<sub>4</sub>. The risks of contamination in this method are minimized and recovery of ammonium was approximately 65%. Besides this, the method can be applied for even low ammonium concentrations.

### *Experimental protocol*

In this method, 200 ml of the filtrate recovered was transferred to a stoppered conical flask of 500 ml capacity (previously washed in chromic acid, rinsed well with deionised water and dried to avoid any risks of contamination). Unlabeled  $^{14}\text{N-NH}_4^+\text{Cl}^-$  (1cc = 25  $\mu\text{g}$  at  $\text{N-NH}_4^+\text{Cl}^-$ ) was added as vector to the filtrate to obtain sufficient nitrogen for detection by emission spectrometry. This was followed by the addition of 100 mg of magnesium oxide which raises the pH to above 9 and causes the release of ammonia from the filtrate. A filter pad impregnated with 0.5 N  $\text{H}_2\text{SO}_4$  was suspended from a hook attached to the stopper of the flask. The flask was then transferred to an oven and heated for 12 hours at a constant temperature of  $60^\circ\text{C}$ . Occasional shaking of the flask enhanced the diffusion of ammonia from the filtrate onto the acid on the filter pad. This was done carefully to ensure that the contents of the flask did not wet the filter pad. At the end of the incubation period, the flasks were removed from the oven and the filter pad was retrieved, dried and stored until isotopic analysis.

In every field trip, ammonium regeneration experiments were carried out on duplicate samples. Recovery and contamination, if any, in the direct diffusion method was checked by running blanks with ammonia liberated from deionised water under identical experimental conditions. Standard solutions of ammonium chloride vector (1cc = 5  $\mu\text{g}$  at  $\text{N-NH}_4^+\text{Cl}^-$ ) were prepared using deionised water freshly each month. Ammonium concentrations prior to tracer addition and after filtration were measured in duplicate.

### 2.4.3 MEASUREMENT OF NITRIFICATION RATES

Estimates of nitrification rates were first made by Rakestraw (1936) and Vaccaro (1962) by colorimetric methods. Later, with the development of isotopic methods,  $^{15}\text{N}$  tracers were applied to the study of nitrification (e.g. Wada and Hattori, 1971; Hattori and Wada, 1971). A review of methods used has been described in detail by Kaplan (1983).

#### 2.4.3.1 Experimental protocol of nitrite extraction

Nitrification rates were measured by oxidation of  $^{15}\text{NH}_4^+$  in dark incubations and the nitrite for measurements of isotope ratios was extracted following the method described by Schell (1978). This involves the extraction of nitrite as a dye (1-benzene-azo-2-naphthol) using an organic solvent.

Samples (1000 ml) were transferred to glass bottles and were incubated with  $^{15}\text{N-NH}_4^+\text{Cl}^-$  in the dark for 24 hours. At the end of the incubation period, samples were filtered onto Whatman GF/F filters (pre-ignited at  $400^\circ\text{C}$  for 4 hours) and 200 ml of the filtrate was recovered for extraction of nitrite. This was placed in a separating funnel of 250 ml capacity. Unlabeled  $^{14}\text{N-Na}^+\text{NO}_2^-$  was added as vector (1cc =  $1.25\ \mu\text{g}$  at  $\text{N-Na}^+\text{NO}_2^-$  and 1cc =  $12\ \mu\text{g}$  at  $\text{N-Na}^+\text{NO}_2^-$  for duplicate samples respectively). Two concentrations of the vector were added to the filtrate as it was difficult to predict the nitrite concentration in the sample after the incubation. This ensured that there was sufficient N for detection by emission spectrometry. To the filtrate, 3 ml of aniline sulfate solution (5 ml/l of aniline sulfate in 1N HCl) was added. In this step, the nitrite in the sample reacts with aniline sulfate to form a diazonium compound. After 5

minutes, 3 ml of  $\beta$ -naphthol (5 g/l of  $\beta$ -naphthol in 3N NaOH) was added and the contents were mixed well to form a complex colour (azo dye: 1-benzene-azo-2-naphthol). The azo-dye formed was then acidified with 1 ml of concentrated HCl to allow its efficient extraction. The extraction was carried out in three steps using the organic solvent carbon tetrachloride ( $\text{CCl}_4$ ). In the first extraction, 5 ml of  $\text{CCl}_4$  was added and the separating funnel was shaken vigorously for about 10 seconds. The phases were allowed to settle and the organic phase was drained into a clean, dry separating funnel avoiding passage of the organic film present between the two phases. This is a potential source of organic nitrogen contamination and its passage, therefore, should be avoided. The following two extractions were carried out in the same way using 3 ml of  $\text{CCl}_4$  each time, and the organic phases were carefully collected into the separating funnel which contained the dye first extracted from the sample.

The dye was then washed with 100 ml of 1N HCl to discard any excess aniline that had also been extracted and the organic phase carefully drained into a small vial. The separating funnel was then rinsed with 10% HCl, deionised water and finally acetone to remove any traces of aniline or dye. The dye recovered was once again transferred to the funnel and washed with 100 ml of 3N NaOH to remove any  $\beta$ -naphthol that was extracted with the dye. The washed dye was drained into the vial (which was rinsed with the solvent) and was allowed to evaporate by keeping the vial in a covered beaker at 40°C. Finally, as the contents in the vial reached dryness, the dye which remained at the bottom of the vial was redissolved in 100  $\mu\text{l}$  of  $\text{CCl}_4$ . The dye and solvent were then adsorbed onto a filter pad of 2.5 cm diameter (Whatman GF/F,

precombusted at 400°C for 4 hours). This procedure was repeated until the dye was completely removed and the filter pad was stored until isotopic analysis.

Recovery of nitrite was determined by measuring the absorption of the dye at 474 nm before the evaporation process. Replicate (five) measurements indicated an average recovery of about 84%. Extraction of nitrite was carried out on duplicate samples. Nitrite blank was obtained by performing the extraction procedure on deionised water samples. Standard solutions of NaNO<sub>2</sub> vector (1cc = 1 µg at N-NaNO<sub>2</sub>) were freshly prepared using deionised water each month. Nitrite concentrations were measured prior to tracer addition and at the end of incubation.

#### 2.4.4 ISOTOPIC ANALYSES BY EMISSION SPECTROMETRY

The determination of <sup>15</sup>N abundance is carried out on nitrogen gas generated from a sample and hence the analysis requires the conversion of nitrogen atoms in the sample to molecular nitrogen gas. <sup>15</sup>N:<sup>14</sup>N isotope ratios can be measured by emission or mass spectrometrical techniques. Emission spectrometry is advantageous over mass spectrometry in that it is less complicated requiring nitrogen gas in microgram amounts (0.2-10 µg) and is less expensive. Also, no high vacuum is required for the preparation of samples. The precision is in the order of 0.01 atom% <sup>15</sup>N compared to that of mass spectrometry which is 0.001 atom% <sup>15</sup>N.

#### 2.4.4.1 Principle of detection

An external energy source excites the nitrogen molecules (the  $^{14}\text{N}$  and  $^{15}\text{N}$  atoms in nitrogen gas pair up to form the nitrogen molecules  $^{14}\text{N}_2$ ,  $^{14}\text{N}^{15}\text{N}$  and  $^{15}\text{N}_2$  i.e.  $^{28}\text{N}$ ,  $^{29}\text{N}$  and  $^{30}\text{N}$ ) in an electrodeless discharge tube. When the excited molecules return to the ground state, the energy difference is emitted as electro-magnetic radiation of specific wavelength, proportional to the mass of the three nitrogen molecules. These radiations are emitted in the ultra-violet region at wavelengths of 297.7 nm, 298.3 nm and 298.9 nm for  $^{28}\text{N}$ ,  $^{29}\text{N}$  and  $^{30}\text{N}$  respectively, and are scanned and resolved by a monochromator. The light intensities of different wavelengths are converted to electrical signals by the use of a photomultiplier and amplifier and are finally recorded. The  $^{15}\text{N}\%$  abundance (concentration of  $^{15}\text{N}$ ) in the sample is then calculated by measuring the peak heights.

$$^{15}\text{N}\% \text{ abundance} = 100 / 2 R + 1$$

where  $R = \text{peak height of } ^{28}\text{N} / \text{peak height of } ^{29}\text{N}$

#### 2.4.4.2 Preparation of samples for emission spectrometry

As mentioned earlier, isotopic analyses require the conversion of the bound nitrogen in the sample to nitrogen gas. This conversion can be done by: (I) Kjeldahl-Rittenberg method; (II) modified Dumas combustion method or (III) Kjeldahl-Dumas method.

In the present study, the modified Dumas combustion method was used for conversion of bound nitrogen to nitrogen gas. Samples containing the extracted dissolved nitrogen (ammonium and nitrite from regeneration and nitrification experiments respectively) and particulate organic nitrogen (nutrient uptake) were processed by this method. This involves the dry combustion of organic and/or inorganic nitrogen together with copper oxide (an oxidant) resulting in the complete reduction to molecular nitrogen. The reaction takes place in vacuum in a sealed tube. The vacuum is necessary so that there is no dilution of the  $^{15}\text{N}$  in the sample with atmospheric nitrogen. Vacuum is also necessary for discharge of the tubes (4 torr).

The filter pad with particulate organic matter was finely ground using a mortar and pestle with approximately 15 mg of copper oxide powder. The ground mixture was then transferred to a discharge tube (volume= 4 cc), with an upper constricted end. Since the nitrogen content of the sample in the discharge tube should yield a pressure of around 4 torr, it is necessary to know the approximate nitrogen content of the sample to at least  $\pm 20\%$ . The total N content required depended on the volume of the discharge tube used and was estimated at approximately 26  $\mu\text{g}$ . The discharge tubes were connected to a vacuum line and evacuated until a vacuum exceeding  $10^{-3}$  torr was achieved. At one time, six tubes could be evacuated on the vacuum system. The adsorbed gases on the inner walls of the tubes were removed by heating with a hand torch. After degassing the tubes (which was ascertained by monitoring the vacuum gauge), the tubes were carefully sealed at the restricted end with a hand torch. The conversion of sample nitrogen to nitrogen gas was then

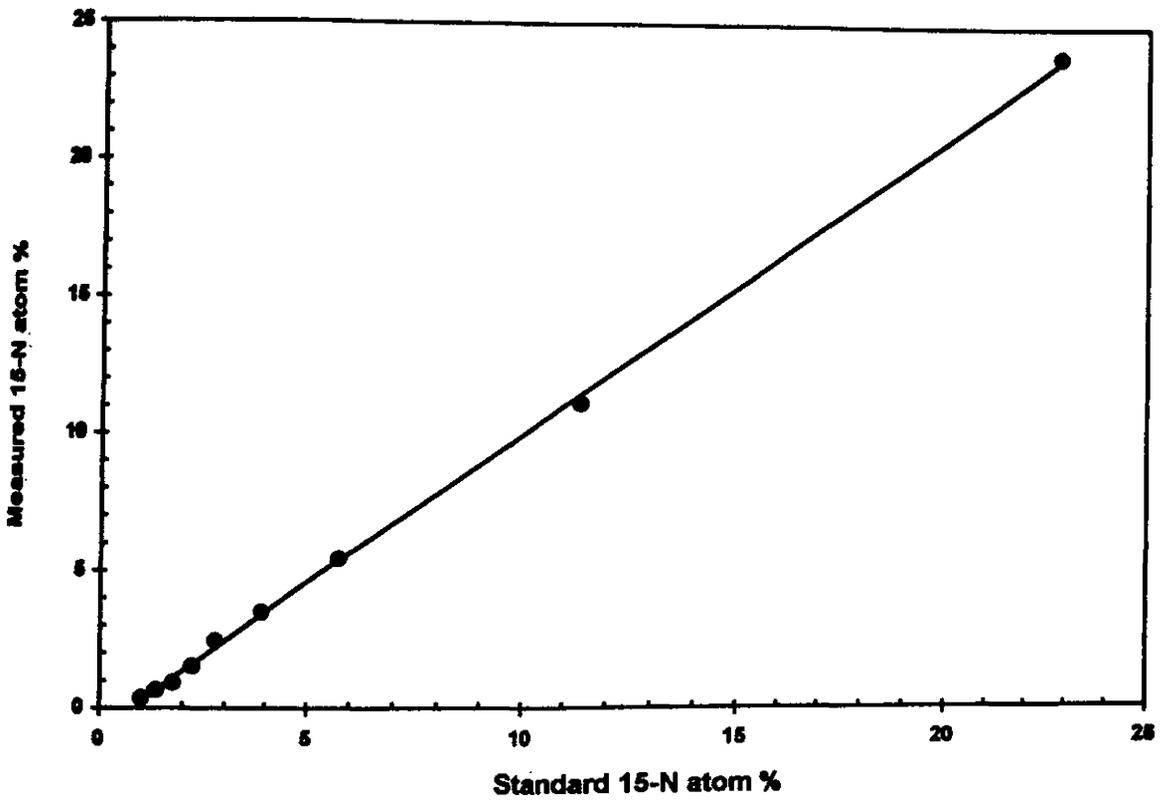


Fig.2.4 Calibration curve for measurement of isotope ratios.

$$\text{Measured 15N atom \%} = (1.0753 * \text{standard15N atom \%}) - 0.799; R^2=0.9995$$

carried out by combusting the evacuated discharge tubes in a muffle furnace at 500°C for 6 hours followed by cooling to room temperature. The other gases produced during combustion *i.e.* CO<sub>2</sub>, H<sub>2</sub>O and oxides of nitrogen were frozen out with liquid nitrogen, allowing the nitrogen gas in the tubes to be analyzed for its N<sup>15</sup> content by emission spectrometry. This was done in a Jasco N-150 Nitrogen analyzer. Although the light intensity of the discharge is highest at a pressure of 1.5 torr (Sommer and Kick, 1965), the discharge lasts longer when more nitrogen is present, and a more stable discharge is obtained around 4 torr. Enrichments were determined using a calibration curve (Fig. 2.4) made using standards provided by the manufacturers. A detailed account on analyses of N<sup>15</sup>/ N<sup>14</sup> ratios has been documented by Fiedler and Proksch (1975).

The discharge tubes, before sample preparation were washed with chromic acid, rinsed well with deionised water and heated in a muffle furnace at 500°C overnight to remove all traces of nitrogen. These were then wrapped in aluminium foil and stored in a desiccator. Copper oxide was also pre-heated at 500°C and stored in a desiccator.

#### 2.4.4.3 Calculations

The study of uptake of nitrogenous nutrients by phytoplankton using <sup>15</sup>N as the isotopic tracer was introduced by Neess *et al.* (1962) and applied to marine systems by Dugdale and Goering (1967). Specific and absolute uptake rates were calculated using the formulae proposed by these authors based on the general principles established for tracer methodology (Sheppard, 1962).

$$\text{Specific uptake rate } v \text{ (h}^{-1}\text{)} = \frac{\text{Atom \% excess in particulate fraction (PF)}}{\text{Atom \% excess in dissolved fraction (DF)}} \times \frac{1}{t}$$

where,

Atom% excess in PF = Measured  $^{15}\text{N}$  % - natural abundance (0.365%)

$$\text{Atom\% excess in DF} = \frac{(^{15}\text{N of sample} + ^{15}\text{N of tracer} \times 100) - 0.365}{\text{Total N (sample + tracer)}}$$

t = duration of the incubation

Absolute uptake rate,  $\rho$  ( $\text{ng at N } \Gamma^1 \text{h}^{-1}$ ) =  $v \times \text{PON content (ng at N } \Gamma^1)$

Where,  $v$  = specific uptake rate

Regeneration rates were calculated using the formula given by Laws (1984) when ammonium concentrations changed between the beginning and end of the incubation.

$$\text{Regeneration rate, } R = \frac{\ln R_t / R_o}{\ln P_t / P_o} \times \frac{(P_o - P_t)}{T}$$

Where,  $R_o$  = Initial atom % excess in DF

$R_t$  = Final atom % excess in DF

$P_o$  = Ammonium concentration at the beginning of the incubation

$P_t$  = Ammonium concentration at the end of the incubation

T = Duration of the incubation

When changes in ammonium concentrations were not detectable after the incubation period, the equation given by Glibert *et al.* (1982b) was used.

$$\text{Regeneration rate, } R_1 = \frac{R_o \times P_o - R_t \times P_t}{UT}$$

$$\text{Where, } U = \frac{(P_o - P_t)}{T} + R$$

### Nitrification rate

Nitrification rates were calculated in the same way as done for specific and absolute transport rates measured with  $^{15}\text{N}$  enriched substrates (Lipschultz, 1984).

Initial atom% excess of  $^{15}\text{N}$  in  $\text{NO}_2$  in the dissolved fraction,

$$^{15}\text{NDI} = \frac{\{(0.00365 \times \text{Nc}) + (0.95 \times \text{Tc}) + (0.00365 \times \text{Ac}) \times 100\}}{\text{TN}} - ^{15}\text{Na}$$

Final atom% excess of  $^{15}\text{N}$  in  $\text{NO}_2$  in the dissolved fraction,

$$^{15}\text{NDF} = 2 \left\{ (^{15}\text{Nex} \times \frac{(\text{Nc} + \text{Vc})}{\text{Nc}} - \frac{(^{15}\text{Na} \times \text{Vc})}{\text{Nc}} - ^{15}\text{Na} \right\}$$

where, Nc = Ambient nitrite concentration ( $\mu\text{g at N } \Gamma^{-1}$ )

Vc = Vector concentration ( $\mu\text{g at N } \Gamma^{-1}$ )

Tc = Tracer concentration ( $\mu\text{g at N } \Gamma^{-1}$ )

Ac = Ambient ammonium concentration ( $\mu\text{g at N } \Gamma^{-1}$ )

TN = Total nitrogen (Nc + Tc + Ac)

$^{15}\text{Na}$  = Natural abundance of  $^{15}\text{N}$  (0.365%)

$^{15}\text{Nex}$  = Atom% excess of  $^{15}\text{N}$  in the azo-dye fraction

Since we are measuring uptake of  $^{15}\text{NH}_4$  into  $^{15}\text{NO}_2$ ,

$$V \text{ (h}^{-1}\text{)} = \frac{^{15}\text{NDF}}{^{15}\text{NDI}} \times \frac{1}{T}$$

where, T = incubation duration (24 hours)

Nitrification rate, R ( $\eta\text{g at N } \Gamma^{-1}\text{h}^{-1}$ ) = (V X Nc) 1000

## 2.5 LITTERFALL STUDIES

The mangrove species selected for litterfall studies were *Rhizophora mucronata*, *Sonneratia alba*, *Avicennia marina* and *A. officinalis*. Litter traps were tied to four trees of each species and the litter was collected at monthly intervals from April 1996 to June 1997. Of the four species studied, *R. mucronata* had the highest litterfall ( $1036 \text{ g m}^{-2} \text{ year}^{-1}$ ). This was followed by *S. alba*, *A. marina* and *A. officinalis* contributing to  $996 \text{ g m}^{-2} \text{ year}^{-1}$ ,  $876 \text{ g m}^{-2} \text{ year}^{-1}$  and  $738 \text{ g m}^{-2} \text{ year}^{-1}$  respectively.

The seasonal variations of total litterfall in the four mangrove species are shown in Fig. 2.5 a-d. Maximum litterfall was recorded in the pre-monsoon season and accounted for 37.1% of the total annual litterfall. The maximum during the pre-monsoon season was closely followed by the monsoon and post-monsoon seasons (33.7 and 29.2% respectively).

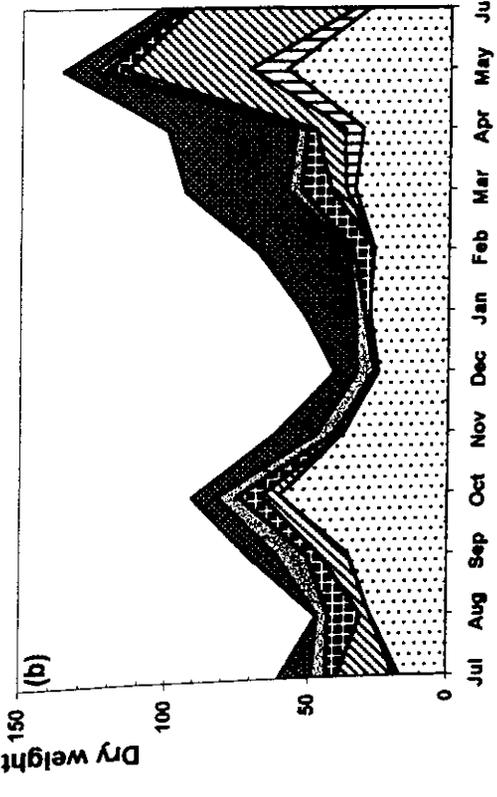
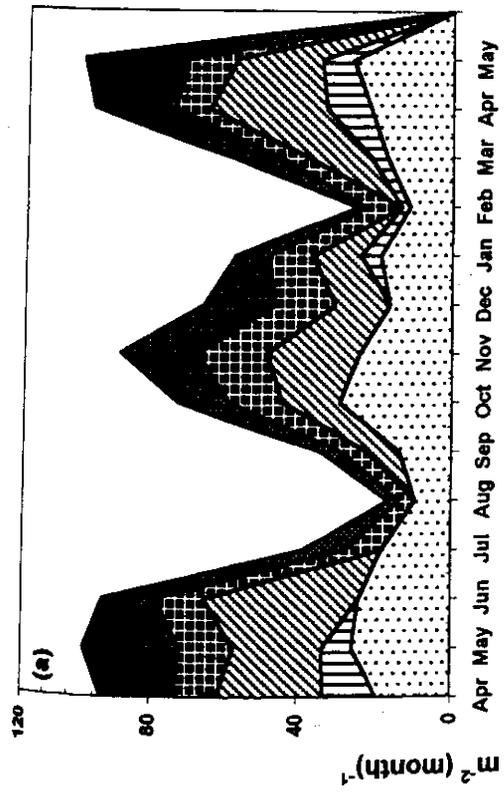


Fig.2.5 Seasonal variation of total litterfall and of the major litter fractions in (a) *Avicennia marina* and (b) *Rhizophora mucronata*. Leaves [stippled], flowers [horizontal lines], twigs [diagonal lines] and miscellaneous [solid black]

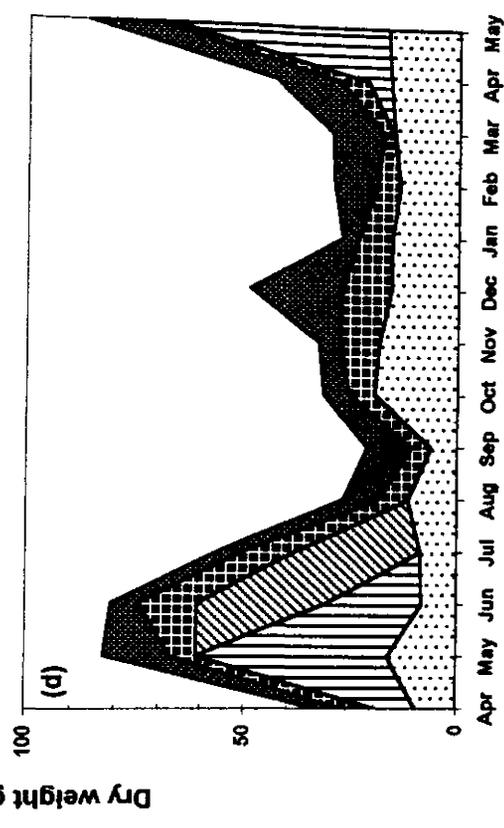
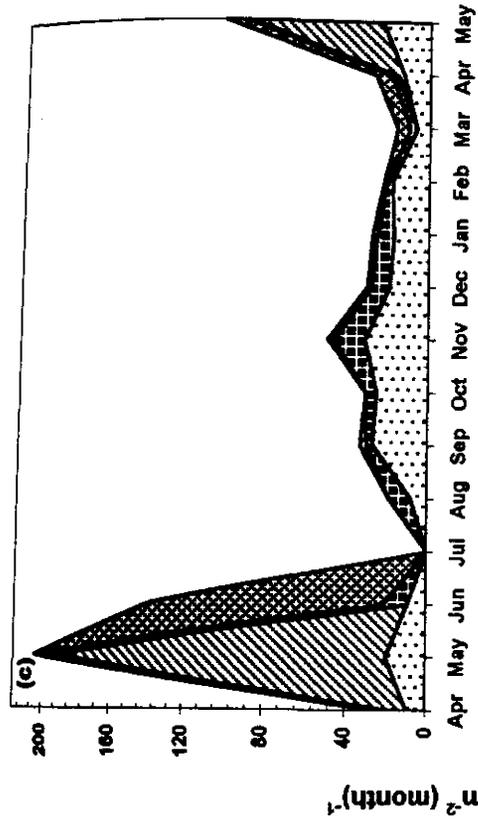


Fig.2.5 Seasonal variation of total litterfall and of the major litter fractions in (c) *Avicennia officinalis* and (d) *Sonneratia alba*. Leaves [stippled], flowers [horizontal lines], twigs [diagonal lines] and miscellaneous [solid black]

## CHAPTER III

### RESULTS

#### 3.1 HYDROLOGICAL PARAMETERS

##### 3.1.1 TEMPERATURE

Water temperature recorded at the three stations varied from 20 to 32.5°C during the study period. The seasonal variations in temperature exhibited a gradual increase from the beginning of the pre-monsoon months to reach a peak at the end of this season (32°C in May) (Fig. 3.1a). A decrease in the water temperature during the monsoon season followed by an increase from the end of this season into the post-monsoon season was evident. The annual averages were of the same order at the reference ( $27.1 \pm 3.0^\circ\text{C}$ ), middle ( $27.4 \pm 3.1^\circ\text{C}$ ) and mouth ( $27.5 \pm 3.0^\circ\text{C}$ ) stations.

##### 3.1.2 SALINITY

The salinity measured in the overall study ranged from 0.11 to 36.89 PSU. The seasonal variations followed a well defined cycle with minimum salinity in the monsoon months, gradually increasing in the post-monsoon to maximum salinity during the pre-monsoon season (Fig. 3.1b). The highest salinity (36.89 PSU) was recorded in this season at the mouth station. There were no wide variations in salinity between the stations. However, as annual averages, the salinity recorded at the mouth station ( $26.88 \pm 13.64$  PSU) was comparatively higher than at the middle ( $24.96 \pm 14.05$  PSU) and reference ( $23.83 \pm 14.98$  PSU) stations, in that order. This was obviously because of the location of the mouth

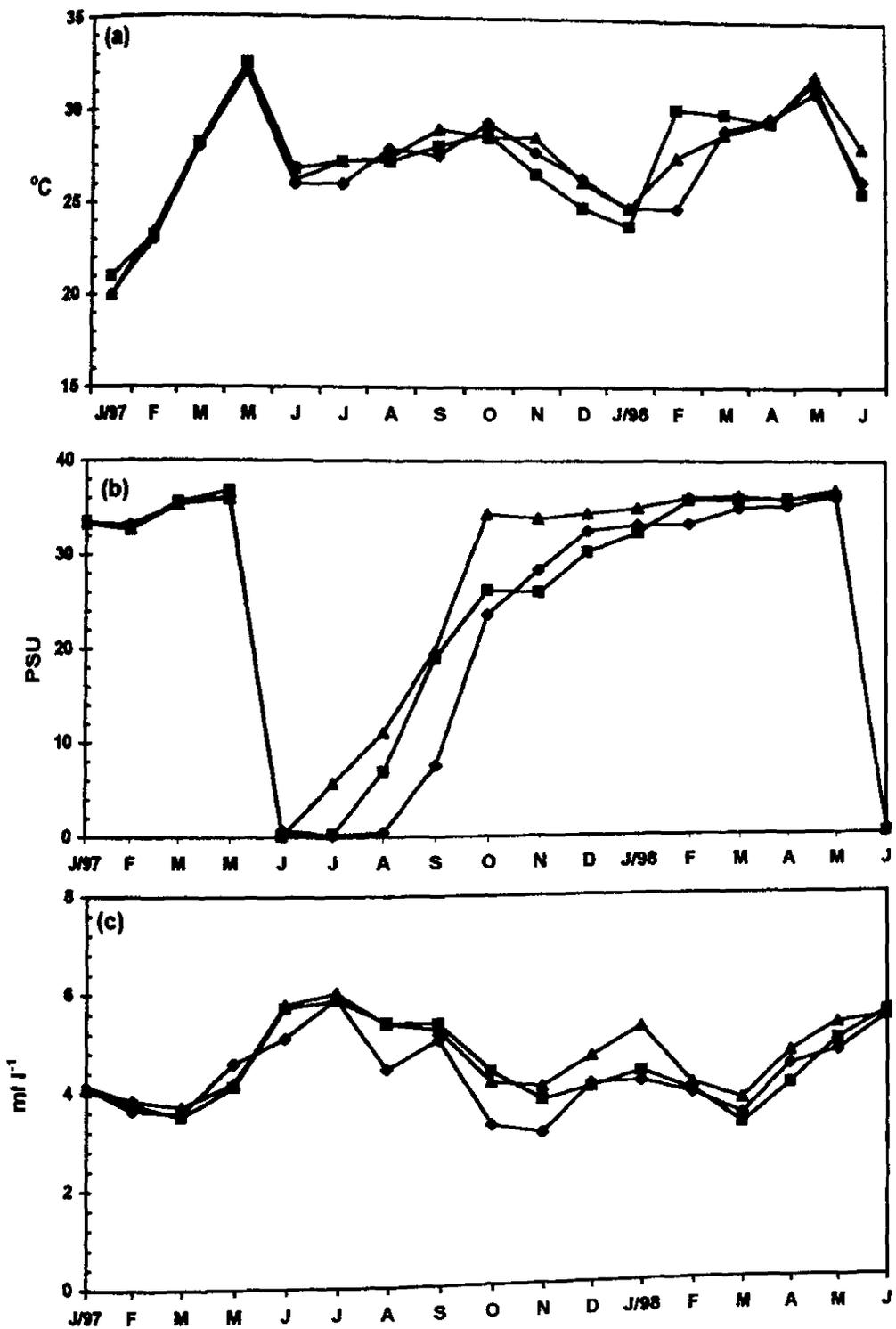


Fig. 3.1 Seasonal variation of (a) water temperature (b) salinity and (c) dissolved oxygen concentrations at reference  $\blacklozenge$ , middle  $\blacksquare$  and mouth  $\blacktriangle$  stations

station towards the sea water end, and the reference station near the fresh water end of the estuary. The middle station, being intermediary, is influenced equally by freshwater and seawater influx and hence showed no extremes in salinity.

### 3.1.3 DISSOLVED OXYGEN

The dissolved oxygen concentrations measured during the study period varied from 3.1 ml/l to 6.0 ml/l. Annual averages did not vary to any great extent and were of the same order at the reference ( $4.3 \pm 0.8$  ml/l), middle ( $4.5 \pm 0.8$  ml/l) and mouth ( $4.7 \pm 0.8$  ml/l) stations. The seasonal variations were well marked with maximum dissolved oxygen concentration in the monsoon season and lower values in the pre- and post-monsoons (Fig. 3.1c). The maximum in the monsoon season was recorded at the mouth station (mean:  $5.6 \pm 0.3$  ml/l). An interesting feature observed was the increase in dissolved oxygen concentration from the reference to the mouth stations during all seasons.

## 3.2 AMBIENT NITROGEN CONCENTRATIONS

### 3.2.1 NITRATE

Concentrations of nitrate measured at the three stations ranged between 0.4 and 19.6  $\mu\text{g at N l}^{-1}$ . As annual averages, the values were of the same order at the reference ( $4.7 \pm 5.3$   $\mu\text{g at N l}^{-1}$ ), middle ( $4.1 \pm 5.2$   $\mu\text{g at N l}^{-1}$ ) and mouth ( $4.7 \pm 4.9$   $\mu\text{g at N l}^{-1}$ ) stations (Table 3.1). The data when tested with ANOVA

showed no significant variations between the stations ( $F=0.083$ ,  $df=2,48$ ;  $P=0.92$ ).

The seasonal changes of nitrate concentrations showed significant variations (Table 3.2) and were marked by a single peak in the monsoon season, characterised by high values in June and July (Fig. 3.2). Towards the end of the monsoon season, concentrations decreased rapidly, down to a lowest value of  $0.4 \mu\text{g at N l}^{-1}$  at the middle station in September. During the post-monsoon season, there were no remarkable variations in the nitrate concentrations at all the three stations. The seasonal average concentrations were higher in the monsoon season, with the maximum values at the reference station ( $10.7 \pm 6.7 \mu\text{g at N l}^{-1}$ ). The nitrate concentrations during the pre- and post-monsoon seasons remained low throughout the study period (Table 3.1). Higher average concentrations were obtained at the reference station during the post-monsoon season ( $2.6 \pm 1.1 \mu\text{g at N l}^{-1}$ ), while at the middle and mouth stations, higher values were recorded in the pre-monsoon season ( $2.0 \pm 1.6 \mu\text{g at N l}^{-1}$  and  $2.6 \pm 1.7 \mu\text{g at N l}^{-1}$  respectively).

Table 3.1 Seasonal and annual averages of nitrate concentrations ( $\mu\text{g at N l}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$2.0 \pm 1.7$	$10.7 \pm 6.7$	$2.6 \pm 1.1$	$4.7 \pm 5.3$
Middle	$2.0 \pm 1.6$	$9.8 \pm 6.8$	$1.2 \pm 0.4$	$4.1 \pm 5.2$
Mouth	$2.6 \pm 1.7$	$10.3 \pm 5.6$	$1.9 \pm 0.8$	$4.7 \pm 4.9$

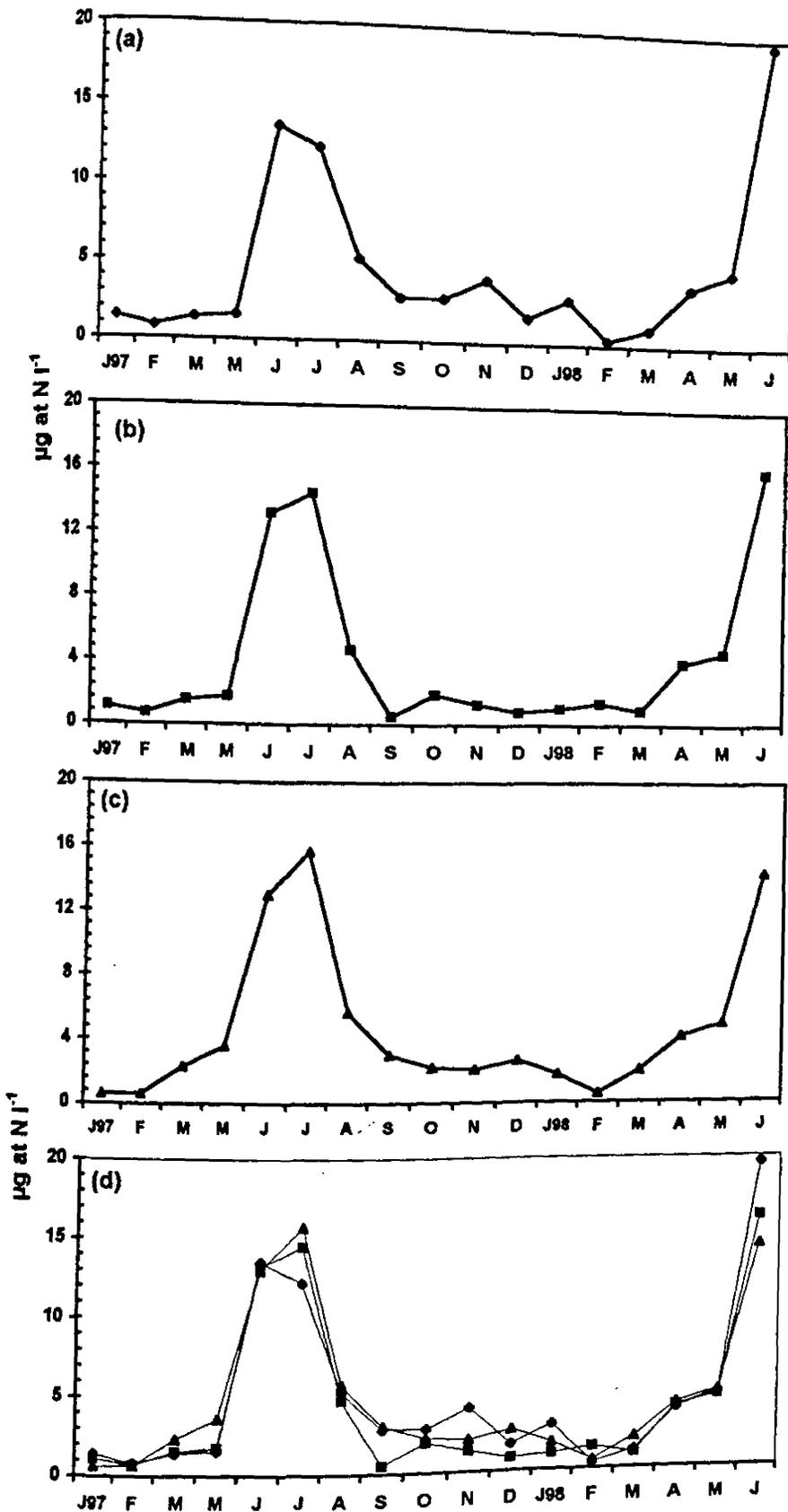


Fig.3.2 Seasonal changes of nitrate concentrations at the (a) reference,  $\blacklozenge$  (b) middle  $\blacksquare$  and (c) mouth  $\blacktriangle$  stations; (d) all stations together

Table 3.2 Results of ANOVA of nitrate concentrations

Source of variation	Stations	F	df	P-values
Between seasons	Reference	8.70	2,14	0.004
	Middle	8.11	2,14	0.005
	Mouth	11.03	2,14	0.001
Between stations	All	0.08	2,48	0.92

### 3.2.2 NITRITE

Nitrite was present in measurable concentrations ( $0.03$  to  $0.8 \mu\text{g at N l}^{-1}$ ) at all stations throughout the year. Compared with nitrate, the concentrations of nitrite varied over a relatively smaller range between the stations. Maximum average concentrations were recorded at the mouth station ( $0.4 \pm 0.3 \mu\text{g at N l}^{-1}$ ), followed by the middle ( $0.2 \pm 0.1 \mu\text{g at N l}^{-1}$ ) and reference ( $0.1 \pm 0.1 \mu\text{g at N l}^{-1}$ ) stations (Table 3.3). Though these variations were less pronounced than those of nitrate, they were still significant, as shown by ANOVA ( $F=7.84$ ,  $df=2,48$ ;  $P=0.001$ ).

ANOVA also showed significant seasonal variations (Table 3.4). The seasonal changes of nitrite (Fig. 3.3) followed a pattern similar to that of nitrate with some minor differences. The seasonal maximum was in the monsoon months with highest values at the mouth station ( $0.6 \pm 0.2 \mu\text{g at N l}^{-1}$ ). The increase to this maximum was rapid from May to June, but the decrease from the maximum during the post-monsoon season was less pronounced and was spread over the whole of this period. A secondary peak, though not prominent, can still be

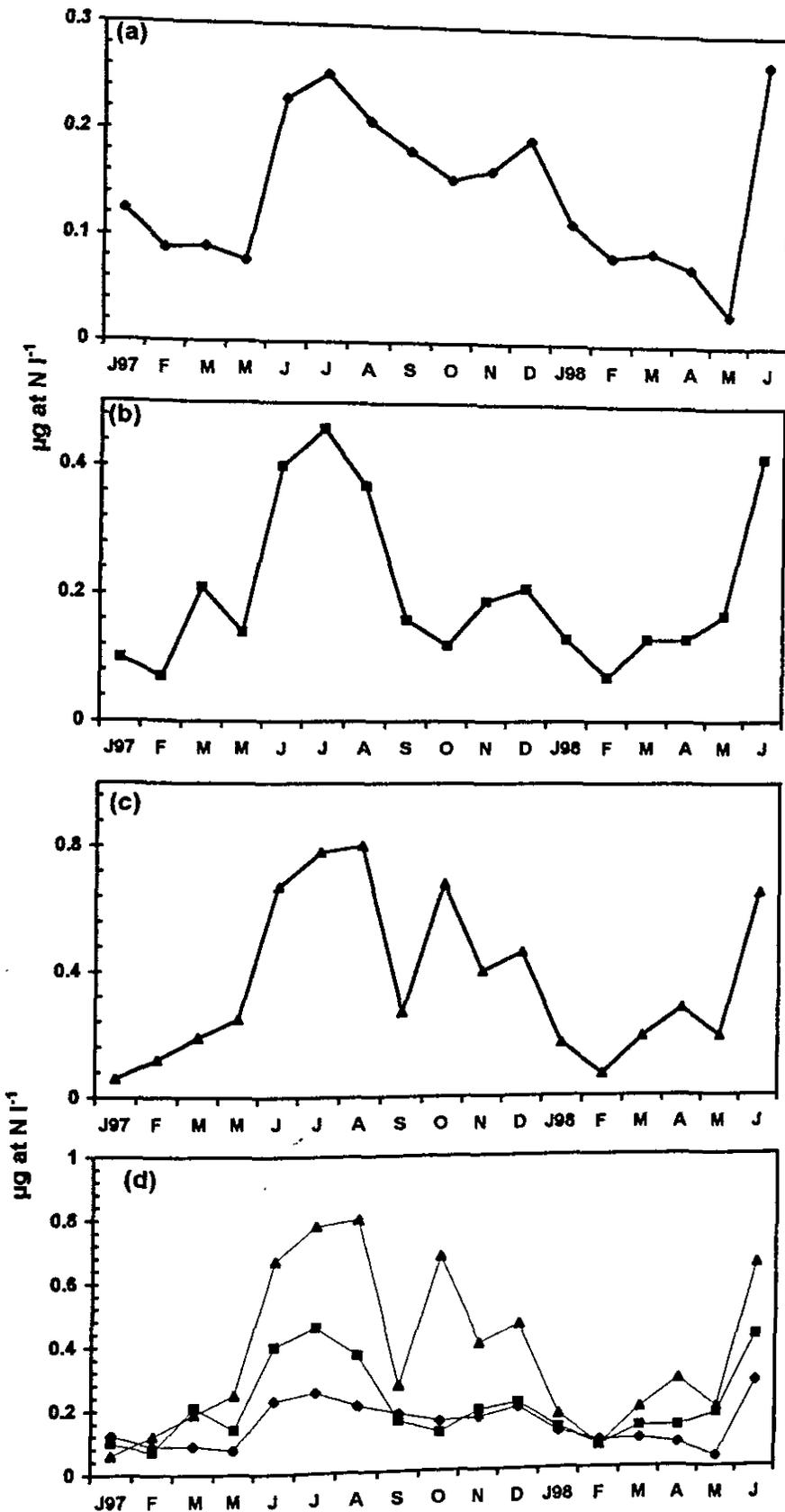


Fig. 3.3 Seasonal changes of nitrite concentrations at the (a) reference ◆ (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

recognised in December. The seasonal minimum was in February at all the three stations with lowest values at the reference station ( $0.1 \pm 0.02 \mu\text{g at N l}^{-1}$ ).

Table 3.3 Seasonal and annual averages of nitrite concentrations ( $\mu\text{g at N l}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post- monsoon	Annual
Reference	$0.1 \pm 0.02$	$0.2 \pm 0.04$	$0.2 \pm 0.03$	$0.1 \pm 0.1$
Middle	$0.1 \pm 0.1$	$0.4 \pm 0.1$	$0.2 \pm 0.1$	$0.2 \pm 0.1$
Mouth	$0.2 \pm 0.1$	$0.6 \pm 0.2$	$0.4 \pm 0.3$	$0.4 \pm 0.3$

Table 3.4 Results of ANOVA of nitrite concentrations

Source of variation	Stations	F	df	P-values
Between seasons	Reference	40.81	2,14	$1.44 \times 10^{-6}$
	Middle	15.92	2,14	0.0003
	Mouth	8.97	2,14	0.0031
Between stations	All	7.84	2,48	0.001

### 3.2.3 AMMONIUM

Ammonium concentrations during the study period ranged from 0.1 to 1.5  $\mu\text{g at N l}^{-1}$ . The average annual concentrations were similar at the reference and middle stations ( $0.6 \pm 0.3 \mu\text{g at N l}^{-1}$  and  $0.6 \pm 0.3 \mu\text{g at N l}^{-1}$ ), but only about half of these ( $0.3 \pm 0.2 \mu\text{g at N l}^{-1}$ ) at the mouth station (Table 3.5). The data, when subjected to statistical analysis, showed that ammonium concentrations varied significantly between the stations ( $F=5.15$ ,  $df=2,48$ ;  $P=0.01$ ).

The seasonal variations were significant only at the reference station (Table 3.6). The seasonal changes of ammonium concentrations (Fig. 3.4) showed a peak in the month of May, followed by a sharp decline at the beginning of the monsoon season at all three stations. It is interesting to note the timing of this peak as opposed to that of nitrate and nitrite, both of which occurred in the monsoon months. The peak in ammonium concentrations in the month of May reflected the high seasonal averages during the pre-monsoon season (Table 3.5). These were similar at the reference and middle stations ( $0.8 \pm 0.3 \mu\text{g at N l}^{-1}$  and  $0.8 \pm 0.4 \mu\text{g at N l}^{-1}$  respectively) and lowest at the mouth station ( $0.4 \pm 0.2 \mu\text{g at N l}^{-1}$ ). Towards the end of the monsoon season, the ammonium concentrations followed an increasing trend until the onset of the post-monsoon, characterised by moderately higher values in September and October. The values gradually increased during the pre-monsoon season to reach the maximum in May.

Table 3.5 Seasonal and annual averages of ammonium concentrations ( $\mu\text{g at N l}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$0.8 \pm 0.3$	$0.5 \pm 0.4$	$0.3 \pm 0.1$	$0.6 \pm 0.3$
Middle	$0.8 \pm 0.4$	$0.4 \pm 0.3$	$0.4 \pm 0.1$	$0.6 \pm 0.3$
Mouth	$0.4 \pm 0.2$	$0.3 \pm 0.2$	$0.2 \pm 0.1$	$0.3 \pm 0.2$

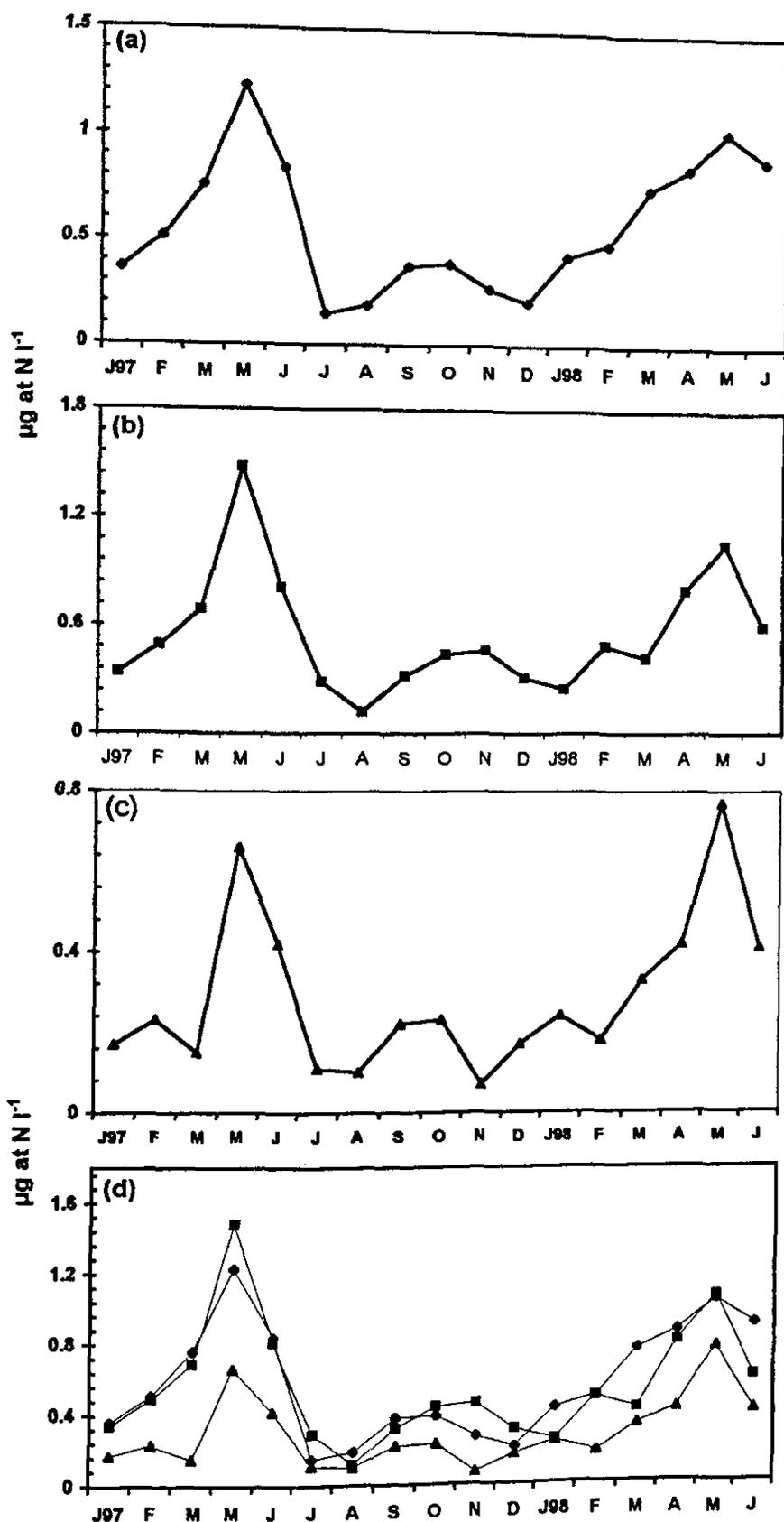


Fig.3.4 Seasonal changes of ammonium concentrations at the (a) reference ◆ (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

Table 3.6 Results of ANOVA of ammonium concentrations

Source of variation	Stations	F	df	P-values
Between seasons	Reference	5.10	2,14	0.022
	Middle	3.54	2,14	0.057
	Mouth	2.17	2,14	0.151
Between stations	All	5.15	2,48	0.01

### 3.2.4 UREA

The concentrations of urea ranged from a low of  $0.1 \mu\text{g at N l}^{-1}$  to a maximum of  $0.7 \mu\text{g at N l}^{-1}$ . Concentrations were identical at the three stations (Table 3.7) and ANOVA confirmed these spatial variations ( $F= 0.73$ ;  $df=2,48$ ;  $P=0.49$ ).

Although the seasonal variations were statistically insignificant (Table 3.8), the monthly changes followed a well defined cycle (Fig. 3.5). A single peak was observed in June at the three stations which was more pronounced at the mouth station compared to the middle and reference stations. In the pre-monsoon season, an increasing trend was observed at the three stations. This increase at the mouth station was punctuated with a sudden decrease in May. During the post-monsoon season, urea concentrations showed no significant increase or decrease except in January at the middle station where concentrations dropped to a minimum of  $0.1 \mu\text{g at N l}^{-1}$ . The seasonal average concentrations (Table 3.7) were maximum in the monsoon season with highest values at the mouth of

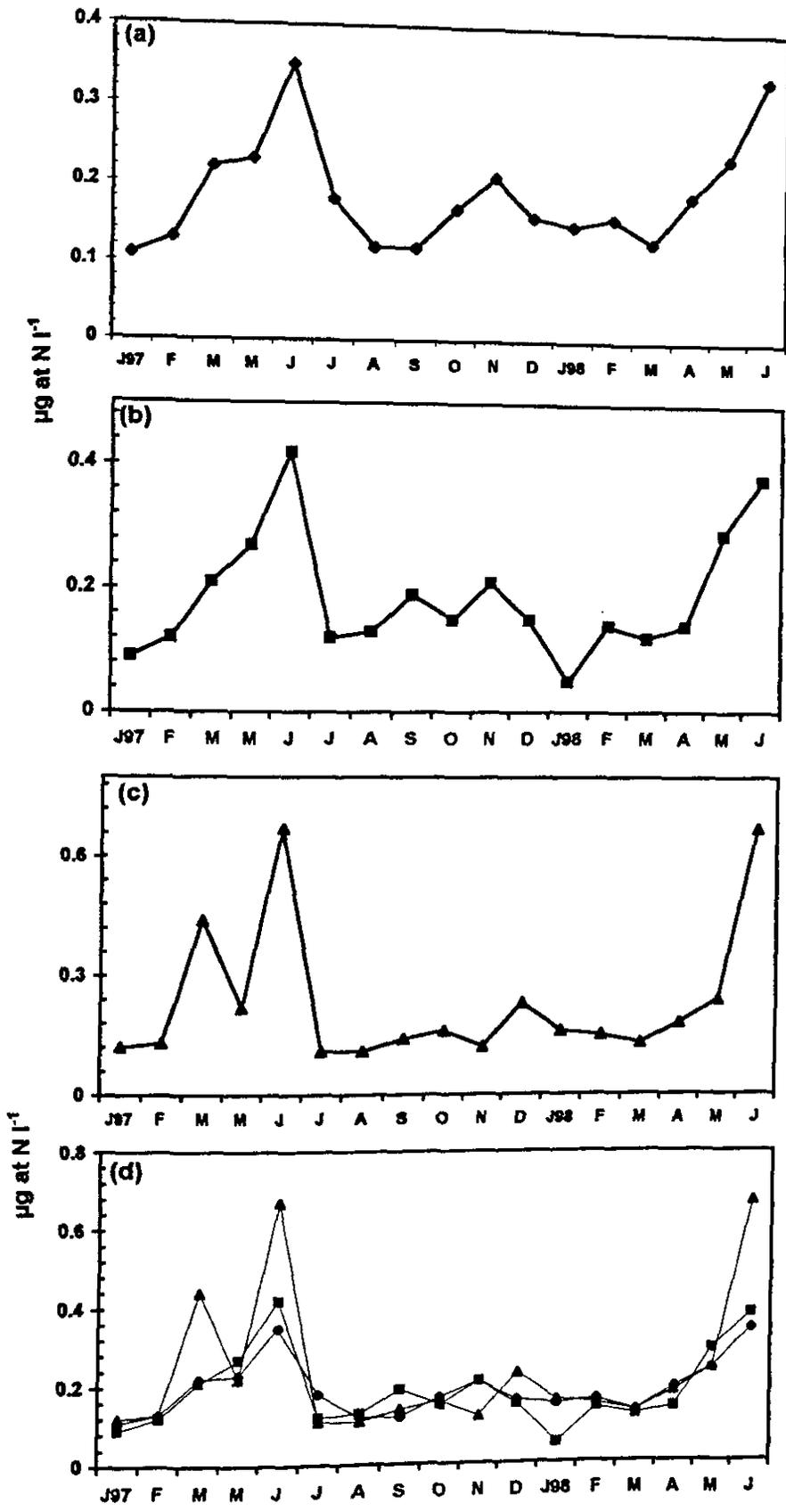


Fig.3.5 Seasonal changes of urea concentrations at the (a) reference ◆ (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

the estuary ( $0.3 \pm 0.3 \mu\text{g at N l}^{-1}$ ). This was followed by the pre-monsoon season and the lowest concentrations were recorded in post-monsoon season with the minimum at the middle station ( $0.1 \pm 0.1 \mu\text{g at N l}^{-1}$ ).

Table 3.7 Seasonal and annual averages of urea concentrations ( $\mu\text{g at N l}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$0.2 \pm 0.1$	$0.2 \pm 0.1$	$0.2 \pm 0.04$	$0.2 \pm 0.1$
Middle	$0.2 \pm 0.1$	$0.3 \pm 0.2$	$0.1 \pm 0.1$	$0.2 \pm 0.1$
Mouth	$0.2 \pm 0.1$	$0.3 \pm 0.3$	$0.2 \pm 0.1$	$0.2 \pm 0.2$

Table 3.8 Results of ANOVA of urea concentrations

Source of variation	Stations	F	df	P-values
Between seasons	Reference	0.95	2,14	0.41
	Middle	1.93	2,14	0.18
	Mouth	1.33	2,14	0.30
Between stations	All	0.73	2,48	0.49

### 3.2.5 PARTICULATE ORGANIC NITROGEN

The particulate organic nitrogen (PON) of surface water samples ranged from 12.1 to 207.9  $\mu\text{g at N l}^{-1}$  over the whole study period. Very high averages were recorded at the reference station ( $85.8 \pm 43.6 \mu\text{g at N l}^{-1}$ ) and the lowest at the mouth station ( $48.6 \pm 51.6 \mu\text{g at N l}^{-1}$ ). Moderately high values ( $72.5 \pm 36.7 \mu\text{g at N l}^{-1}$ ) were recorded at the middle station (Table 3.9). ANOVA confirmed these spatial variations ( $F=3.06$ ,  $df=2,48$ ;  $P=0.05$ ).

Significant variations were also seen between the seasons (Table 3.10). The seasonal variations showed a peak in the middle of the monsoon season at all three stations, followed by a decreasing trend towards the end of this season (Fig. 3.6). There were no remarkable variations in the PON concentrations during the post-monsoon season. However, in the pre-monsoon months, the concentrations increased gradually to reach the peak in July. Maximum seasonal averages were recorded in the monsoon season (Table 3.9) with highest values at the reference station ( $137.4 \pm 41.5 \mu\text{g at N l}^{-1}$ ), while lowest concentrations were recorded during the post-monsoon season at the mouth station ( $17.7 \pm 4.0 \mu\text{g at N l}^{-1}$ ).

Table 3.9 Seasonal and annual averages of PON concentrations ( $\mu\text{g at N l}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$62.6 \pm 23.0$	$137.4 \pm 41.5$	$66.7 \pm 18.9$	$85.8 \pm 43.6$
Middle	$61.2 \pm 20.2$	$112.1 \pm 43.0$	$48.8 \pm 6.2$	$72.5 \pm 36.7$
Mouth	$39.9 \pm 42.5$	$100.1 \pm 63.9$	$17.7 \pm 4.0$	$48.6 \pm 51.6$

Table 3.10 Results of ANOVA of PON concentrations

Source of variation	Stations	F	df	P-values
Between seasons	Reference	11.55	2,14	0.001
	Middle	8.08	2,14	0.005
	Mouth	7.24	2,14	0.007
Between stations	All	3.06	2,48	0.05

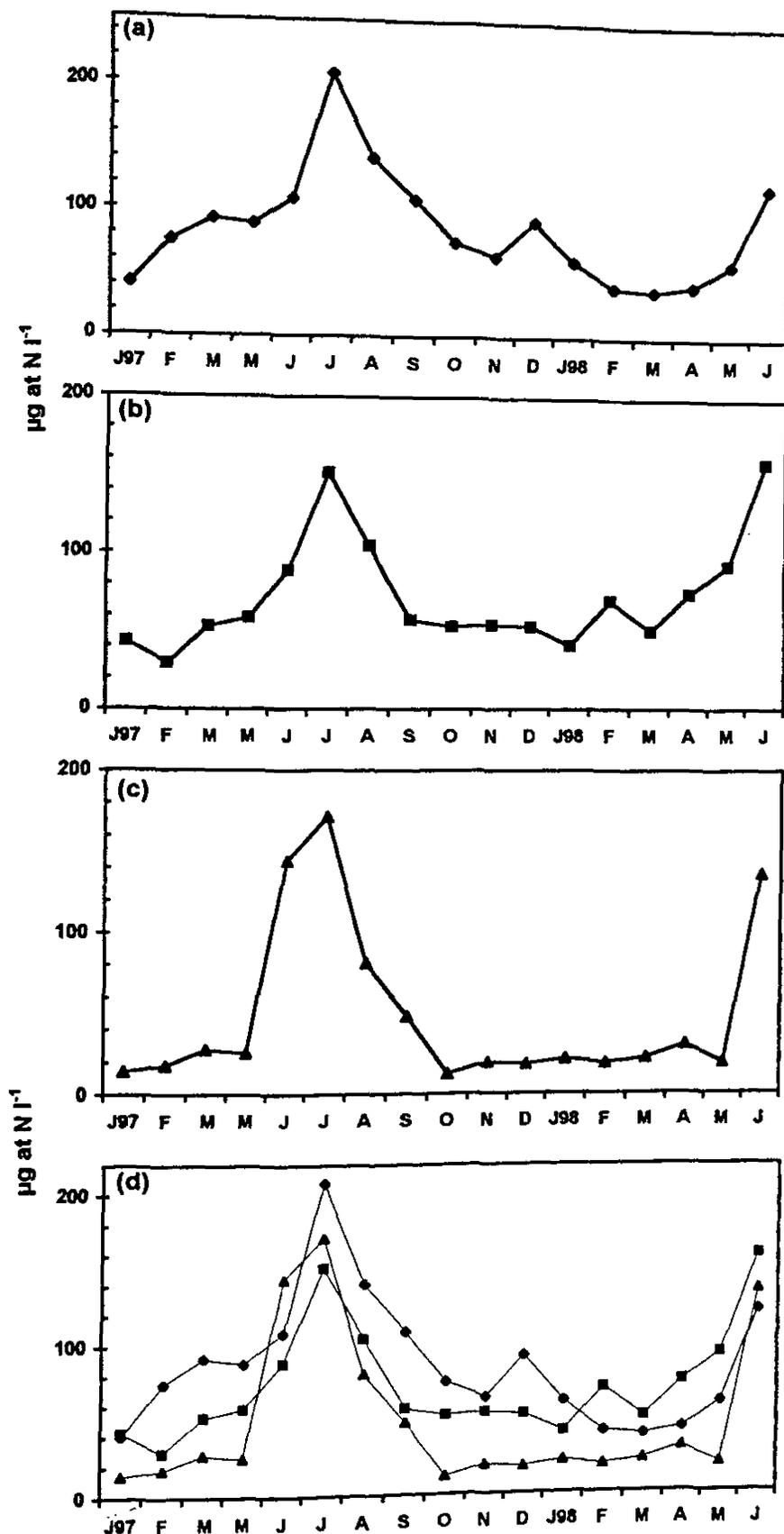


Fig.3.6 Seasonal changes of particulate organic nitrogen concentrations at the (a) reference◆, (b) middle■ and (c) mouth ▲ stations; (d) all stations

### 3.3 BIOLOGICAL PARAMETERS

#### 3.3.1 CHLOROPHYLL-A

Surface chlorophyll-*a* concentrations varied in a wide range from 0.1 to 21.6  $\mu\text{g (chl-a) l}^{-1}$  during the study period. The average concentrations at the middle and mouth stations were identical ( $8.7 \pm 5.4$  and  $8.7 \pm 5.2 \mu\text{g (chl-a) l}^{-1}$  respectively), while a slightly lower value was recorded at the reference station ( $7.6 \pm 4.8 \mu\text{g (chl-a) l}^{-1}$ ) (Table 3.11). Tests with ANOVA showed no significant variations between the stations ( $F=0.26$ ,  $df=2,48$ ;  $P=0.77$ ).

The seasonal variations were significant only at the mouth station (Table 3.12). The seasonality of chlorophyll-*a* concentrations showed a distinct peak in the month of May at all three stations (Fig. 3.7). The intensity of this maximum was however the highest at the middle station ( $21.6 \mu\text{g (chl-a) l}^{-1}$ ). The high concentrations in the pre-monsoon months were followed by a sharp decline at the beginning of the monsoon season, with minimum values in July. During this season, chlorophyll-*a* concentrations decreased from the reference to the mouth station (Table 3.11). Towards the end of the monsoon months, in August, concentrations increased rapidly to reach high values in September at all three stations. As seasonal averages, high values were recorded in the post-monsoon season, with the seasonal maximum at the mouth station ( $11.9 \pm 3.8 \mu\text{g (chl-a) l}^{-1}$ ).

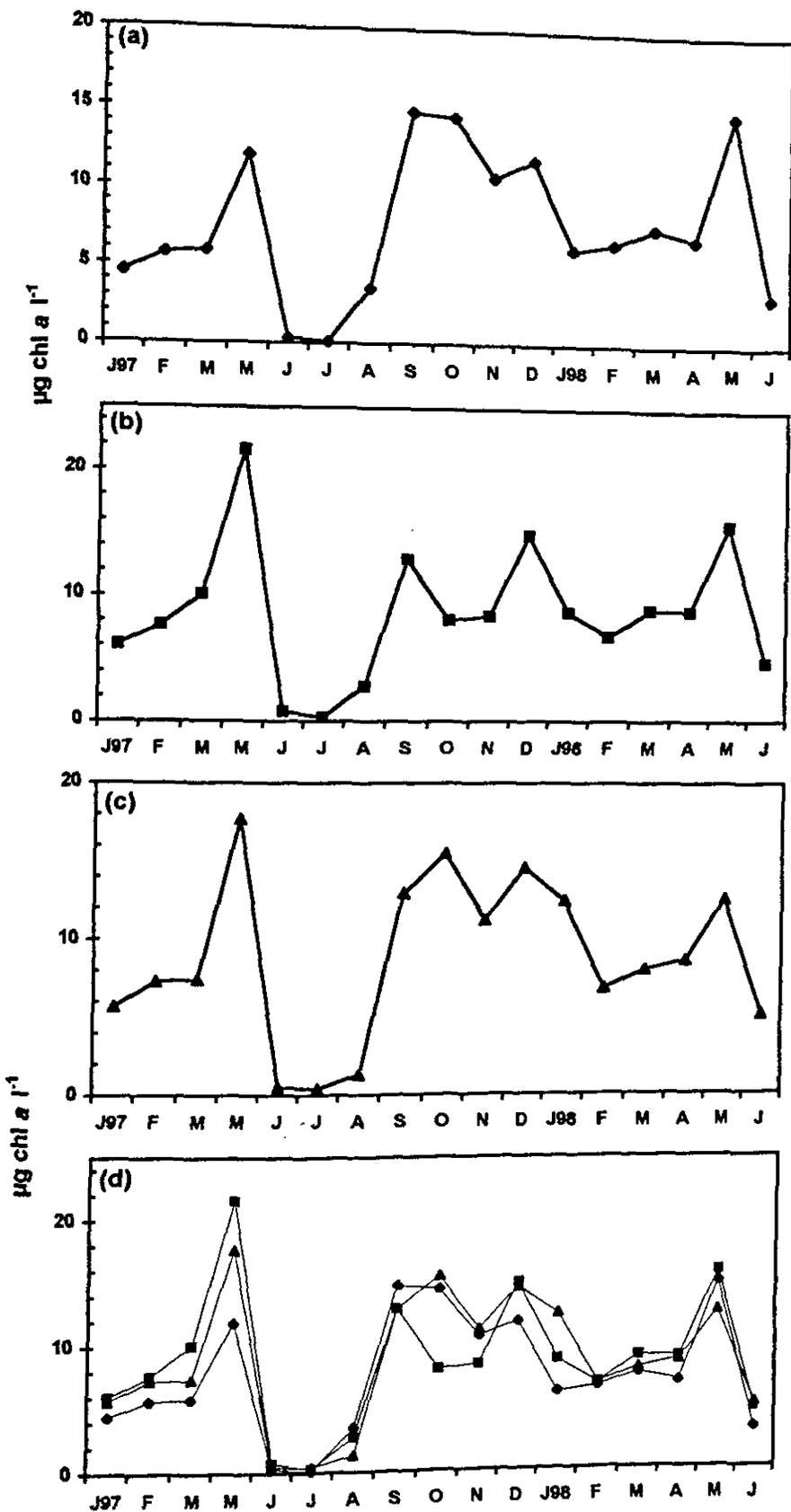


Fig.3.7 Seasonal changes of total chlorophyll-a concentrations at the (a) reference  $\blacklozenge$ , (b) middle  $\blacksquare$  and (c) mouth  $\blacktriangle$  stations; (d) all stations together

Table 3.11 Seasonal and annual averages of chlorophyll-a concentrations ( $\mu\text{g chl-a l}^{-1}$ )

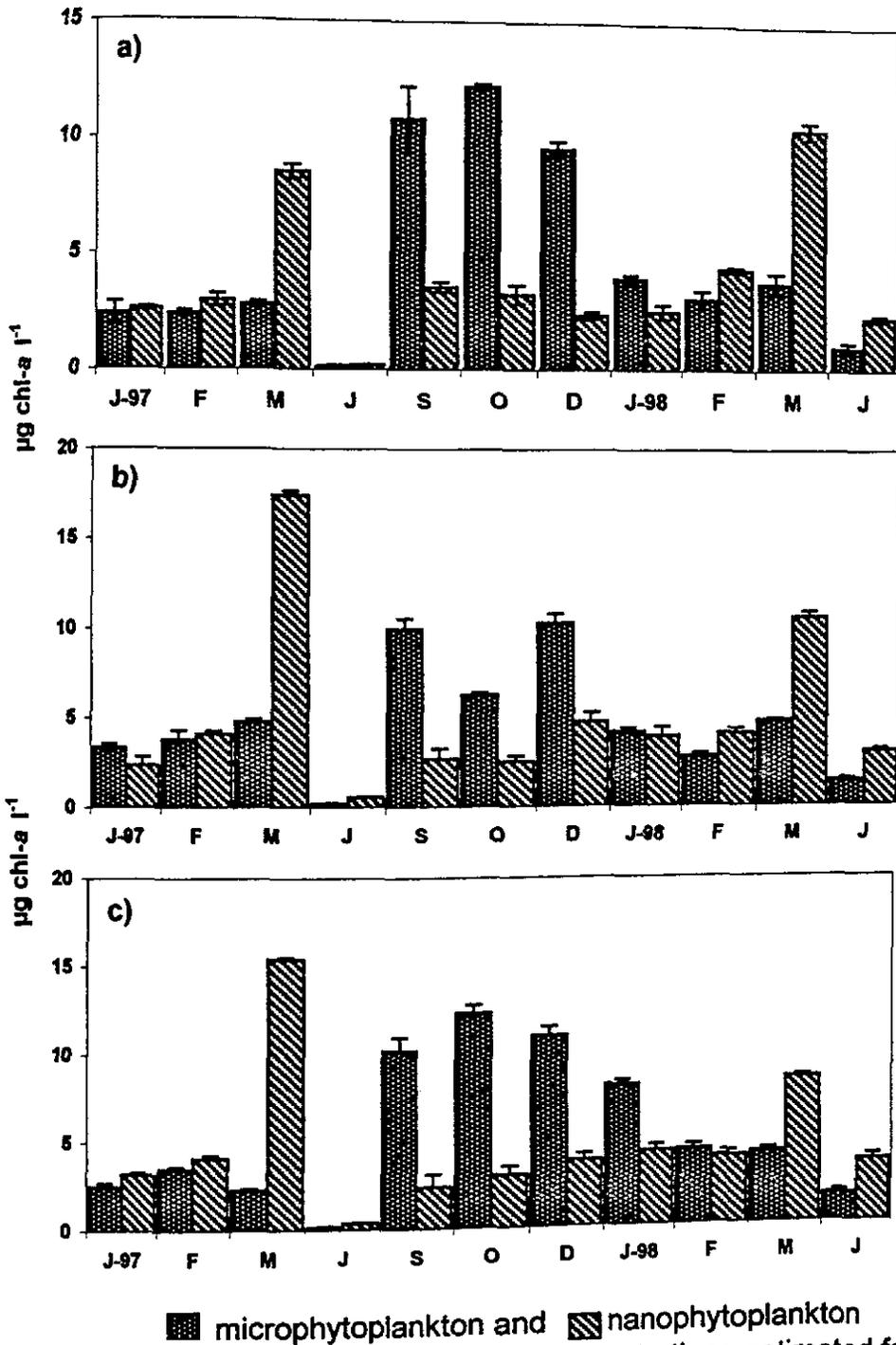
Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	8.5 $\pm$ 3.5	4.4 $\pm$ 6.0	9.5 $\pm$ 4.1	7.6 $\pm$ 4.8
Middle	11.4 $\pm$ 5.4	4.3 $\pm$ 5.1	9.2 $\pm$ 3.4	8.7 $\pm$ 5.4
Mouth	9.8 $\pm$ 4.0	4.0 $\pm$ 5.3	11.9 $\pm$ 3.8	8.7 $\pm$ 5.2

Table 3.12 Results of ANOVA of chlorophyll-a concentrations

Source of variation	Stations	F	df	P-values
Between seasons	Reference	1.84	2,14	0.20
	Middle	3.21	2,14	0.07
	Mouth	4.35	2,14	0.03
Between stations	All	0.26	2,48	0.77

### 3.3.2 CHLOROPHYLL-A OF SIZE FRACTIONS

The seasonal distribution of chlorophyll-a estimated for the two fractions, viz. the microphytoplankton (200-20  $\mu\text{m}$ ) and the nanophytoplankton (20-0.8  $\mu\text{m}$ ) are shown in Fig. 3.8. In the overall study, a comparison of the chlorophyll-a concentrations during the three seasons (Fig. 3.8) showed a maximum contribution of the nanophytoplankton in the monsoon season (76%). Although the percentage contribution of the nanophytoplankton was high during the monsoon season, the total density of the population during these months was low in comparison to the pre-monsoon season. Of the total population in the pre-monsoon season, the nanophytoplankton contributed to 66%, while, in the post-monsoon months, their population was low (19%). The microphytoplankton



■ microphytoplankton and ▨ nanophytoplankton

Fig. 3.8 Seasonal distribution of chlorophyll a concentrations estimated for the two size fractions at the (a) reference, (b) middle and (c) mouth stations

dominated the population in the post-monsoon season, contributing to 81% of the total chlorophyll-a but accounted for relatively less percentages, 37% and 24% in the pre-monsoon and monsoon seasons respectively.

### 3.3.3 PHYTOPLANKTON CELL COUNTS AND SPECIES DISTRIBUTION

Throughout the study cycle, diatoms constituted the major group of phytoplankton (58.7%). Flagellates and blue green algae were recorded in more or less equal proportions (20.8% and 20.5 % respectively). The percentage contribution of the different groups of phytoplankton is given in Table 3.13 a.

Table 3.13 a Percentage contribution of different groups of phytoplankton to the total population

STATIONS	DIATOMS	BLUE GREEN ALGAE	FLAGELLATES
Reference	56.7%	21.6%	21.7%
Middle	60.2%	18.0%	21.8%
Mouth	59.3%	21.9%	18.8%

Succession of species was observed at all the stations (Fig. 3.9 a-c). During the pre-monsoon months, centric diatoms and blue green algae dominated the population. Of the total population of the centric forms (60.94%), *Coscinodiscus marginatum* was abundant. During the initial pre-monsoon months (February, March), a mixed diatom population comprising of *Nitzschia closterium*, *Rhizosolenia setigera*, *Chaetoceros debilis*, *C. indicus*, *Merismopedia glauca* were recorded. The blue green algae that thrived during this period accounted for 39.05% of the phytoplankton crop. The occurrence of *Trichodesmium erythraeum* blooms appeared to be a recurring phenomenon in the month of

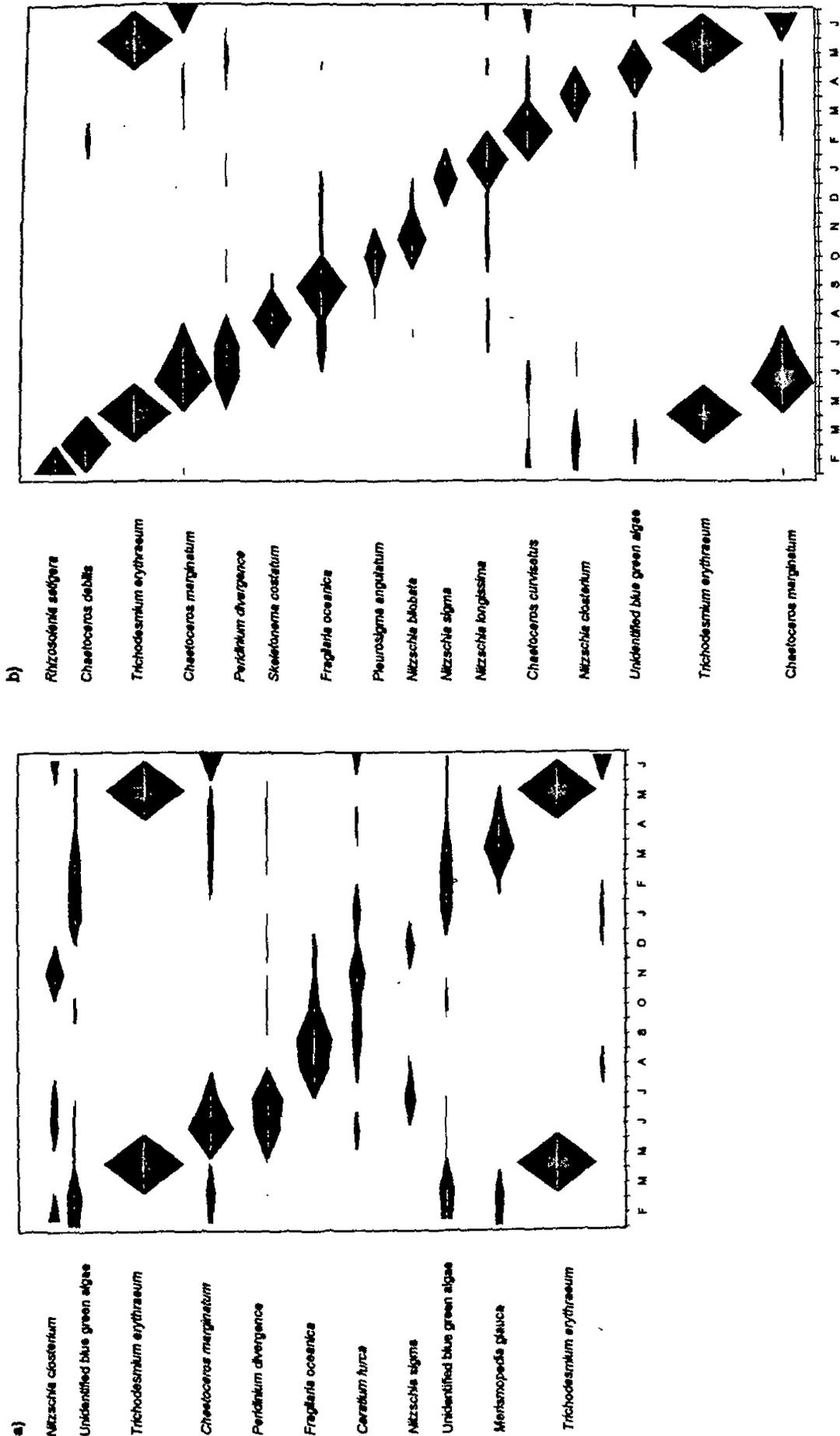


Fig. 3.9 Seasonal changes in the relative abundance of phytoplankton species at the (a) reference and (b) middle stations. Abundance expressed as % of the total number of cells identified

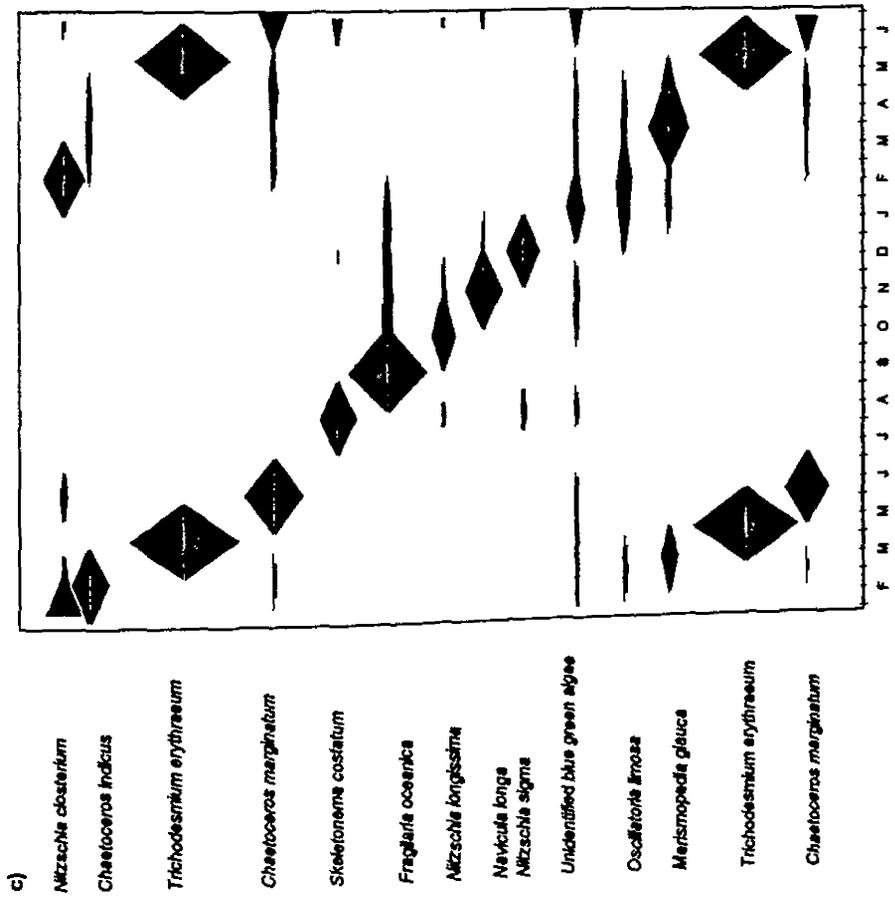


Fig. 3.9 Seasonal changes in the relative abundance of phytoplankton species at the (c) mouth station. Abundance expressed as % of the total number of cells identified

May and significantly increased the total phytoplankton cell counts. Other blue green forms also present were *Oscillatoria limosa* and *Trichodesmium thiebautii*, though in less numbers.

With the onset of the monsoon season and the subsequent reduction in the salinity levels, the high salinity tolerant forms were immediately replaced with species such as *Chaetoceros marginatum*, *Peridinium divergence* and *Skeletonema costatum* in June and July. In the late monsoon months (August and September), a gradual decline in rainfall resulted in an increase in the salinity regime in the estuary. During this period, *Fragilaria oceanica* dominated the population and was recorded at all the stations. The flagellate forms contributed 27.7% to the phytoplankton crop during this period and the abundant species were *Ceratium furca*, *C. horridum*, *Dinophysis miles* and *Gonyaulax tamarensis*.

During the post-monsoon period (October and January), the percentage ratio among the taxa varied: diatoms (65.23%) and flagellates (26.81%) dominated the community, while blue green algae were less abundant (7.13%). Of the total diatom population, the pennate forms accounted for 76.34%. Among these, *Nitzschia sigma*, *N. bilobata*, *N. longissima*, *Pleurosigma angulatum* and *Fragilaria oceanica* were important. A list of the species recorded at Achara estuary is given in Table 3.13 b.

**Table 3.13 List of phytoplankton species recorded at Achara estuary**

**Diatoms**

*Amphiprora gigantea* Grunow var. *sulcata*  
(O'Meara) Cleve  
*Amphora ostrearia*  
*Amphora lineolata*  
*Asterionella japonica* Cleve  
*Bacillaria paradoxa* Gmelin  
*Biddulphia favus*  
*Biddulphia sinensis*  
*Chaetoceros curvisetus* Cleve  
*Chaetoceros debilis*  
*Chaetoceros affinis*  
*Chaetoceros marginatus* Ehrenberg  
*Chaetoceros indicus* sp. nov.  
*Coscinodiscus perforatus* var. *Pavillardi*  
(forti) Hustedt  
*Coscinodiscus gigas* Ehrenberg  
*Coscinodiscus marginatum*  
*Coscinodiscus rothii* (Ehrenberg) Grunow,  
var. *subsalsa* (Juhliln-Dannfelt). Hustedt  
*Ditylum brightwellii* (West) Grunow  
*Fragilaria oceanica* Cleve  
*Fragilaria cylindrus* Cleve  
*Gyrosigma balticum* (Ehrenberg) Rabenhorst  
*Grammatophora undulata* Ehrenberg  
*Leptocylindrus danicus* Cleve  
*Melosira moniliformis* (Muller) Agardh  
*Navicula salinarum*  
*Navicula hennedyii*  
*Navicula longa* (Gregory) Ralfs  
*Nitzschia bilobata* W.Smith  
*Nitzschia closterium* (Ehrenberg) W.Smith  
*Nitzschia delicatissima*  
*Nitzschia longissima* (Brebisson) Ralfs  
*Nitzschia panduriformis* Grunow  
*Nitzschia seriata* Cleve  
*Nitzschia sigma* W.Smith var. *indica* Karsten  
*Pinnularia alpina* W.Smith  
*Pleurosigma angulatum* W.Smith

*Pleurosigma carinatum* Donkin  
*Pleurosigma normanii* Ralfs  
*Rhizosolenia alata* Brightwell f. *indica*  
*Rhizosolenia robusta* Norma  
*Rhizosolenia setigera* Brightwell  
*Rhizosolenia fragilissima*  
*Schroederella delicatula* (Pergallo) Pavillard  
*Skeletonema costatum* (Greville) Cleve  
*Striatella unipunctata*  
*Surirella fluminesis* Grunow  
*Thalassionema nitzschioides* Grunow  
*Thalassiothrix frauenfeldii* Irunow  
*Thalassiothrix longissima*  
*Triceratium favus* Ehrenberg

**Flagellates**

*Ceratium extensum*  
*Ceratium tripos* (O.F.Muller) Nitzsch  
*Ceratium furca* Ehrenberg  
*Ceratium horridum* (Cleve) Gran  
*Ceratium lineatum* Ehrenberg  
*Dinophysis caudata* Kent  
*Dinophysis miles*  
*Dunaliella tetriolecta*  
*Gonyaulax tamarensis*  
*Olisthodiscus* sp.  
*Peridinium balticum*  
*Peridinium chattoni*  
*Peridinium divergence* Ehrenberg  
*Peridinium triquestrum*  
*Platymonas* sp.  
*Prorocentrum micans* Ehrenberg  
*Parphyridium* sp.

**Blue Green Algae**

*Merismopedia glauca*  
*Trichodesmium erythraeum* Ehrenberg  
*Trichodesmium thiebautii* Gomont  
*Oscillatoria limosa*  
Unidentified blue green algae

Maximum cell counts were recorded at the middle station ( $525 \pm 367 \times 10^3$  cells/l), followed by the reference ( $515 \pm 396 \times 10^3$  cells/l) and mouth ( $382 \pm 267 \times 10^3$  cells/l) stations (Table 3.14). The cell counts tested with ANOVA showed significant spatial variations ( $F=4.65$ ;  $df=2,45$ ;  $P=0.01$ ).

At the reference station, diatoms contributed significantly to the phytoplankton population (56.7%) of which, *Fragilaria oceanica* dominated. Other abundant species were *Chaetoceros marginatum*, *Nitzschia closterium* and *Coscinodiscus rothii*. *Trichodesmium erythraeum* occurred in large numbers in the month of May ( $1780 \times 10^3$  cells/l). The flagellates accounted for 21.7% of the total population and *Ceratium furca* was the most abundant.

At the middle station, diatoms formed 60.2% of the population. Among the diatom population, *Fragilaria oceanica* was the most abundant species with a density of  $448 \times 10^3$  cells/l. *Nitzschia closterium* and *N. sigma* were also abundantly found. Blooms of the cyanophyte, *Trichodesmium erythraeum* were recorded in May ( $1725 \times 10^3$  cells/l). The flagellate population was composed of *Ceratium furca* ( $284 \times 10^3$  cells/l) and *Dinophysis miles* ( $115 \times 10^3$  cells/l).

The phytoplankton population at the mouth station was largely composed of diatoms (59.3%). The dominant species were *Fragilaria oceanica* ( $394 \times 10^3$  cells/l), *N. closterium* and *N. longissima*. Among the blue green algae, *Trichodesmium erythraeum* and was recorded in high numbers in the month of

May. The total contribution of this species was lower compared with the reference and middle stations ( $1205 \times 10^3$  cells/l). The flagellate population was low (18.8%).

Though statistically the seasonal variations were insignificant at all the stations (Table 3.15), the seasonal changes showed high cell counts in the pre- and post-monsoon seasons and lowest densities during the monsoon season (Fig. 3.10a). The trend followed a different cycle for each station. At the reference station, two distinct peaks were observed. The first one was in the month of May (1997 and 1998) and the second one was more spread out, from the end of the monsoon season into the post-monsoon season. The seasonal trend at the middle station showed the pre-monsoon peaks, but no post-monsoon high. The changes at the mouth station were erratic with alternating increases and decreases, resulting in four peaks. As seasonal averages, maximum cell counts were recorded in the post-monsoon season at the middle station ( $700 \pm 144 \times 10^3$  cells/l) and minimum during the monsoon season at the mouth station ( $180 \pm 282 \times 10^3$  cells/l) (Table 3.14).

The Shannon-Weaver diversity indices ranged from 0 to 5.3 bits and the seasonal variations showed higher species diversity in the post-monsoon season at the three stations (Fig. 3.10b). Maximum diversity index was obtained in the month of August at all the stations. The diversity indices were low in the monsoon season and minimum values were obtained at the mouth station in

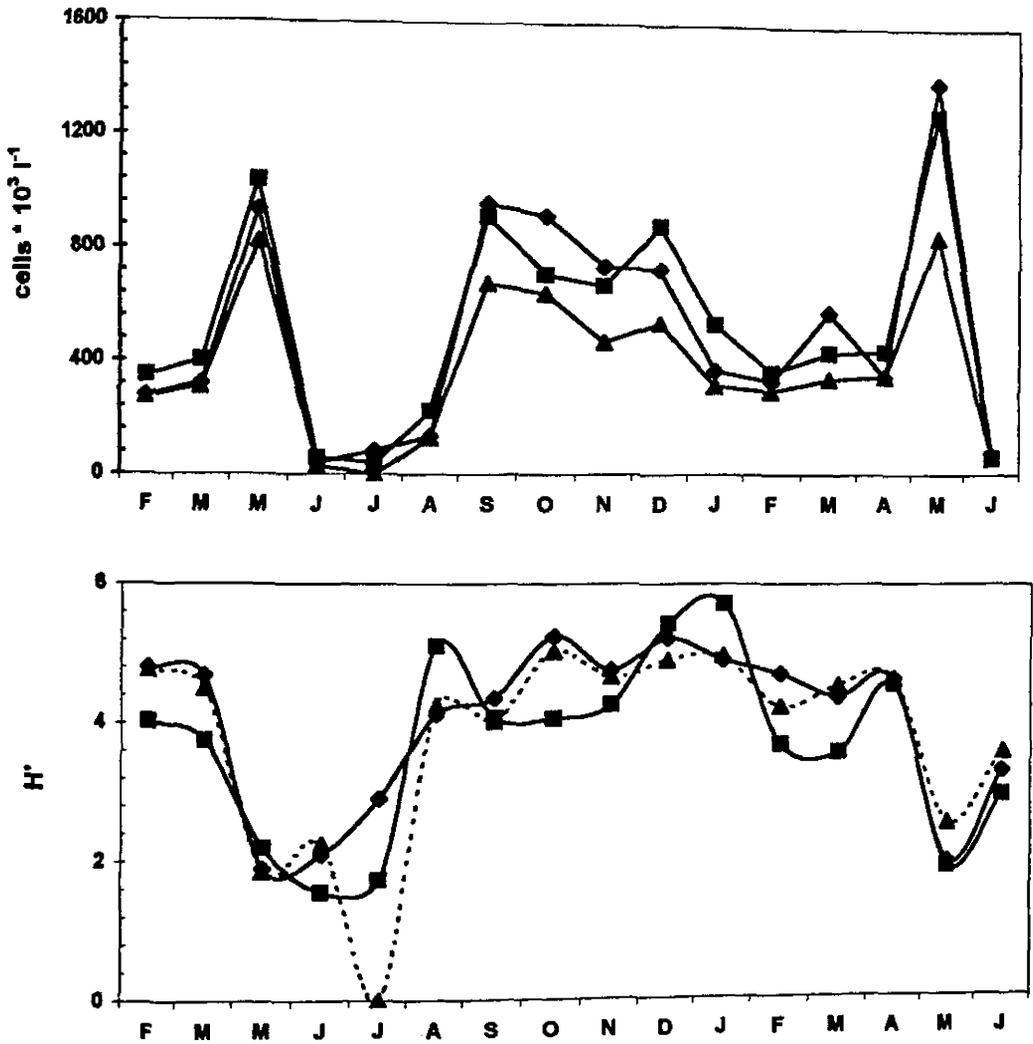


Fig. 3.10 Seasonal and spatial changes of (a) phytoplankton cell counts and (b) diversity indices at the reference  $\blacklozenge$ , middle  $\blacksquare$  and mouth  $\blacktriangle$  stations

July (0 bits). Among the three stations, maximum species diversity was recorded at the reference station (4.4 bits), followed by the middle (4.2 bits) and mouth (4.0 bits) stations.

Table 3.14 Seasonal and annual averages of phytoplankton cell counts (cells x  $10^3$  l)

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	600 ± 424	257 ± 393	689 ± 230	515 ± 396
Middle	616 ± 382	260 ± 373	700 ± 144	525 ± 367
Mouth	646 ± 257	180 ± 282	492 ± 137	382 ± 267

Table 3.15 Results of ANOVA of phytoplankton cell counts

Source of variation	Stations	F	df	P-values
Between seasons	Reference	1.77	2,13	0.21
	Middle	1.65	2,13	0.23
	Mouth	2.25	2,13	0.14
Between stations	All	4.65	2,45	0.01

### 3.4 REGENERATION OF NITROGEN

#### 3.4.1 AMMONIFICATION RATES

Ammonium regeneration rates measured in the present study ranged from 10 to 1500  $\eta\text{g}$  at  $\text{N l}^{-1} \text{h}^{-1}$ . Annual averages between the stations did not vary considerably. Maximum rates were recorded at the middle station ( $410 \pm 500 \eta\text{g}$  at  $\text{N l}^{-1} \text{h}^{-1}$ ), followed by the reference station ( $360 \pm 430 \eta\text{g}$  at  $\text{N l}^{-1} \text{h}^{-1}$ ) and the lowest rates at the mouth station ( $220 \pm 260 \eta\text{g}$  at  $\text{N l}^{-1} \text{h}^{-1}$ ) (Table 3.16). ANOVA showed that spatial variations were not significant ( $F=0.93$ ,  $df=2,45$ ;  $P=0.4$ ).

The seasonal changes of regeneration rates showed significant variations (Table 3.17) and were marked by a pre-monsoon peak at all stations (Fig. 3.11). The regeneration rates dropped to a minimum at the beginning of the monsoon season, in the month of June, and then increased gradually through the post-monsoon months. From February onwards, rates increased rather rapidly to reach the pre-monsoon peak in May. Although the intensities of these peaks were highest at the middle station, the general trend followed was more or less the same at all three stations. With the exception of the reference and mouth stations, the middle station showed a minor increase in October ( $200 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ), followed by a decrease in November. The seasonal averages were maximum in the pre-monsoon season, with highest rates at the middle station ( $870 \pm 440 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ), followed by the reference ( $680 \pm 480 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ) and mouth ( $440 \pm 270 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ) stations. The rates during the post-monsoon season were low in comparison with the pre-monsoon season and the lowest rates were recorded in the monsoon season (Table 3.16).

Table 3.16 Seasonal and annual averages of ammonium regeneration rates ( $\text{ng at N } \Gamma^1 \text{ h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$680 \pm 480$	$40 \pm 30$	$200 \pm 30$	$360 \pm 430$
Middle	$870 \pm 440$	$30 \pm 10$	$100 \pm 50$	$410 \pm 500$
Mouth	$440 \pm 270$	$30 \pm 10$	$90 \pm 30$	$220 \pm 260$

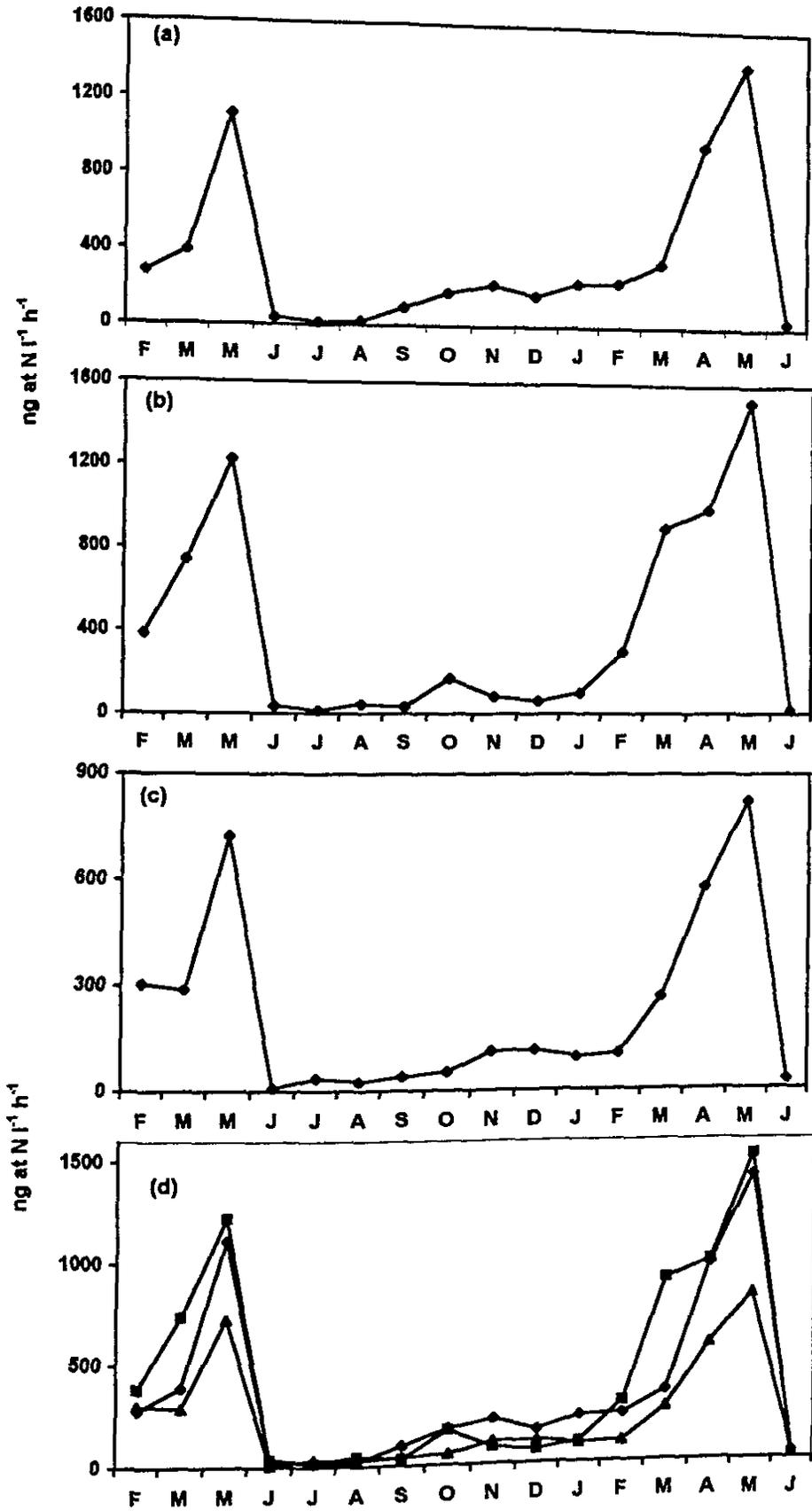


Fig. 3.11 Seasonal changes of ammonification rates at the (a) reference ◆, (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

Table 3.17 ANOVA results of ammonium regeneration rates

Source of variation	Stations	F	df	P-values
Between seasons	Reference	6.29	2,13	0.01
	Middle	14.41	2,13	0.001
	Mouth	8.81	2,13	0.004
Between stations	All	0.93	2,45	0.4

### 3.4.2 NITRIFICATION RATES

Nitrification rates in the water column varied from 0.1 to 96.7  $\eta\text{g at N l}^{-1} \text{ h}^{-1}$  during the study period. The averages between the reference and middle stations differed only marginally ( $49.9 \pm 28.5 \eta\text{g at N l}^{-1} \text{ h}^{-1}$  and  $47.3 \pm 29.2 \eta\text{g at N l}^{-1} \text{ h}^{-1}$  respectively), while at the mouth station, lower nitrification rates were recorded ( $30.6 \pm 19.3 \eta\text{g at N l}^{-1} \text{ h}^{-1}$ ) (Table 3.18). ANOVA showed that the spatial variations in nitrification rates were not significant ( $F=1.63$ ,  $df=2,33$ ;  $P=0.21$ )

Significant seasonal variations in nitrification rates were observed at the three stations (Table 3.19). The trend showed increasing rates from minimum values in the monsoon season, to moderately high values in the post-monsoon and reaching finally maximum nitrification activity in the pre-monsoon season (Fig. 3.12). Maximum nitrification rates were recorded in the month of May at all three stations, with highest rates at the reference station ( $96.7 \eta\text{g at N l}^{-1} \text{ h}^{-1}$ ). This also reflects in the high seasonal averages during the pre-monsoon season, with maximum nitrification activity at the reference station ( $77.8 \pm 17.5 \eta\text{g at N l}^{-1}$

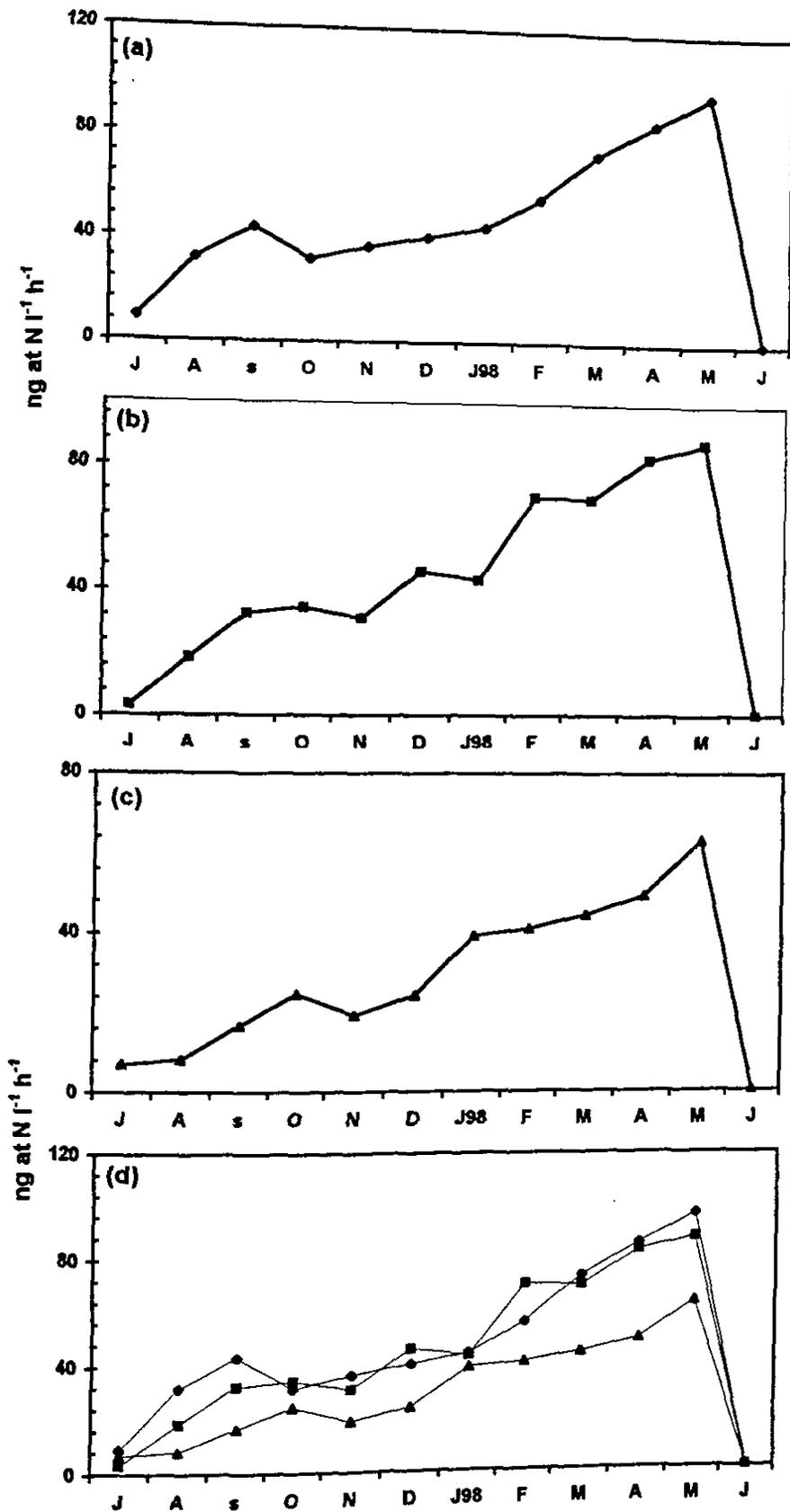


Fig. 3.12 Seasonal changes of nitrification rates at the (a) reference  $\blacklozenge$ , (b) middle  $\blacksquare$  and (c) mouth  $\blacktriangle$  stations; (d) all stations together

$h^{-1}$ ). The middle station showed similar values ( $77.6 \pm 9.1 \text{ } \mu\text{g at N } \Gamma^{-1} h^{-1}$ ), while, at the mouth station, rates during this season were much lower ( $49.4 \pm 9.8 \text{ } \mu\text{g at N } \Gamma^{-1} h^{-1}$ ). In the entire study, lowest nitrification rates were recorded during the monsoon season, with minimum rates at the mouth station ( $8.0 \pm 6.8 \text{ } \mu\text{g at N } \Gamma^{-1} h^{-1}$ ). Moderately high nitrification rates, with comparable values at the reference and middle stations (Table 3.18) were recorded in the post-monsoon season.

Table 3.18 Seasonal and annual averages of nitrification rates ( $\mu\text{g at N } \Gamma^{-1} h^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual average
Reference	$77.8 \pm 17.5$	$21.3 \pm 20.0$	$38.2 \pm 5.4$	$49.9 \pm 28.5$
Middle	$77.6 \pm 9.1$	$13.6 \pm 15.0$	$38.7 \pm 7.2$	$47.3 \pm 29.2$
Mouth	$49.4 \pm 9.8$	$8.0 \pm 6.8$	$26.8 \pm 8.6$	$30.6 \pm 19.3$

Table 3.19 ANOVA results of nitrification rates

Source of variation	Stations	F	df	P-values
Between seasons	Reference	13.74	2,9	$1.84 \times 10^{-3}$
	Middle	34.73	2,9	$5.87 \times 10^{-5}$
	Mouth	23.79	2,9	$2.55 \times 10^{-4}$
Between stations	All	1.63	2,33	0.21

### 3.5 NITROGEN UPTAKE STUDIES

#### 3.5.1 UNFRACTIONATED UPTAKE

##### 3.5.1.1 Nitrate

##### Specific nitrate uptake rate ( $v\text{NO}_3^-$ )

The specific uptake rates of nitrate in the overall study ranged from  $1.3 \times 10^{-5}$  to  $593 \times 10^{-5} h^{-1}$ . Between the three stations, the maximum annual average was at

the mouth station ( $233 \times 10^{-5} \pm 194 \times 10^{-5} \text{ h}^{-1}$ ), followed, in order by the reference ( $191 \times 10^{-5} \pm 175 \times 10^{-5} \text{ h}^{-1}$ ) and middle ( $164 \times 10^{-5} \pm 153 \times 10^{-5} \text{ h}^{-1}$ ) stations (Table 3.20). ANOVA showed that the variations in  $\nu\text{NO}_3^-$  between the stations were not significant ( $F=0.63$ ,  $df=2,45$ ;  $P=0.54$ ).

Nitrate uptake rates varied significantly between the seasons (Table 3.21). The seasonal changes of  $\nu\text{NO}_3^-$  (Fig. 3.13) showed remarkably higher rates in the post-monsoon compared to the pre-monsoon season, and the lowest rates in the monsoon season. One of the remarkable features in the seasonality is the sharp increase to peak values from the lowest monsoon values. In the post-monsoon season, as seasonal averages, rates remained high at the mouth and reference stations (Table 3.20). At the middle station, however, the  $\nu\text{NO}_3^-$  decreased in the month of November to give a lower mean uptake rate ( $323 \times 10^{-5} \pm 67.8 \times 10^{-5} \text{ h}^{-1}$ ). Rates continued to decrease through the pre-monsoon season, except at the mouth station where it was interrupted by a rise in March-May. A similar trend in rates was apparent during this season at the reference and middle stations, with the  $\nu\text{NO}_3^-$  decreasing to a minimum in July. The minimum for the mouth station was however earlier (June) and extended up to August.

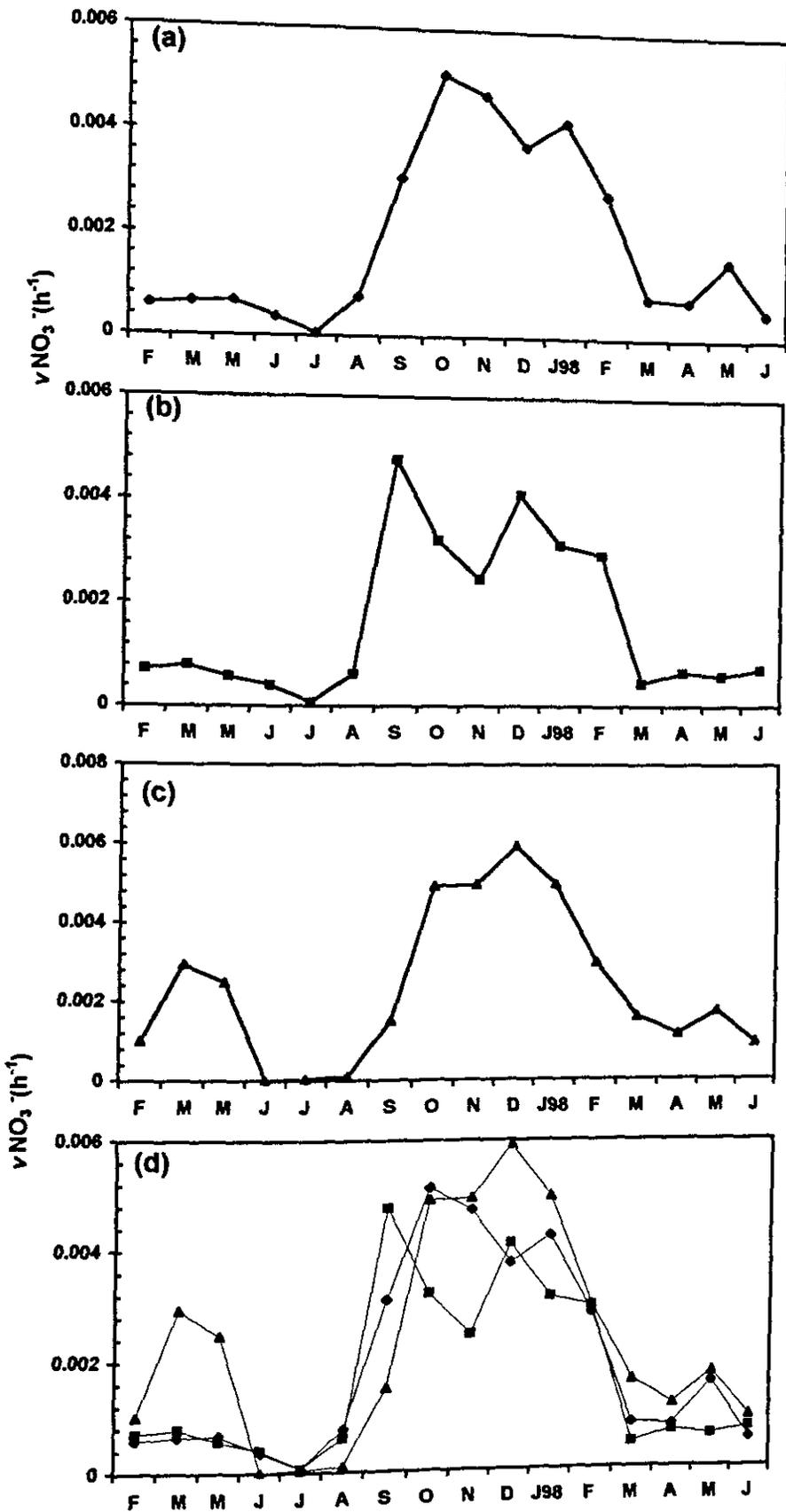


Fig. 3.13 Seasonal changes of specific nitrate uptake rates at the (a) reference ◆ (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

Table 3.20 Seasonal and annual averages of specific nitrate uptake rates ( $\text{h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$113 \times 10^{-5} \pm 82.1 \times 10^{-5}$	$97 \times 10^{-5} \pm 122 \times 10^{-5}$	$447 \times 10^{-5} \pm 59.6 \times 10^{-5}$	$191 \times 10^{-5} \pm 175 \times 10^{-5}$
Middle	$96.3 \times 10^{-5} \pm 88.4 \times 10^{-5}$	$132 \times 10^{-5} \pm 195 \times 10^{-5}$	$323 \times 10^{-5} \pm 67.8 \times 10^{-5}$	$164 \times 10^{-5} \pm 153 \times 10^{-5}$
Mouth	$199 \times 10^{-5} \pm 81.5 \times 10^{-5}$	$52.1 \times 10^{-5} \pm 67 \times 10^{-5}$	$519 \times 10^{-5} \pm 49.1 \times 10^{-5}$	$233 \times 10^{-5} \pm 194 \times 10^{-5}$

Table 3.21 ANOVA results of specific nitrate uptake rates

Source of variation	Stations	F	df	P-values
Between seasons	Reference	20.47	2,13	$9.61 \times 10^{-5}$
	Middle	4.23	2,13	$3.84 \times 10^{-2}$
	Mouth	49.86	2,13	$7.99 \times 10^{-7}$
Between stations	All	0.63	2,45	0.54

### Absolute nitrate uptake rate ( $\rho\text{NO}_3^-$ )

Like  $\nu\text{NO}_3^-$ , the absolute nitrate uptake rates also varied through more than two orders of magnitude (from 1.8 to 385.0  $\text{ng at N l}^{-1} \text{h}^{-1}$ ). Exceedingly higher annual averages were recorded at the reference station ( $143.7 \pm 132.5 \text{ ng at N l}^{-1} \text{h}^{-1}$ ) followed by moderately higher values at the middle station ( $98.8 \pm 81.7 \text{ ng at N l}^{-1} \text{h}^{-1}$ ) and the lowest values at the mouth station ( $56.7 \pm 39.1 \text{ ng at N l}^{-1} \text{h}^{-1}$ ) (Table 3.22). ANOVA showed that the rates between the stations varied significantly ( $F=3.53$ ,  $df=2,45$ ;  $P=3.78 \times 10^{-2}$ )

Although statistically significant seasonal variations were observed only at the reference station (Table 3.23), the overall seasonal trend followed a well defined

cycle at the three stations. The trend (Fig. 3.14) shared one common feature with that of specific uptake rate *i.e.* high absolute uptake rates in the post-monsoon season. The highest average absolute uptake rate during this season was obtained at the reference station ( $324.1 \pm 53.5 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and the lowest at the mouth station ( $91.8 \pm 22 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). The  $\text{pNO}_3^-$  at the middle station showed fluctuations, with an average ( $162.7 \pm 40.6 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) remaining at an intermediate level. The alternating increase and decrease observed from November to March at this station resulted in two peaks of more or less similar intensity in the months of December and February. Seasonal averages were minimum in the pre-monsoon season with the lowest values at the mouth station ( $46.1 \pm 21.9 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ), while during the monsoon season, rates were moderate (Table 3.22).

Table 3.22 Seasonal and annual averages of absolute nitrate uptake rates ( $\text{ng at N l}^{-1} \text{ h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$62.5 \pm 31.5$	$113 \pm 130.2$	$324.1 \pm 53.5$	$143.7 \pm 132.5$
Middle	$61.5 \pm 65.3$	$100 \pm 104.2$	$162.7 \pm 40.6$	$98.8 \pm 81.7$
Mouth	$46.1 \pm 21.9$	$43.4 \pm 54.7$	$91.8 \pm 22.0$	$56.7 \pm 39.1$

Table 3.23 ANOVA results of absolute nitrate uptake rates

Source of variation	Stations	F	df	P-values
Between seasons	Reference	14.3	2,13	$5.21 \times 10^{-4}$
	Middle	2.29	2,13	0.14
	Mouth	2.63	2,13	0.11
Between stations	All	3.53	2,45	$3.78 \times 10^{-2}$

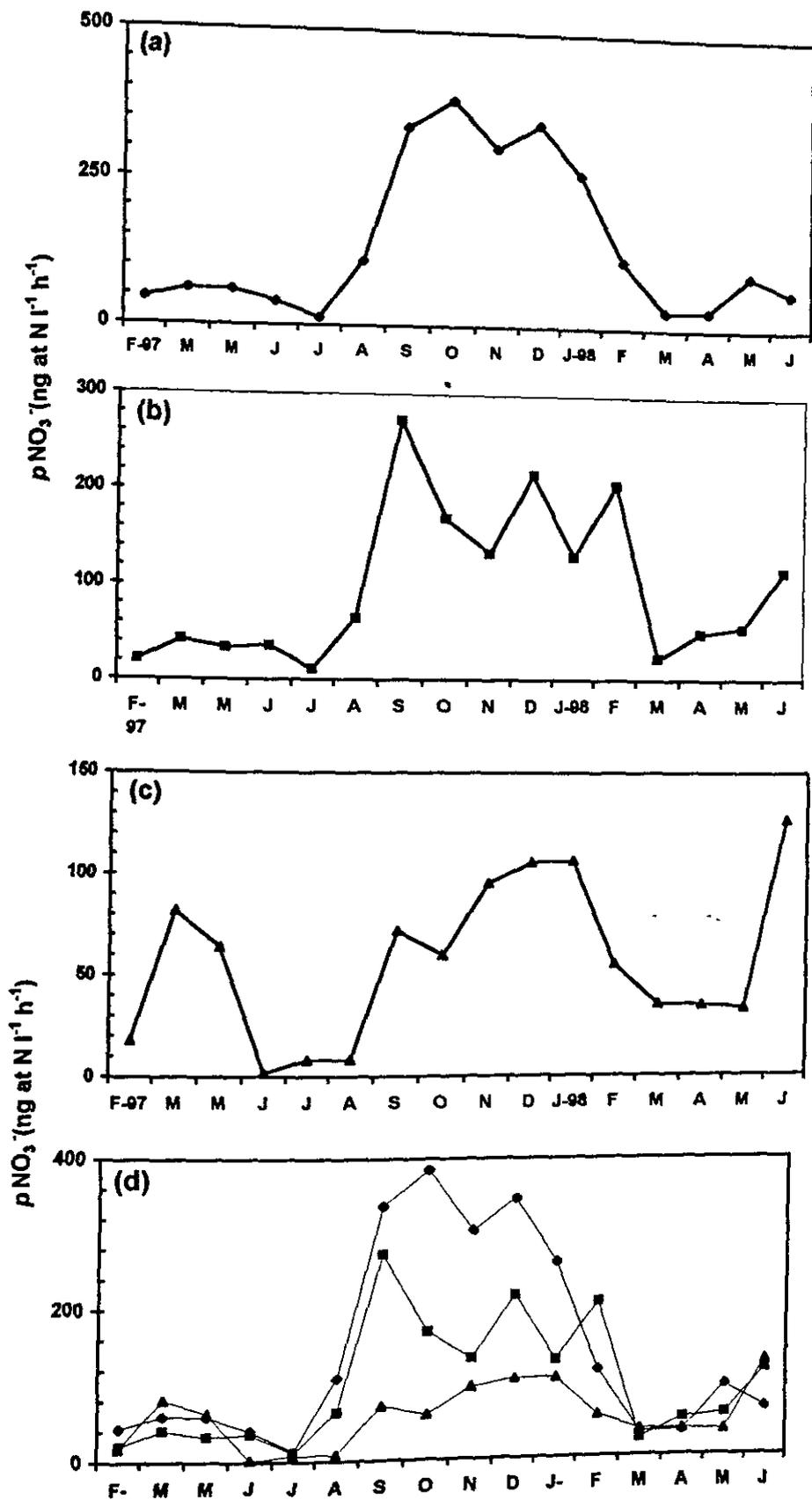


Fig. 3.14 Seasonal changes of absolute nitrate uptake rates at the (a) reference  $\blacklozenge$ , (b) middle  $\blacksquare$  and (c) mouth  $\blacktriangle$  stations; (d) all stations together

### 3.4.1.2 Nitrite

#### Specific nitrite uptake rate ( $v\text{NO}_2^-$ )

The specific nitrite uptake rates in the present study were 10 times lower than those of nitrate uptake and ranged from  $6.4 \times 10^{-6}$  to  $8.65 \times 10^{-4} \text{ h}^{-1}$ . The annual averages varied in a narrow range, from  $2.1 \times 10^{-4} \pm 1.6 \times 10^{-4} \text{ h}^{-1}$  to  $3.0 \times 10^{-4} \pm 2.0 \times 10^{-4} \text{ h}^{-1}$  at the mouth and middle stations respectively (Table 3.24). The spatial differences were found to be insignificant ( $F=0.73$ ,  $df=2,45$ ;  $P=0.49$ ).

Although statistically significant seasonal variations were observed only at the middle and mouth stations (Table 3.25), the seasonal changes showed high uptake rates during the post-monsoon season and lower rates in the pre-monsoon and monsoon seasons. The monthly variations of nitrite uptake rates (Fig. 3.15) followed a similar trend as that of nitrate uptake. The post-monsoon high ( $5.1 \times 10^{-4} \pm 2.3 \times 10^{-4} \text{ h}^{-1}$ ) was characterised by a peak in November at the middle station. Though the rates at the mouth station during this season were comparatively lower ( $3.2 \times 10^{-4} \pm 1.9 \times 10^{-4} \text{ h}^{-1}$ ), high rates were obtained in the months of November and December. The reference station, with moderately high rates during this season ( $4.3 \times 10^{-4} \pm 2.3 \times 10^{-4} \text{ h}^{-1}$ ), showed high values in October and November, though the seasonal maximum occurred in the preceding month (September). The uptake rates during the monsoon and pre-monsoon seasons were low compared with the post-monsoon season. The  $v\text{NO}_2^-$  in the overall study was the lowest during the monsoon season with very

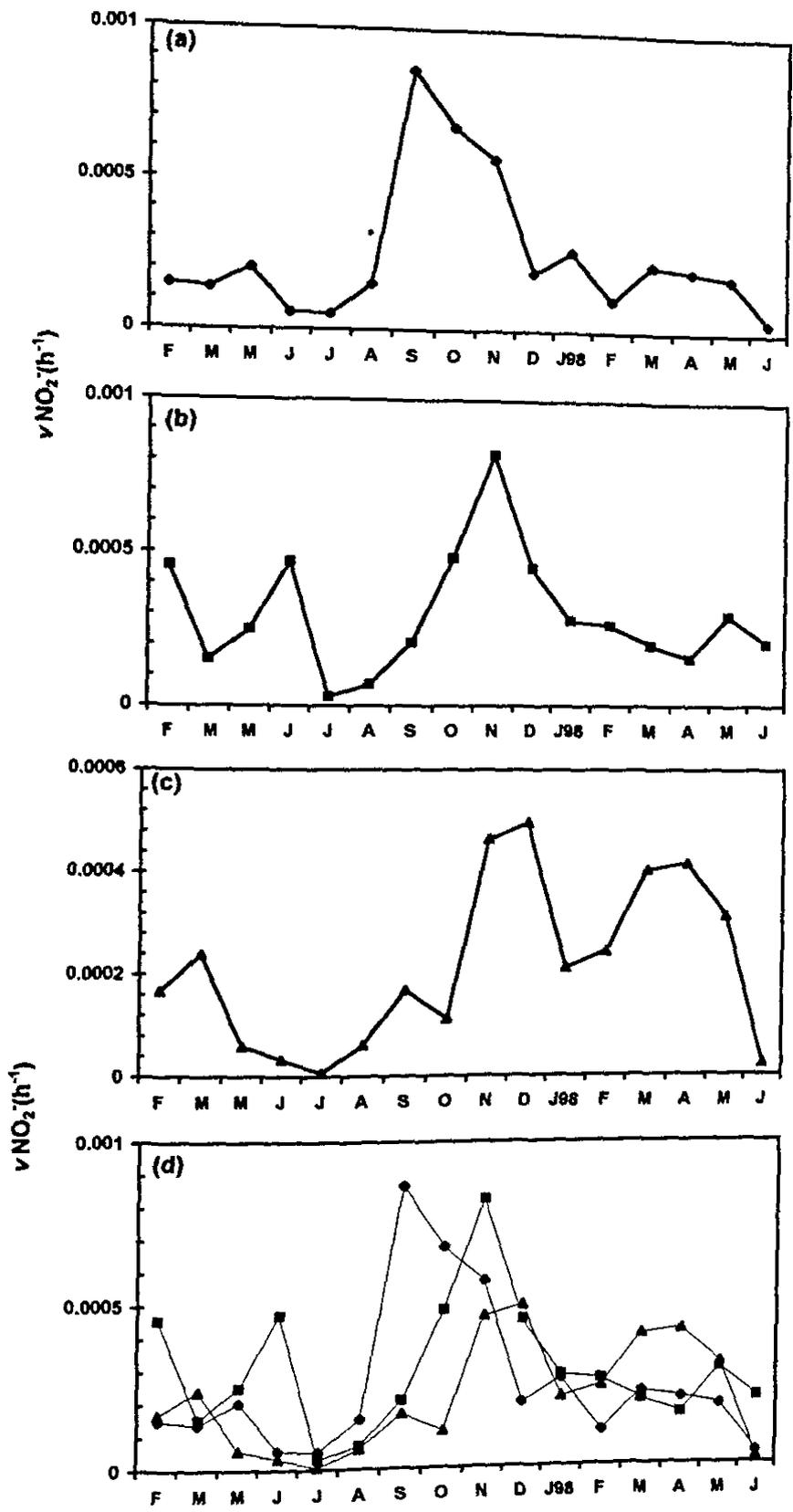


Fig. 3.15 Seasonal changes of specific nitrite uptake rates at the (a) reference ◆ (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

low rates at the mouth station ( $5.8 \times 10^{-5} \pm 6.5 \times 10^{-5} \text{ h}^{-1}$ ). At the middle station, however, there was an exceptional increase in  $\nu\text{NO}_2^-$  ( $4.7 \times 10^{-4} \text{ h}^{-1}$ ) in the month of June. The pre-monsoon season recorded moderately high values (Table 3.24) with maximum rates at the mouth station ( $2.6 \times 10^{-4} \pm 1.3 \times 10^{-4} \text{ h}^{-1}$ ) shown by the secondary maximum in March and April.

Table 3.24 Seasonal and annual averages of specific nitrite uptake rates ( $\text{h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$1.7 \times 10^{-4} \pm 4.3 \times 10^{-5}$	$2.3 \times 10^{-4} \pm 3.6 \times 10^{-4}$	$4.3 \times 10^{-4} \pm 2.3 \times 10^{-4}$	$2.6 \times 10^{-4} \pm 2.4 \times 10^{-4}$
Middle	$2.5 \times 10^{-4} \pm 1.0 \times 10^{-4}$	$2 \times 10^{-4} \pm 1.7 \times 10^{-4}$	$5.1 \times 10^{-4} \pm 2.3 \times 10^{-4}$	$3.0 \times 10^{-4} \pm 2.0 \times 10^{-4}$
Mouth	$2.6 \times 10^{-4} \pm 1.3 \times 10^{-4}$	$5.8 \times 10^{-5} \pm 6.5 \times 10^{-5}$	$3.2 \times 10^{-4} \pm 1.9 \times 10^{-4}$	$2.1 \times 10^{-4} \pm 1.6 \times 10^{-4}$

Table 3.25 ANOVA results of specific nitrite uptake rate

Source of variation	Stations	F	df	P-values
	Reference	1.61	2,13	0.24
Between seasons	Middle	4.65	2,13	$3.0 \times 10^{-2}$
	Mouth	5.42	2,13	$1.9 \times 10^{-2}$
Between stations	All	0.73	2,45	0.49

### Absolute nitrite uptake rate ( $\rho\text{NO}_2^-$ )

The absolute nitrite uptake rates ranged from 1.1 to 93.9  $\text{ng at N l}^{-1} \text{ h}^{-1}$ . Maximum annual averages were recorded at the reference station ( $20.9 \pm 23.0 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). The  $\rho\text{NO}_2^-$  at the middle station was almost of the same order ( $19.2 \pm 12.3 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ), while it was at its lowest at the mouth station ( $5.5 \pm$

3.3  $\eta\text{g at N l}^{-1} \text{h}^{-1}$ ) (Table 3.26). The data when tested with ANOVA showed significant spatial variations ( $F=4.93$ ,  $df=2,45$ ;  $P=0.01$ ).

Although the seasonal variations in absolute nitrite uptake rates were statistically not significant (Table 3.27), the monthly changes (Fig. 3.16) were similar to those of absolute nitrate uptake. High rates were obtained in the post-monsoon season at the reference station ( $30.5 \pm 16.4 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ) and the lowest rates in the monsoon season at the mouth station ( $4.4 \pm 2.6 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ). The seasonal changes of  $\rho\text{NO}_2^-$  at the reference and middle stations followed almost similar trends to that of  $\nu\text{NO}_2^-$ . At the reference station, the low absolute uptake rates in the pre-monsoon season persisted through the early monsoon months before rising to a peak value in September, which also is the cause of the high average  $\rho\text{NO}_2^-$  for this season (Table 3.26). At the middle station, two maximas were evident, one in June ( $41.7 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ) and the other in November ( $44.6 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ). The seasonal changes at the mouth station were marked by alternating highs and lows, with the highest peak in the month of April ( $12.5 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ).

Table 3.26 Seasonal and annual averages of absolute nitrite uptake rates ( $\eta\text{g at N l}^{-1} \text{h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$10.7 \pm 4.2$	$27.4 \pm 37.8$	$30.5 \pm 16.4$	$20.9 \pm 23.0$
Middle	$14.8 \pm 6.5$	$19.6 \pm 16.6$	$26.4 \pm 13.7$	$19.2 \pm 12.3$
Mouth	$6.2 \pm 3.7$	$4.4 \pm 2.6$	$5.9 \pm 3.7$	$5.5 \pm 3.3$

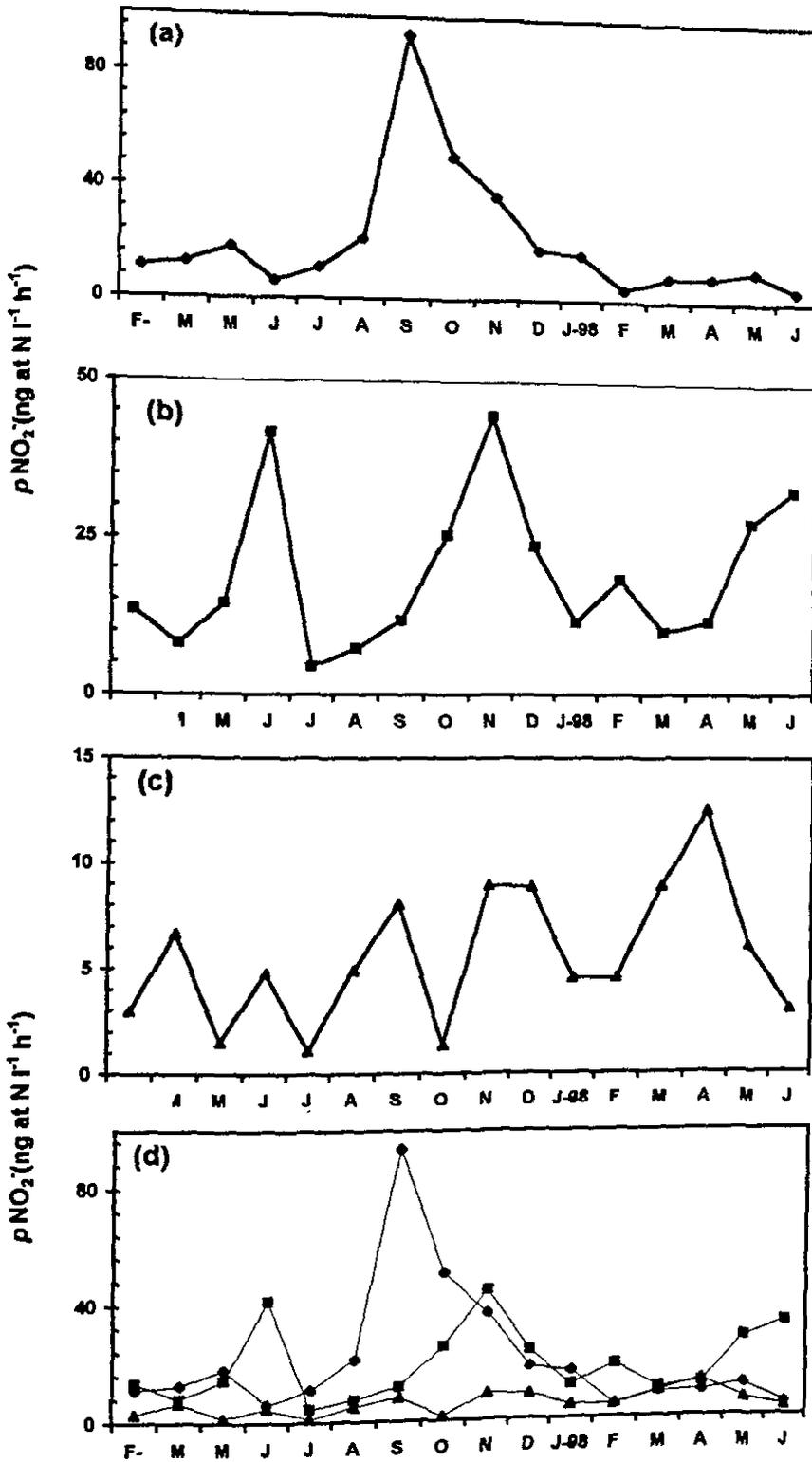


Fig. 3.16 Seasonal changes of absolute nitrite uptake rates at the (a) reference ◆, (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

Table 3.27 ANOVA results of absolute nitrite uptake rates

Source of variation	Stations	F	df	P-values
Between seasons	Reference	1.28	2,13	0.31
	Middle	1.16	2,13	0.35
	Mouth	0.44	2,13	0.66
Between stations	All	4.93	2,45	0.01

### 3.4.1.3 Ammonium

#### Specific ammonium uptake rate ( $\nu\text{NH}_4^+$ )

Specific ammonium uptake rates were comparatively higher than nitrate and nitrite specific uptake rates during the study period and ranged from  $3.6 \times 10^{-5}$  to  $1.4 \times 10^{-2} \text{ h}^{-1}$ . Maximum annual averages were recorded at the reference station ( $5.1 \times 10^{-3} \pm 5.1 \times 10^{-3} \text{ h}^{-1}$ ), followed by the middle station ( $3.7 \times 10^{-3} \pm 3.3 \times 10^{-3} \text{ h}^{-1}$ ) and the mouth station ( $1.1 \times 10^{-3} \pm 1.2 \times 10^{-3} \text{ h}^{-1}$ ) (Table 3.28). Tests with ANOVA revealed that the station-wise variations were significant ( $F=5.17$ ,  $df=2,45$ ;  $P=9.56 \times 10^{-3}$ )

The seasonal changes also varied significantly at all three stations (Table 3.29). The seasonality of  $\nu\text{NH}_4^+$  showed a pre-monsoon high in the month of May at all stations (Fig. 3.17). The same was observed in the next annual cycle, with the peak marked by high values in April and May. During the intervening seasons, rates did not vary much, but marginally higher rates were obtained in the post-monsoon season (Table 3.28). The magnitude of the peaks during the pre-

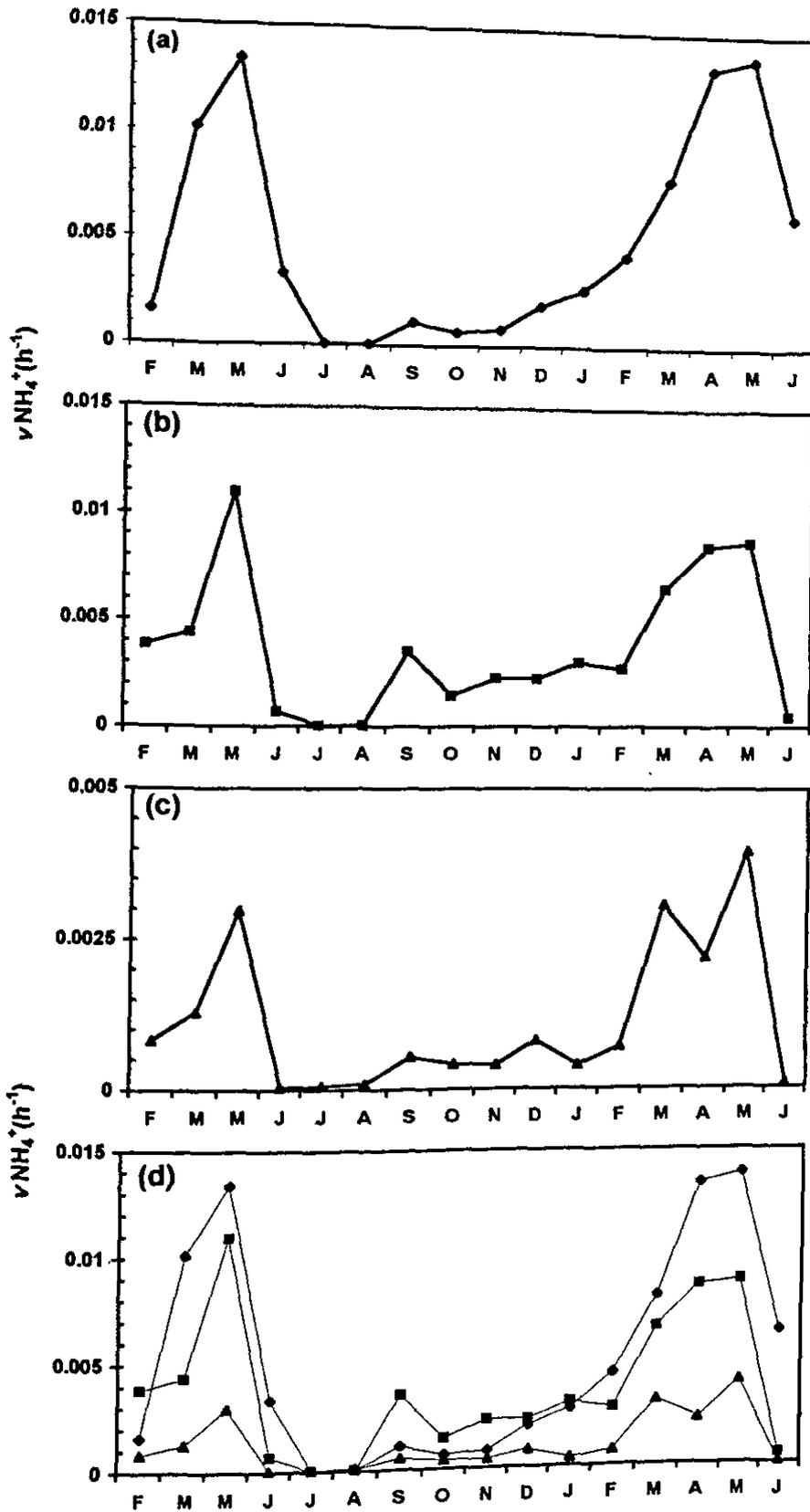


Fig. 3.17 Seasonal changes of specific ammonium uptake rates at the (a) reference◆, (b) middle ■ and (c) mouth▲ stations; (d) all stations together

monsoon season decreased from the reference to the mouth station. This is reflected in the maximum average rates recorded at the reference station ( $9.3 \times 10^{-3} \pm 4.8 \times 10^{-3} \text{ h}^{-1}$ ), followed by the middle ( $6.5 \times 10^{-3} \pm 3.0 \times 10^{-3} \text{ h}^{-1}$ ) and mouth ( $2.1 \times 10^{-3} \pm 1.3 \times 10^{-3} \text{ h}^{-1}$ ) stations. Following the peak in May, a sudden decrease in rates was observed at the beginning of the monsoon season. Values remained very low in this period with a minimum rate of  $3.6 \times 10^{-5} \text{ h}^{-1}$  in August at the middle station. The post-monsoon season was marked by a gradual increase to reach the pre-monsoon peak of the next annual cycle.

Table 3.28 Seasonal and annual averages of specific ammonium uptake rates ( $\text{h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$9.3 \times 10^{-3} \pm 4.8 \times 10^{-3}$	$2.2 \times 10^{-3} \pm 2.7 \times 10^{-3}$	$1.5 \times 10^{-3} \pm 9.8 \times 10^{-4}$	$5.1 \times 10^{-3} \pm 5.1 \times 10^{-3}$
Middle	$6.5 \times 10^{-3} \pm 3.0 \times 10^{-3}$	$9.5 \times 10^{-4} \pm 1.5 \times 10^{-3}$	$2.3 \times 10^{-3} \pm 5 \times 10^{-4}$	$3.7 \times 10^{-3} \pm 3.3 \times 10^{-3}$
Mouth	$2.1 \times 10^{-3} \pm 1.3 \times 10^{-3}$	$1.6 \times 10^{-4} \pm 2.1 \times 10^{-4}$	$5.0 \times 10^{-4} \pm 2.0 \times 10^{-4}$	$1.1 \times 10^{-3} \pm 1.2 \times 10^{-3}$

Table 3.29 ANOVA results of specific ammonium uptake rates

Source of variation	Stations	F	df	P-values
Between seasons	Reference	8.15	2,13	$5.08 \times 10^{-3}$
	Middle	10.3	2,13	$2.09 \times 10^{-3}$
	Mouth	9.04	2,13	$3.47 \times 10^{-3}$
Between stations	All	5.17	2,45	$9.56 \times 10^{-3}$

### Absolute ammonium uptake rate ( $\rho\text{NH}_4^+$ )

Absolute ammonium uptake rates varied in an enormous range, from 3.8 to 1188.4  $\text{ng at N l}^{-1} \text{h}^{-1}$ . As annual averages, spatial variations of  $\rho\text{NH}_4^+$  were similar to those of  $v\text{NH}_4^+$  *i.e.* rates decreased from the reference to the mouth stations ( $366.5 \pm 375.1 \text{ ng at N l}^{-1} \text{h}^{-1}$  to  $27.9 \pm 27.2 \text{ ng at N l}^{-1} \text{h}^{-1}$  respectively) (Table 3.30). Statistical analysis confirmed these significant variations ( $F=6.93$ ,  $df=2,45$ ;  $P=2.39 \times 10^{-3}$ ).

Like that of specific uptake rate, the variations between the seasons were also significant at all the stations (Table 3.31). The overall seasonal trend (Fig. 3.18) was more or less similar to that of  $v\text{NH}_4^+$  exhibiting higher rates in the pre-monsoon months and comparatively lower rates during the intervening seasons at all the stations. The pre-monsoon rates were maximum at the reference station ( $591.9 \pm 409.5 \text{ ng at N l}^{-1} \text{h}^{-1}$ ) and minimum at the mouth station ( $49.9 \pm 28.1 \text{ ng at N l}^{-1} \text{h}^{-1}$ ). The high  $\rho\text{NH}_4^+$  in the month of May dropped rapidly in June and reached minimum values in August at all three stations. During the monsoon season, however, an increase in September was noticed at the three stations. This was followed by a decrease in the post-monsoon season in the month of October. A comparison of the seasonal averages (Table 3.30) in the monsoon and post-monsoon periods revealed higher rates in the monsoon season at the reference and mouth stations ( $254.0 \pm 321.0 \text{ ng at N l}^{-1} \text{h}^{-1}$  and  $12.2 \pm 7.8 \text{ ng at N l}^{-1} \text{h}^{-1}$  respectively), while higher rates in the post-monsoon season were recorded at the middle station ( $111.4 \pm 23.4 \text{ ng at N l}^{-1} \text{h}^{-1}$ ).

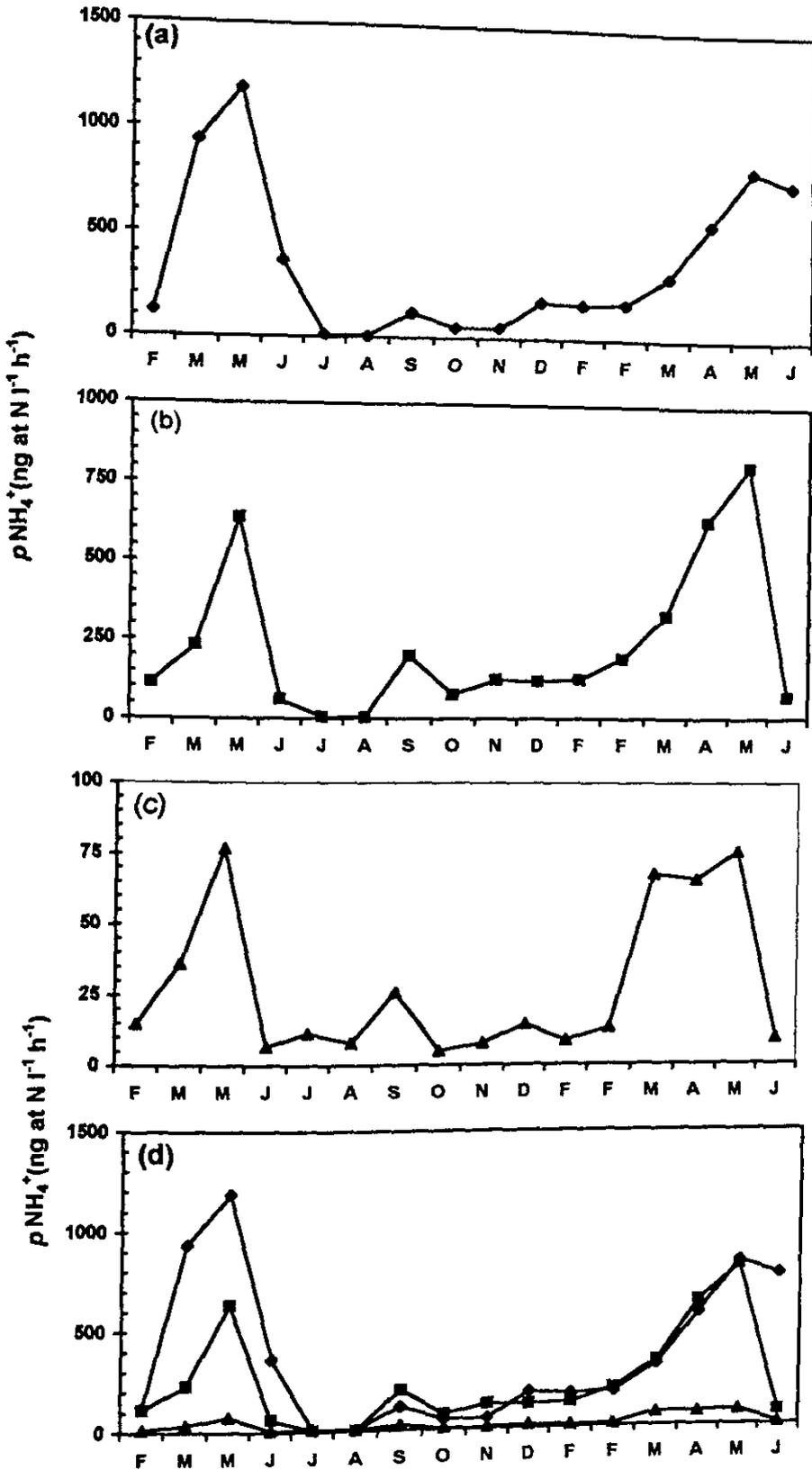


Fig. 3.18 Seasonal changes of absolute ammonium uptake rates at the (a) reference ◆ (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

Table 3.30 Seasonal and annual averages of absolute ammonium uptake rates ( $\text{ng at N } \Gamma^{-1} \text{ h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	591.9 $\pm$ 409.5	254.0 $\pm$ 321.0	112.7 $\pm$ 71.0	366.5 $\pm$ 375.1
Middle	421.3 $\pm$ 269.4	68.7 $\pm$ 80.2	111.4 $\pm$ 23.4	233.6 $\pm$ 245.6
Mouth	49.9 $\pm$ 28.1	12.2 $\pm$ 7.8	8.9 $\pm$ 3.9	27.9 $\pm$ 27.2

Table 3.31 ANOVA results of absolute ammonium uptake rates

Source of variation	Stations	F	df	P-values
Between seasons	Reference	3.07	2,13	$8.10 \times 10^{-2}$
	Middle	6.21	2,13	$1.28 \times 10^{-2}$
	Mouth	7.85	2,13	$5.80 \times 10^{-3}$
Between stations	All	6.93	2,45	$2.39 \times 10^{-3}$

#### 3.4.1.4 Urea

##### Specific urea uptake rate ( $v$ urea)

Specific urea uptake rates in the study period varied in a narrow range, from undetectable levels to  $0.009 \text{ h}^{-1}$ . Annual averages were lower than  $v\text{NH}_4^+$  and  $v\text{NO}_3^-$ , but higher than  $v\text{NO}_2^-$  and indicates that urea stood third with respect to uptake rate after ammonium and nitrate. Highest uptake rates were recorded at the mouth station ( $5.1 \times 10^{-4} \pm 3.1 \times 10^{-4} \text{ h}^{-1}$ ), followed by the middle station ( $4.3 \times 10^{-4} \pm 2.5 \times 10^{-4} \text{ h}^{-1}$ ) and marginally lower rates at the reference station ( $3.9 \times$

$10^{-4} \pm 2.3 \times 10^{-4} \text{ h}^{-1}$ ) (Table 3.32). The rates did not vary significantly between the stations ( $F=0.83$ ,  $df=2,45$ ;  $P=0.44$ ).

The seasonal variations were significant at all the stations (Table 3.33). The seasonality of urea specific uptake rates (Fig. 3.19) exhibited a minimum during the monsoon season, characterised by low values in June, July and August at the reference and middle stations. The minimum at the mouth station, however, extended up to September. Seasonal averages during this season were of the same order at the reference ( $1.5 \times 10^{-4} \pm 2.6 \times 10^{-4} \text{ h}^{-1}$ ), middle ( $1.5 \times 10^{-4} \pm 1.7 \times 10^{-4} \text{ h}^{-1}$ ) and mouth ( $1.6 \times 10^{-4} \pm 2.6 \times 10^{-4} \text{ h}^{-1}$ ) stations. At the end of this season, in September, a sharp increase in rates was observed at the reference and middle stations. The changes during the pre-monsoon season were characterised by a peak in the month of May at all the stations. Spatially and seasonally, higher averages at the mouth ( $6.9 \times 10^{-4} \pm 1.6 \times 10^{-4} \text{ h}^{-1}$ ) and reference ( $5.1 \times 10^{-4} \pm 1.3 \times 10^{-4} \text{ h}^{-1}$ ) stations were obtained during the pre-monsoon season, while at the middle station, higher rates were obtained during the post-monsoon season (Table 3.32). The changes in this season showed a fluctuating trend and were more pronounced at the middle station. This led to two peaks, one being in October and the other in January, thus resulting in the high seasonal average. The post-monsoon high at the mouth station was characterised by high values in the months of October, November and December.

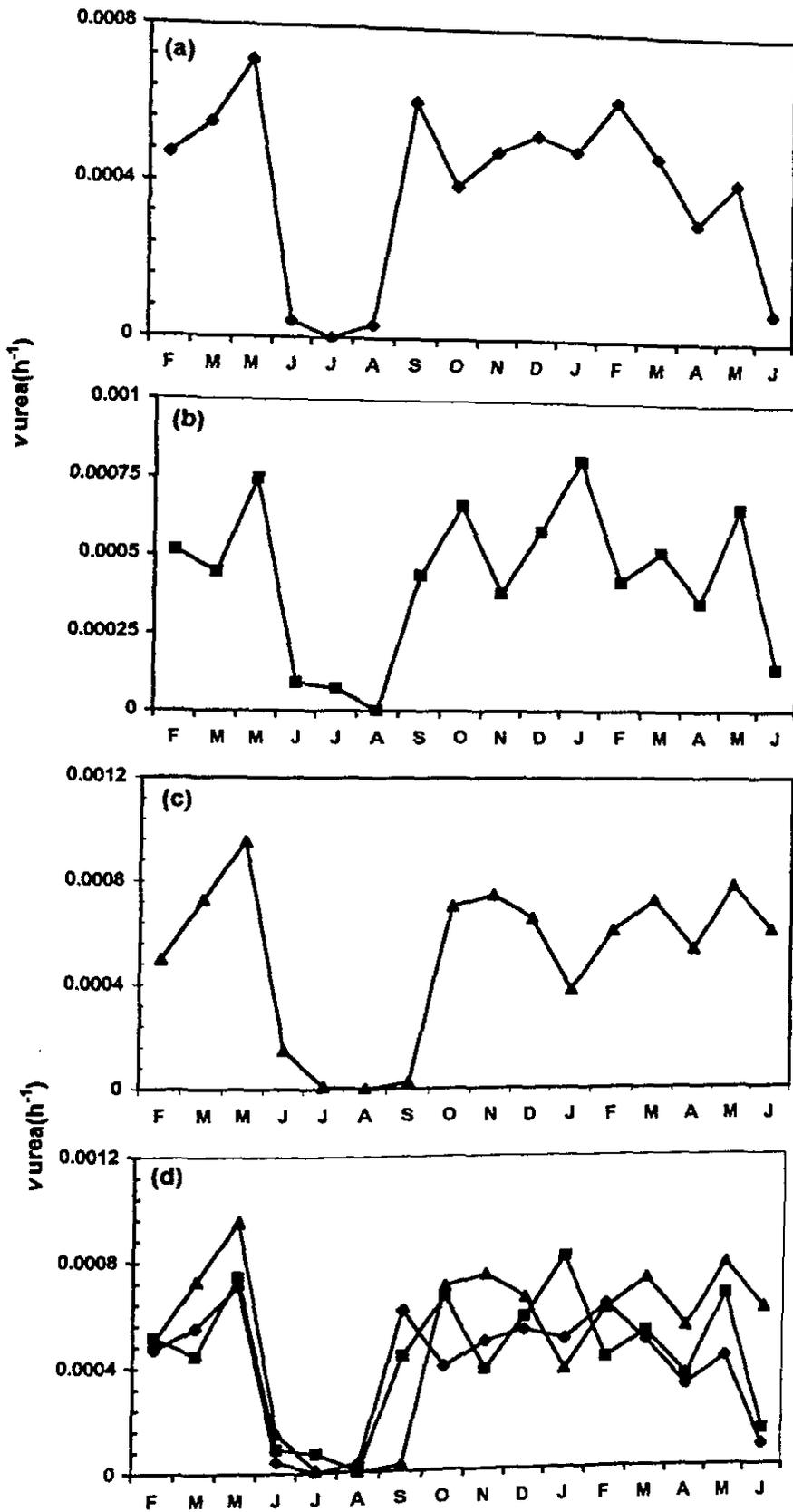


Fig. 3.19 Seasonal changes of specific urea uptake rates at the (a) reference◆, (b) middle■ and (c) mouth▲ stations; (d) all stations together

Table 3.32 Seasonal and annual averages of specific urea uptake rates ( $\text{h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$5.1 \times 10^{-4} \pm 1.3 \times 10^{-4}$	$1.5 \times 10^{-4} \pm 2.6 \times 10^{-4}$	$4.8 \times 10^{-4} \pm 5.8 \times 10^{-5}$	$3.9 \times 10^{-4} \pm 2.3 \times 10^{-4}$
Middle	$5.2 \times 10^{-4} \pm 1.4 \times 10^{-4}$	$1.5 \times 10^{-4} \pm 1.7 \times 10^{-4}$	$6.1 \times 10^{-4} \pm 1.8 \times 10^{-4}$	$4.3 \times 10^{-4} \pm 2.5 \times 10^{-4}$
Mouth	$6.9 \times 10^{-4} \pm 1.6 \times 10^{-4}$	$1.6 \times 10^{-4} \pm 2.6 \times 10^{-4}$	$6.2 \times 10^{-4} \pm 1.7 \times 10^{-4}$	$5.1 \times 10^{-4} \pm 3.1 \times 10^{-4}$

Table 3.33 ANOVA results of specific urea uptake rates

Source of variation	Stations	F	df	P-values
	Reference	7.06	2,13	$8.40 \times 10^{-3}$
Between seasons	Middle	11.75	2,13	$1.22 \times 10^{-3}$
	Mouth	11.7	2,13	$1.24 \times 10^{-3}$
Between stations	All	0.83	2,45	0.44

### Absolute urea uptake rate ( $\rho$ urea)

Absolute urea uptake rates ranged from undetectable levels to  $82.7 \text{ ng at N } \Gamma^{-1} \text{ h}^{-1}$ . The annual averages did not differ to a great extent. Highest rates were recorded at the reference station ( $28.6 \pm 20.4 \text{ ng at N } \Gamma^{-1} \text{ h}^{-1}$ ). This was followed by the middle ( $25.7 \pm 14.9 \text{ ng at N } \Gamma^{-1} \text{ h}^{-1}$ ) and mouth ( $17.0 \pm 18.8 \text{ ng at N } \Gamma^{-1} \text{ h}^{-1}$ ) stations (Table 3.34). There were no significant variations between the stations ( $F=1.76$ ,  $df=2,45$ ;  $P=0.18$ )

Though statistically the seasonal variations were insignificant at all the stations (Table 3.35), the seasonal changes showed a somewhat similar trend as that of specific uptake rate with lower values during the monsoon season in

comparison to the pre- and post-monsoon seasons (Fig. 3.20). Lowest seasonal average during this season was obtained at the mouth station ( $4.1 \pm 6.1 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ). The minimum in the monsoon season occurred in different months at each station. At the reference station, the monsoon low was characterised by low rates in June, July and August. Minimum rates were recorded at the middle station in the month of August, while at the mouth station, this was noticed in August and September. The pre-monsoon peak at the three stations was more pronounced at the reference station ( $63.0 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ). This peak was not well marked in the next annual cycle with the exception of the middle station ( $61.4 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ). The absolute uptake rates followed almost similar trends at the reference and middle stations and a completely different trend at the mouth station. Here, rates were much lower during all the months with an exceptional increase in June ( $82.7 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ). Annual average rates at the reference and middle stations were maximum during the post-monsoon season ( $35.1 \pm 9.3 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$  and  $30.1 \pm 6.4 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ). However, at the mouth station, higher rates were recorded during the pre-monsoon season (Table 3.34).

Table 3.34 Seasonal and annual averages of absolute urea uptake rates ( $\text{ng at N } \Gamma^1 \text{ h}^{-1}$ )

Stations	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	$33.1 \pm 17.9$	$17.1 \pm 27.7$	$35.1 \pm 9.3$	$28.6 \pm 20.4$
Middle	$28.7 \pm 15.2$	$17.8 \pm 18.6$	$30.1 \pm 6.4$	$25.7 \pm 14.9$
Mouth	$26.6 \pm 25.1$	$4.1 \pm 6.1$	$11.4 \pm 3.2$	$17.0 \pm 18.8$

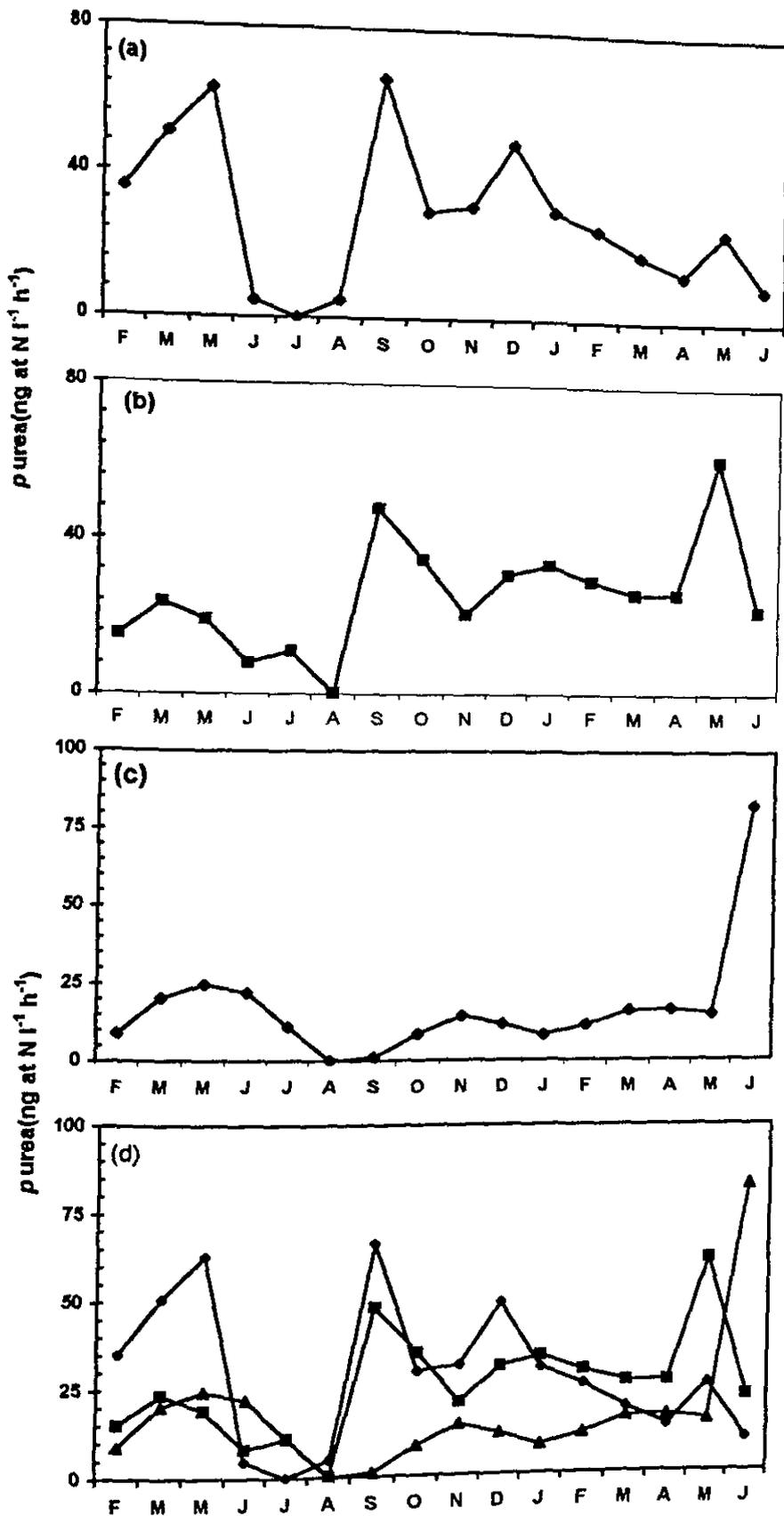


Fig. 3.20 Seasonal changes of absolute urea uptake rates at the (a) reference◆, (b) middle ■ and (c) mouth ▲ stations; (d) all stations together

Table 3.35 ANOVA results of absolute urea uptake rates

Source of variation	Stations	F	df	P-values
Between seasons	Reference	1.21	2,13	0.33
	Middle	1.02	2,13	0.39
	Mouth	1.30	2,13	0.31
Between stations	All	1.76	2,45	0.18

### 3.5.2 SIZE-FRACTIONATED UPTAKE

#### 3.5.2.1 Nitrate

##### Specific nitrate uptake rate ( $v\text{NO}_3^-$ )

During the seasonal cycle of the size-fractionated uptake study, the larger fraction showed higher specific uptake rates (range:  $5.7 \times 10^{-5}$  to  $0.008 \text{ h}^{-1}$ ) than the smaller fraction (range: 0 to  $0.00013 \text{ h}^{-1}$ ). The station-wise annual averages were maximum at the middle station for the larger fraction ( $0.0028 \pm 0.0031 \text{ h}^{-1}$ ) and at the mouth station for the smaller fraction ( $8.2 \times 10^{-5} \pm 6 \times 10^{-5} \text{ h}^{-1}$ ) (Table 3.36). The rates did not differ considerably between the stations for both the fractions as shown by the insignificant *P*-values (Table 3.37).

The larger fraction showed significant seasonal variations at all the stations, while the smaller fraction showed significant variations only at the middle and mouth stations (Table 3.37). The seasonal changes at all the three stations were well marked for the larger fraction as opposed to the smaller fraction (Fig. 3.21). Highest uptake for the 200-20  $\mu\text{m}$  fraction was in the post-monsoon season at the middle station ( $0.0075 \text{ h}^{-1}$ ). For the 20-0.8  $\mu\text{m}$  fraction, high rates were

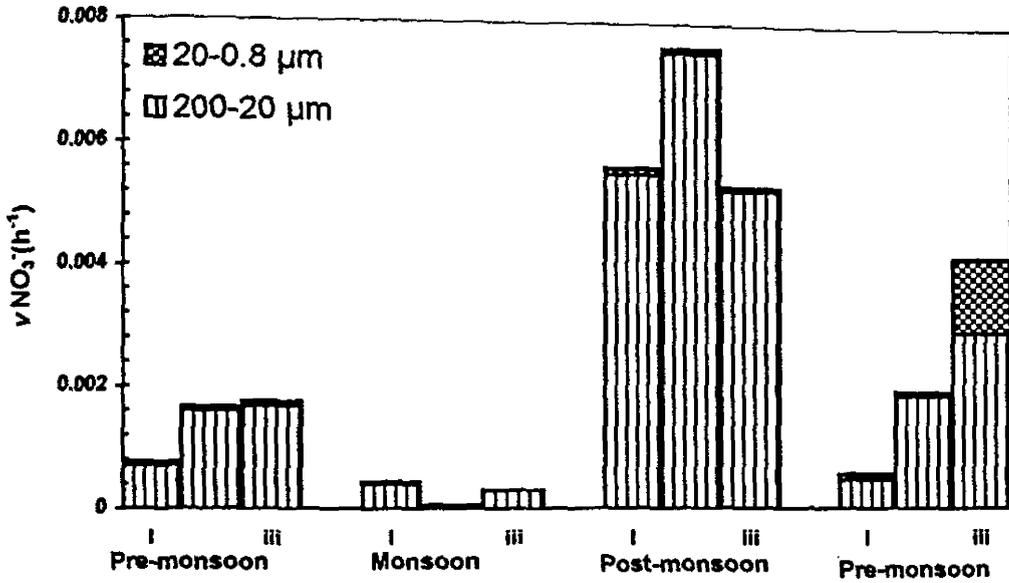


Fig. 3.21 Seasonal changes of specific nitrate uptake rates by two size fractions at the (i) reference, (ii) middle and (iii) mouth stations

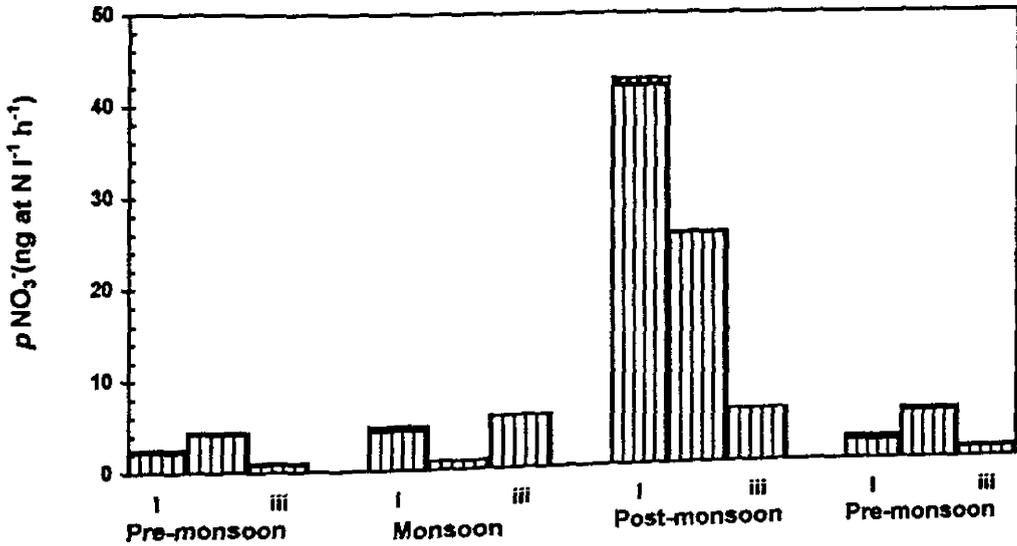


Fig. 3.22 Seasonal changes of absolute nitrate uptake rates by two size fractions at the (i) reference, (ii) middle and (iii) mouth stations

recorded in the pre-monsoon season at the mouth station and in the post-monsoon season at the reference station. The monsoon season recorded the lowest uptake rates for both the fractions (Table 3.36).

Table 3.36 Seasonal and annual averages of specific nitrate uptake rates ( $\text{h}^{-1}$ )

Stations	Size( $\mu\text{m}$ )	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	200-20	$0.0006 \pm 0.0003$	$0.0004 \pm 5.9 \times 10^{-5}$	$0.0055 \pm 0.0007$	$0.001 \pm 0.0023$
	20-0.8	$8.5 \times 10^{-5} \pm 5.8 \times 10^{-5}$	$4.2 \times 10^{-5} \pm 4.2 \times 10^{-5}$	$0.0001 \pm 1.94 \times 10^{-5}$	$8.1 \times 10^{-5} \pm 4.9 \times 10^{-5}$
Middle	200-20	$0.0017 \pm 0.0009$	$5.7 \times 10^{-5} \pm 4.0 \times 10^{-5}$	$0.0075 \pm 0.0013$	$0.0028 \pm 0.0031$
	20-0.8	$7.2 \times 10^{-5} \pm 8.5 \times 10^{-6}$	$0 \pm 0$	$8.7 \times 10^{-5} \pm 2.3 \times 10^{-5}$	$5.8 \times 10^{-5} \pm 3.8 \times 10^{-5}$
Mouth	200-20	$0.0024 \pm 0.0006$	$0.0003 \pm 0.0001$	$0.0053 \pm 6.9 \times 10^{-5}$	$0.0026 \pm 0.0019$
	20-0.8	$0.0001 \pm 3.2 \times 10^{-5}$	$0 \pm 0$	$7.6 \times 10^{-5} \pm 2.7 \times 10^{-5}$	$8.2 \times 10^{-5} \pm 6 \times 10^{-5}$

Table 3.37 ANOVA results of specific nitrate uptake rates

Source of variation	Station	Size fraction: 200-20 $\mu\text{m}$			Size fraction: 20-0.8 $\mu\text{m}$		
		F	df	P-value	F	df	P-value
Between seasons	Reference	140.34	2,5	$4.05 \times 10^{-5}$	1.03	2,5	0.421
	Middle	42.87	2,5	$7.13 \times 10^{-4}$	31.71	2,5	0.001
	Mouth	55.43	2,5	$3.87 \times 10^{-4}$	14.31	2,5	0.009
Between stations	All	0.36	2,21	0.70	0.61	2,21	0.552

### Absolute nitrate uptake rate ( $\rho\text{NO}_3$ )

Like specific uptake rates, absolute uptake rates differed in the two fractions, with the larger fraction taking up more nitrate than the smaller fraction. The rates ranged widely for the larger fraction (0.8 to  $41.8 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ), while for the smaller fraction, rates ranged from 0 to  $0.9 \text{ ng at N l}^{-1} \text{ h}^{-1}$ . The annual averages

were maximum at the reference station for both the fractions ( $12.6 \pm 18.2 \text{ ng at N l}^{-1} \text{ h}^{-1}$  for the 200-20  $\mu\text{m}$  fraction and  $0.5 \pm 0.3 \text{ ng at N l}^{-1} \text{ h}^{-1}$  for the 20-0.8  $\mu\text{m}$  fraction). Minimum rates for both the fractions were recorded at the mouth station (Table 3.38). Statistical analysis revealed significant spatial variations only for the smaller fraction (Table 3.39).

The seasonal variations were significant at all stations for the larger fraction and at the middle and mouth stations for the smaller fraction (Table 3.39). The seasonal pattern (Fig. 3.22) showed maximum absolute uptake rates during the post-monsoon season for the larger fraction ( $41.8 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). The seasonal maximum for the smaller fraction was also during the post-monsoon season at the reference station ( $0.9 \pm 0.2 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). In this season, highest rates were recorded at the reference station, followed by the middle and mouth stations for both the fractions (Table 3.38). The seasonal minimum was in the monsoon season for both the fractions. For the smaller fraction, there was no detectable uptake at the middle and mouth stations, while for the larger fraction, minimum rates were recorded at the middle station ( $0.3 \pm 0.2 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ).

Table 3.38 Seasonal and annual averages of absolute nitrate uptake rates ( $\text{ng at N l}^{-1} \text{h}^{-1}$ )

Stations	Size( $\mu\text{m}$ )	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	200-20	2.1 $\pm$ 1.0	4.3 $\pm$ 0.6	41.8 $\pm$ 4.5	12.6 $\pm$ 18.2
	20-0.8	0.4 $\pm$ 0.3	0.5 $\pm$ 0.5	0.9 $\pm$ 0.2	0.5 $\pm$ 0.3
Middle	200-20	4.6 $\pm$ 2.3	0.3 $\pm$ 0.2	25.5 $\pm$ 4.4	8.8 $\pm$ 10.7
	20-0.8	0.3 $\pm$ 0.1	0 $\pm$ 0	0.1 $\pm$ 0.03	0.2 $\pm$ 0.1
Mouth	200-20	0.89 $\pm$ 0.12	1.0 $\pm$ 0.4	5.8 $\pm$ 0.1	2.1 $\pm$ 2.4
	20-0.8	0.17 $\pm$ 0.05	0 $\pm$ 0	0.02 $\pm$ 0.01	0.1 $\pm$ 0.1

Table 3.39 ANOVA results of absolute nitrate uptake rates

Source of variation	Station	Size fraction: 200-20 $\mu\text{m}$			Size fraction: 20-0.8 $\mu\text{m}$		
		F	df	P-value	F	df	P-value
Between seasons	Reference	242.04	2,5	$1.06 \times 10^{-5}$	1.72	2,5	0.270
	Middle	54.51	2,5	$4.03 \times 10^{-4}$	26.30	2,5	0.002
	Mouth	436.84	2,5	$2.44 \times 10^{-6}$	15.59	2,5	0.007
Between stations	All	1.49	2,21	0.248	10.26	2,21	0.0008

### 3.5.2.2 Nitrite

#### Specific nitrite uptake rate ( $\nu\text{NO}_2^-$ )

Specific nitrite uptake rates were about 10 times lower than that of nitrate and higher uptake rates were recorded for the larger fraction (range: 0 to  $0.0008 \text{ h}^{-1}$ ) compared to the smaller fraction (range: 0 to  $0.0002 \text{ h}^{-1}$ ). As station-wise variations were concerned, maximum rates were recorded at the middle station for the smaller fraction ( $6.9 \times 10^{-5} \pm 7.4 \times 10^{-5} \text{ h}^{-1}$ ) and a minimum at the mouth station ( $1.4 \times 10^{-5} \pm 2.7 \times 10^{-5} \text{ h}^{-1}$ ), while for the larger fraction, values were

identical at the three stations (Table 3.40). The data when tested with ANOVA showed no significant variations between the stations for both the fractions (Table 3.41).

Although the rates varied in a narrow range, ANOVA showed significant seasonal variations for both fractions, except for the smaller fraction at the mouth station (Table 3.41). The pattern more or less resembled that of nitrate specific uptake, with maximum uptake rates during the post-monsoon season and lowest rates in the monsoon season (Fig. 3.23). The maximum for the 200-20  $\mu\text{m}$  fraction was recorded during the post-monsoon season at the middle station ( $0.0008 \text{ h}^{-1}$ ). For the 20-0.8  $\mu\text{m}$  fraction, identical rates were recorded at the reference and middle stations ( $0.0002 \text{ h}^{-1}$ ) during this period. Low uptake rates were recorded in the monsoon and pre-monsoon seasons (Table 3.40). Both the fractions showed a seasonal minimum in the monsoon season at the mouth station, where uptake rates were not measurable. During the pre-monsoon season, uptake rates were also not measurable for the smaller fraction at the reference station.

Table 3.40 Seasonal and annual averages of specific nitrite uptake rates ( $\text{h}^{-1}$ )

Stations	Size ( $\mu\text{m}$ )	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	200-20	$6.2 \times 10^{-5} \pm 3.5 \times 10^{-5}$	$7.7 \times 10^{-5} \pm 3.0 \times 10^{-5}$	$0.0005 \pm 0.0002$	$0.0002 \pm 0.0002$
	20-0.8	$0 \pm 0$	$0 \pm 0$	$0.0002 \pm 6.0 \times 10^{-6}$	$4.4 \times 10^{-5} \pm 8.1 \times 10^{-5}$
Middle	200-20	$7.3 \times 10^{-5} \pm 2.2 \times 10^{-5}$	$3.1 \times 10^{-5} \pm 2.2 \times 10^{-5}$	$0.0008 \pm 1.5 \times 10^{-5}$	$0.0002 \pm 0.0003$
	20-0.8	$3.0 \times 10^{-5} \pm 1.6 \times 10^{-5}$	$4.1 \times 10^{-5} \pm 4.8 \times 10^{-6}$	$0.0002 \pm 7.9 \times 10^{-5}$	$6.9 \times 10^{-5} \pm 7.4 \times 10^{-5}$
Mouth	200-20	$3.4 \times 10^{-5} \pm 1.8 \times 10^{-5}$	$0 \pm 0$	$0.0007 \pm 8.9 \times 10^{-5}$	$0.0002 \pm 0.0003$
	20-0.8	$2.9 \times 10^{-5} \pm 3.4 \times 10^{-5}$	$0 \pm 0$	$0 \pm 0$	$1.4 \times 10^{-5} \pm 2.7 \times 10^{-5}$

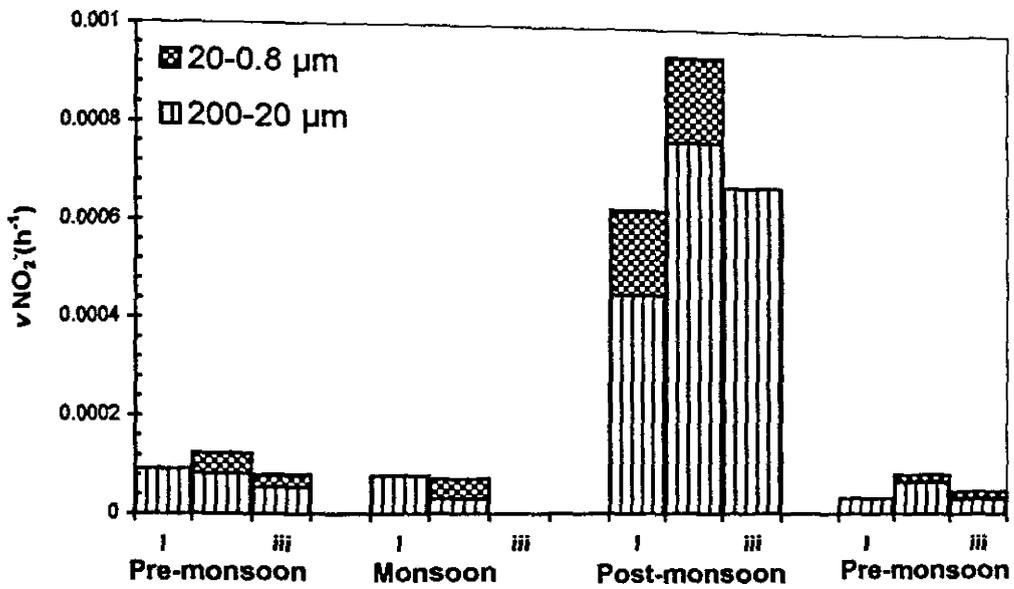


Fig. 3.23 Seasonal changes of specific nitrite uptake rates by two size fractions at the (i) reference, (ii) middle and (iii) mouth stations

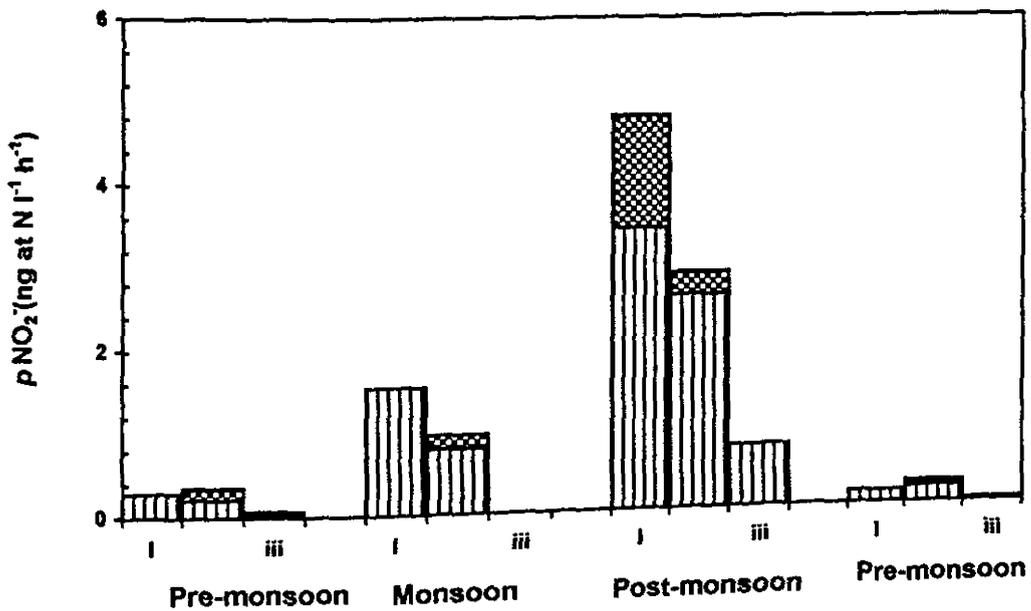


Fig. 3.24 Seasonal changes of absolute nitrite uptake rates by two size fractions at the (i) reference, (ii) middle and (iii) mouth stations

Table 3.41 ANOVA results of specific nitrite uptake rates

Source of variation	Station	Size fraction: 200-20 $\mu\text{m}$			Size fraction: 20-0.8 $\mu\text{m}$		
		F	df	P-value	F	df	P-value
Between seasons	Reference	18.19	2,5	0.0051	3237.89	2,5	$1.65 \times 10^{-8}$
	Middle	865.32	2,5	$4.45 \times 10^{-7}$	11.13	2,5	0.014
	Mouth	179.21	2,5	$2.22 \times 10^{-5}$	1.18	2,5	0.381
Between stations	All	0.141	2,21	0.869	1.41	2,21	0.266

### Absolute nitrite uptake rate ( $\rho\text{NO}_2$ )

Absolute nitrite uptake rates ranged from 0 to 3.4  $\eta\text{g at N l}^{-1} \text{h}^{-1}$  for the larger fraction and 0 to 1.4  $\eta\text{g at N l}^{-1} \text{h}^{-1}$  for the smaller fraction. For the 200-20  $\mu\text{m}$  fraction, maximum rates were obtained at the reference station ( $1.4 \pm 1.5 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ), followed by the middle station ( $1.0 \pm 1.1 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ) and the lowest were at the mouth station ( $0.2 \pm 0.3 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ). The 20-0.8  $\mu\text{m}$  fraction also showed decreasing rates from the reference ( $0.3 \pm 0.6 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ) to the mouth station ( $0.02 \pm 0.04 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ) (Table 3.42). The spatial variations were not statistically different (Table 3.43).

The variations between the seasons were significant at all stations for the larger fraction, but only at the reference station for the smaller fraction (Table 3.43). The seasonal changes revealed a slightly different pattern from that of specific uptake (Fig. 3.24). High absolute uptake rates were recorded in the post-monsoon season for both the fractions, with the exception of the mouth station

for the smaller fraction (no measurable absolute uptake). During the monsoon season, rates were moderately high at the reference ( $1.6 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and middle ( $0.8 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) stations for the larger fraction and minimum at the mouth station for both the fractions. The pre-monsoon season was characterised by lower rates at all stations (Table 3.42).

Table 3.42 Seasonal and annual averages of absolute nitrite uptake rates ( $\text{ng at N l}^{-1} \text{ h}^{-1}$ )

Stations	Size( $\mu\text{m}$ )	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	200-20	$0.2 \pm 0.1$	$1.6 \pm 0.7$	$3.4 \pm 1.2$	$1.4 \pm 1.5$
	20-0.8	$0 \pm 0$	$0 \pm 0$	$1.4 \pm 0.1$	$0.3 \pm 0.6$
Middle	200-20	$0.2 \pm 0.1$	$0.8 \pm 0.02$	$2.6 \pm 0.1$	$1.0 \pm 1.1$
	20-0.8	$0.1 \pm 0.1$	$0.2 \pm 0.02$	$0.3 \pm 0.1$	$0.2 \pm 0.1$
Mouth	200-20	$0.01 \pm 0.008$	$0 \pm 0$	$0.7 \pm 0.1$	$0.2 \pm 0.3$
	20-0.8	$0.04 \pm 0.05$	$0 \pm 0$	$0 \pm 0$	$0.02 \pm 0.04$

Table 3.43 ANOVA results of absolute nitrite uptake rates

Source of variation	Station	Size fraction: 200-20 $\mu\text{m}$			Size fraction: 20-0.8 $\mu\text{m}$		
		F	df	P-value	F	df	P-value
Between seasons	Reference	18.34	2,5	0.0049	3496.73	2,5	$1.36 \times 10^{-8}$
	Middle	1324.26	2,5	$1.54 \times 10^{-7}$	4.25	2,5	0.083
	Mouth	198.17	2,5	$1.73 \times 10^{-5}$	0.997	2,5	0.432
Between stations	All	2.38	2,21	0.1167	1.52	2,21	0.243

### 3.5.2.3 Ammonium

#### Specific ammonium uptake rate ( $v_{\text{NH}_4^+}$ )

The uptake rates showed that the smaller fraction utilised ammonium extremely well, with rates that ranged from  $1.6 \times 10^{-5}$  to  $0.016 \text{ h}^{-1}$ . Although lower rates were recorded for the larger fraction (range: 0 to  $0.0087 \text{ h}^{-1}$ ), ammonium was still taken up rapidly and ranked next to nitrate. Maximum annual average rates were obtained at the middle station ( $0.004 \pm 0.004 \text{ h}^{-1}$ ) for the larger fraction and at the reference station for the smaller fraction ( $0.009 \pm 0.007 \text{ h}^{-1}$ ). Minimum rates for both the fractions were recorded at the mouth station (Table 3.44). ANOVA showed significant variations for the larger fraction, with rates varying widely between the stations, while for the smaller fraction, rates did not show a large spatial difference (Table 3.45).

The seasonal variations were significant at all stations for both the fractions (Table 3.45). The seasonal pattern was well marked, with maximum rates during the pre-monsoon season and lowest rates in the monsoon season (Fig. 3.25). The seasonal maximum was recorded at different stations for the two size fractions, *i.e.*, at the reference station for the smaller fraction ( $0.016 \pm 0.009 \text{ h}^{-1}$ ) and at the middle station for the larger fraction ( $0.008 \pm 0.001 \text{ h}^{-1}$ ).

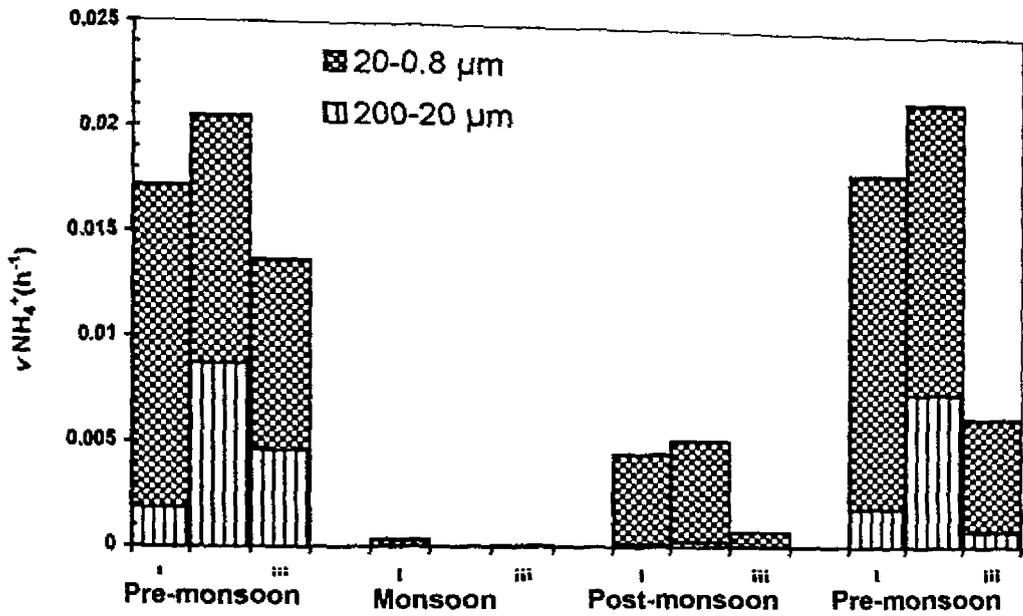


Fig. 3.25 Seasonal changes of specific ammonium uptake rates by two size fractions at the (i) reference, (ii) middle and (iii) mouth stations

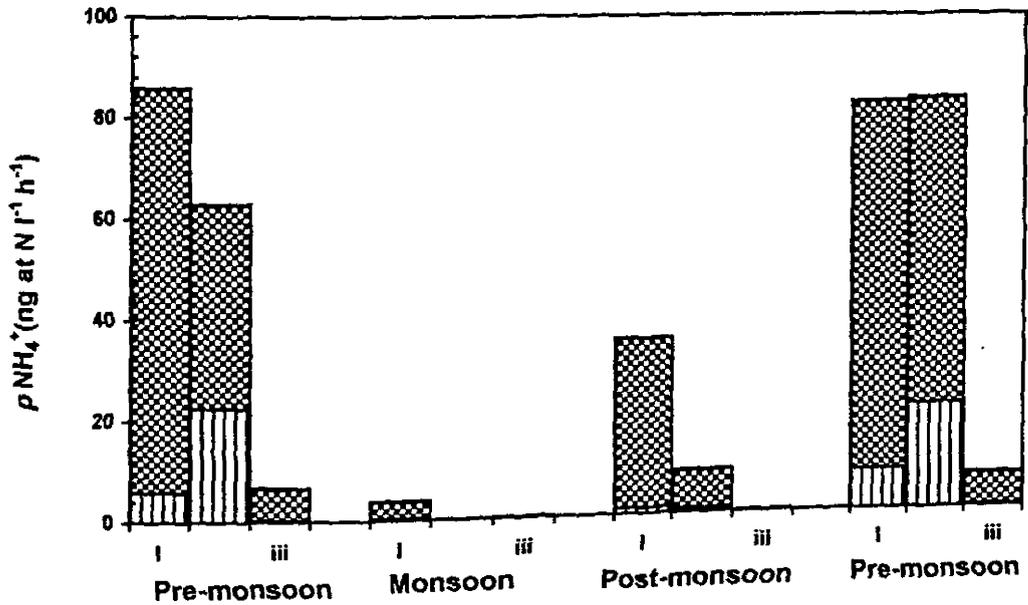


Fig. 3.26 Seasonal changes of absolute ammonium uptake rates by two size fractions at the (i) reference, (ii) middle and (iii) mouth stations

Table 3.44 Seasonal and annual averages of specific ammonium uptake rates ( $h^{-1}$ )

Stations	Size ( $\mu m$ )	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	200-20	$0.0019 \pm 0.0002$	$2.8 \times 10^{-5} \pm 2.2 \times 10^{-5}$	$0.0001 \pm 0.0002$	$0.001 \pm 0.001$
	20-0.8	$0.016 \pm 0.001$	$0.0003 \pm 0.0001$	$0.004 \pm 0.0004$	$0.009 \pm 0.007$
Middle	200-20	$0.008 \pm 0.001$	$0 \pm 0$	$0.0002 \pm 4.3 \times 10^{-5}$	$0.004 \pm 0.004$
	20-0.8	$0.013 \pm 0.003$	$1.6 \times 10^{-5} \pm 3.9 \times 10^{-6}$	$0.005 \pm 5.3 \times 10^{-5}$	$0.008 \pm 0.006$
Mouth	200-20	$0.0007 \pm 0.0001$	$1.8 \times 10^{-5} \pm 2.5 \times 10^{-6}$	$2.0 \times 10^{-5} \pm 8.6 \times 10^{-6}$	$0.0003 \pm 0.0004$
	20-0.8	$0.005 \pm 0.0008$	$7.0 \times 10^{-5} \pm 8.6 \times 10^{-6}$	$0.0007 \pm 1.5 \times 10^{-5}$	$0.003 \pm 0.003$

Table 3.45 ANOVA results of specific ammonium uptake rates

Source of variation	Station	Size fraction: 200-20 $\mu m$			Size fraction: 20-0.8 $\mu m$		
		F	df	P-value	F	df	P-value
Between seasons	Reference	147.76	2,5	$3.57 \times 10^{-5}$	359.21	2,5	$3.97 \times 10^{-6}$
	Middle	111.06	2,5	$7.19 \times 10^{-5}$	32.80	2,5	0.0013
	Mouth	53.51	2,5	0.00042	56.44	2,5	0.00037
Between stations	All	4.97	2,21	0.017	2.76	2,21	0.086

#### Absolute ammonium uptake rate ( $\rho NH_4^+$ )

Absolute ammonium uptake rates ranged from 0 to 22.4  $\mu g$  at  $N \ l^{-1} \ h^{-1}$  for the larger fraction and from 0.1 to 80.0  $\mu g$  at  $N \ l^{-1} \ h^{-1}$  for the smaller fraction. Like specific uptake, rates were significantly higher for the smaller fraction. As annual averages, maximum rates were recorded at the reference station for the 20-0.8  $\mu m$  fraction ( $48.1 \pm 33.4 \mu g$  at  $N \ l^{-1} \ h^{-1}$ ) and at the middle station for the 200-20  $\mu m$  fraction ( $11.0 \pm 11.5 \mu g$  at  $N \ l^{-1} \ h^{-1}$ ). Lowest rates for both the fractions were

at the mouth station (Table 3.46). ANOVA revealed significant spatial variations for both the fractions (Table 3.47)

The seasonal variations were also significant (Table 3.47). The seasonal pattern (Fig. 3.26) was similar to that of specific uptake with maximum rates in the pre-monsoon season, followed by the post-monsoon and minimum in the monsoon season. The pre-monsoon maximum was at the reference station for the smaller fraction ( $77.3 \pm 4.5 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ) and at the middle station for the larger fraction ( $21.7 \pm 2.3 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ). The monsoon low was recorded at the middle station for both the fractions (Table 3.46).

Table 3.46 Seasonal and annual averages of absolute ammonium uptake rates ( $\text{ng at N } \Gamma^1 \text{ h}^{-1}$ )

Stations	Size ( $\mu\text{m}$ )	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	200-20	6.9 $\pm$ 1.3	0.3 $\pm$ 0.2	1.0 $\pm$ 1.31	3.8 $\pm$ 3.5
	20-0.8	77.3 $\pm$ 4.5	3.6 $\pm$ 1.6	34.3 $\pm$ 3.4	48.1 $\pm$ 33.4
Middle	200-20	21.7 $\pm$ 2.3	0 $\pm$ 0	0.8 $\pm$ 0.14	11.0 $\pm$ 11.5
	20-0.8	51.3 $\pm$ 15.1	0.07 $\pm$ 0.02	7.8 $\pm$ 0.16	27.6 $\pm$ 27.4
Mouth	200-20	0.2 $\pm$ 0.03	0.05 $\pm$ 0.01	0.02 $\pm$ 0.01	0.1 $\pm$ 0.1
	20-0.8	6.5 $\pm$ 0.6	0.20 $\pm$ 0.02	0.2 $\pm$ 0.01	3.4 $\pm$ 3.4

Table 3.47 ANOVA results of absolute ammonium uptake rates

Source of variation	Station	Size fraction: 200-20 $\mu\text{m}$			Size fraction: 20-0.8 $\mu\text{m}$		
		F	df	P-value	F	df	P-value
Between seasons	Reference	27.23	2,5	0.002	256.15	2,5	$9.18 \times 10^{-8}$
	Middle	147.89	2,5	$3.56 \times 10^{-5}$	16.61	2,5	0.0062
	Mouth	85.31	2,5	0.00014	200.63	2,5	$1.68 \times 10^{-5}$
Between stations	All	5.13	2,21	0.015	6.41	2,21	0.0067

#### 3.4.2.4 Urea

##### Specific urea uptake rate ( $v_{\text{urea}}$ )

Like ammonium, urea was also significantly taken up by the smaller fraction with specific uptake rates ranging from  $1.9 \times 10^{-5}$  to  $0.0008 \text{ h}^{-1}$ . However, rates were 10 times lower than those of specific ammonium uptake. For the larger fraction, rates ranged from 0 to  $0.0004 \text{ h}^{-1}$ . As station-wise variations were concerned, maximum rates were recorded for the larger fraction at the middle station ( $0.0003 \pm 0.0002 \text{ h}^{-1}$ ) and a minimum at the mouth station ( $9.3 \times 10^{-5} \pm 6.0 \times 10^{-5}$ ), while, for the smaller fraction, values were identical at the middle and mouth stations (Table 3.48). Statistical analysis revealed significant variations only for the larger fraction (Table 3.49).

The variations between the seasons were significant for both the fractions (Table 3.49). The seasonal pattern resembled that of ammonium, with higher uptake in the pre-monsoon season, moderately high in the post-monsoon

season and lowest in the monsoon season for both the fractions (Fig. 3.27). The pre-monsoon maximum was recorded at the middle station for both the fractions ( $0.0004 \pm 0.0001 \text{ h}^{-1}$  for the larger fraction and  $0.0006 \pm 9.2 \times 10^{-5} \text{ h}^{-1}$  for the smaller fraction). Specific uptake rates during the monsoon season were very low.

Table 3.48 Seasonal and annual averages of specific urea uptake rates ( $\text{h}^{-1}$ )

Stations	Size ( $\mu\text{m}$ )	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	200-20	$0.0002 \pm 3.1 \times 10^{-5}$	$0 \pm 0$	$6.9 \times 10^{-5} \pm 3.3 \times 10^{-5}$	$0.0001 \pm 0.0001$
	20-0.8	$0.0006 \pm 0.0003$	$5.0 \times 10^{-5} \pm 6.8 \times 10^{-6}$	$0.0001 \pm 3.9 \times 10^{-5}$	$0.0003 \pm 0.0003$
Middle	200-20	$0.0004 \pm 0.0001$	$1.5 \times 10^{-5} \pm 9.3 \times 10^{-6}$	$0.0003 \pm 2.0 \times 10^{-5}$	$0.0003 \pm 0.0002$
	20-0.8	$0.0006 \pm 9.2 \times 10^{-5}$	$1.9 \times 10^{-5} \pm 8.1 \times 10^{-6}$	$0.0002 \pm 2.7 \times 10^{-5}$	$0.0004 \pm 0.0003$
Mouth	200-20	$0.0001 \pm 1.5 \times 10^{-5}$	$0 \pm 0$	$0.0001 \pm 2.1 \times 10^{-5}$	$9.3 \times 10^{-5} \pm 6.0 \times 10^{-5}$
	20-0.8	$0.0005 \pm 0.0001$	$3.2 \times 10^{-5} \pm 3.7 \times 10^{-6}$	$0.0004 \pm 0.0001$	$0.0004 \pm 0.0002$

Table 3.49 ANOVA results of specific urea uptake rates

Source of variation	Station	Size fraction: 200-20 $\mu\text{m}$			Size fraction: 20-0.8 $\mu\text{m}$		
		F	df	P-value	F	df	P-value
Between seasons	Reference	42.84	2,5	0.00071	6.79	2,5	0.037
	Middle	8.99	2,5	0.022	44.76	2,5	0.0006
	Mouth	54.58	2,5	0.0004	14.41	2,5	0.008
Between stations	All	3.79	2,21	0.039	0.025	2,21	0.975

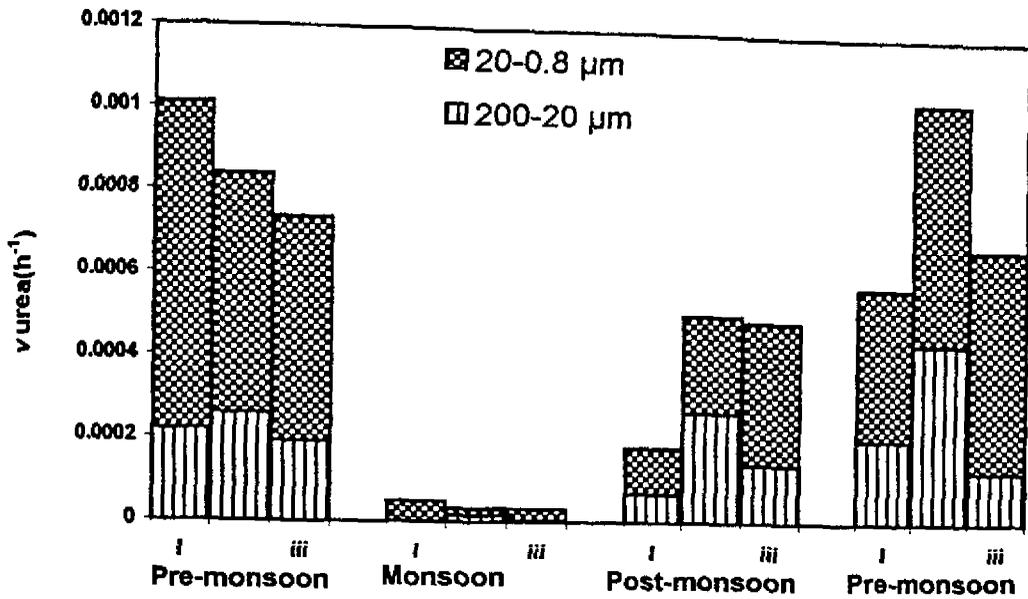


Fig. 3.27 Seasonal changes of specific urea uptake rates by two size fractions at the (i) reference, (ii) middle and (iii) mouth stations

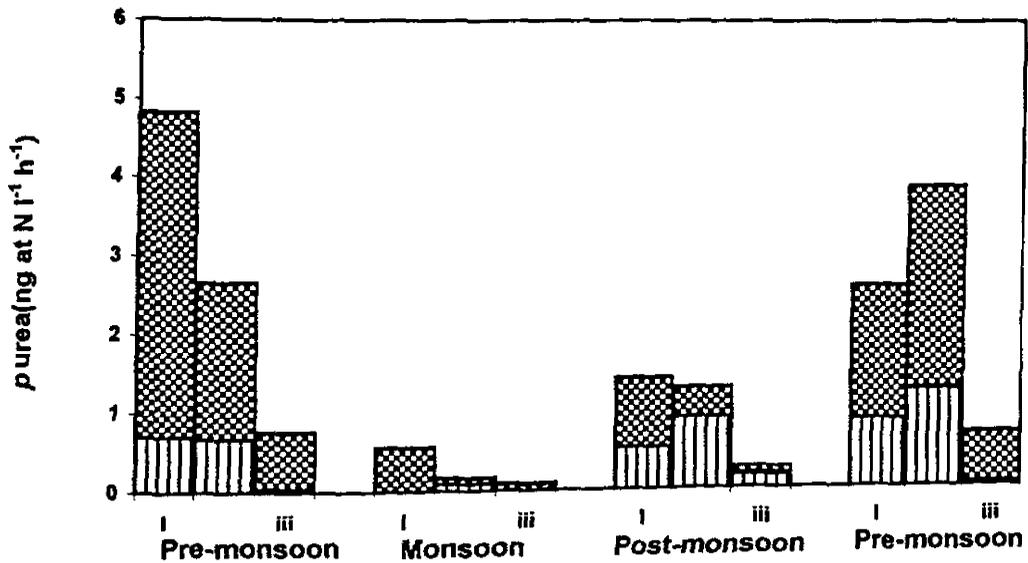


Fig. 3.28 Seasonal changes of absolute urea uptake rates by two size fractions at the (i) reference, (ii) middle and (iii) mouth stations

**Absolute urea uptake rate ( $\rho_{urea}$ )**

The absolute uptake rates ranged from 0 to 0.91  $\eta\text{g at N l}^{-1} \text{h}^{-1}$  for the larger fraction and 0.08 to 4.1  $\eta\text{g at N l}^{-1} \text{h}^{-1}$  for the smaller fraction. Maximum rates for the larger fraction were recorded at the middle station ( $0.7 \pm 0.5 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ) and for the smaller fraction, highest rates were obtained at the reference station ( $1.8 \pm 1.5 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ). Lowest absolute uptake rates were recorded at the mouth station for both the fractions (Table 3.50). ANOVA showed significant spatial variations for both the fractions (Table 3.51).

The seasonal variations were significant for both fractions, except at the reference station for the smaller fraction (Table 3.51). The seasonal changes are presented in Fig. 3.28 and the seasonal maximum for both the fractions was recorded during the pre-monsoon season at the middle station ( $1.0 \pm 0.4 \eta\text{g at N l}^{-1} \text{h}^{-1}$  for the larger fraction and at the reference station for the smaller fraction ( $2.9 \pm 1.4 \eta\text{g at N l}^{-1} \text{h}^{-1}$ ). Rates during the monsoon season were considerably low (Table 3.50).

Table 3.50 Seasonal and annual averages of absolute urea uptake rates ( $\eta$  at  $\text{N l}^{-1} \text{h}^{-1}$ )

Stations	Size ( $\mu\text{m}$ )	Pre-monsoon	Monsoon	Post-monsoon	Annual
Reference	200-20	0.8 $\pm$ 0.2	0 $\pm$ 0	0.5 $\pm$ 0.3	0.5 $\pm$ 0.4
	20-0.8	2.9 $\pm$ 1.4	0.6 $\pm$ 0.1	0.9 $\pm$ 0.3	1.8 $\pm$ 1.5
Middle	200-20	1.0 $\pm$ 0.4	0.1 $\pm$ 0.1	0.9 $\pm$ 0.1	0.7 $\pm$ 0.5
	20-0.8	2.3 $\pm$ 0.55	0.1 $\pm$ 0.03	0.4 $\pm$ 0.1	1.3 $\pm$ 1.2
Mouth	200-20	0.04 $\pm$ 0.002	0 $\pm$ 0	0.2 $\pm$ 0.02	0.1 $\pm$ 0.1
	20-0.8	0.7 $\pm$ 0.1	0.1 $\pm$ 0.01	0.1 $\pm$ 0.04	0.4 $\pm$ 0.3

Table 3.51 ANOVA results of absolute urea uptake rates

Source of variation	Station	Size fraction: 200-20 $\mu\text{m}$			Size fraction: 20-0.8 $\mu\text{m}$		
		F	df	P-value	F	df	P-value
Between seasons	Reference	14.26	2,5	0.0086	3.88	2,5	0.096
	Middle	6.50	2,5	0.041	23.99	2,5	0.0027
	Mouth	132.92	2,5	$4.63 \times 10^{-5}$	29.69	2,5	0.0017
Between stations	All	7.86	2,21	0.0028	3.34	2,21	0.055

In conclusion, the two fractions had an uptake preference: The smaller fraction (nanophytoplankton) utilised the reduced forms, namely ammonium and urea extremely well, while the larger fraction (microphytoplankton) preferred the oxidized forms, nitrate and nitrite. Comparing the uptake rates of the different nutrients by the larger fraction, nitrate was taken up at a higher rate, with the maximum mean annual uptake ( $0.0024 \pm 0.0024 \text{ h}^{-1}$ ), followed closely by ammonium ( $0.0018 \pm 0.0029 \text{ h}^{-1}$ ). Nitrite and urea uptake rates were ten times lower than those of nitrate and ammonium. For the smaller fraction, the

variations in uptake rates of the different nutrients were more pronounced and the order of preference was: ammonium > urea > nitrate > nitrite. Ammonium uptake rates ( $0.0065 \pm 0.0062 \text{ h}^{-1}$ ) were 10 times higher than that of urea ( $0.0004 \pm 0.0003 \text{ h}^{-1}$ ), and 100 times that of nitrate and nitrite ( $7.0 \times 10^{-5} \pm 5.0 \times 10^{-5} \text{ h}^{-1}$  and  $4.3 \times 10^{-5} \pm 6.6 \times 10^{-5} \text{ h}^{-1}$ ).

### 3.6 SALIENT FEATURES OF RESULTS

#### 3.6.1 AMBIENT NITROGEN CONCENTRATIONS

- Nitrate was the most abundant nutrient, contributing to as much as 72% to the measured dissolved nitrogen pool ( $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+ + \text{urea-N}$ ). This was followed by ammonium (16%) and nitrite and urea which contributed to 6% each.
- Nitrate concentrations ranged from 0.4 to 19.6  $\mu\text{g at N l}^{-1}$  (average: 4.5  $\mu\text{g at N l}^{-1}$ ). The spatial variations were not significant and concentrations were high at all three stations (reference station:  $4.7 \pm 5.3 \mu\text{g at N l}^{-1}$ , middle station:  $4.1 \pm 5.2 \mu\text{g at N l}^{-1}$  and mouth station:  $4.7 \pm 4.9 \mu\text{g at N l}^{-1}$ ). The seasonal variations were well defined, marked by a high average in the monsoon season (average: 10.3  $\mu\text{g at N l}^{-1}$ ). The concentrations in the pre- and post-monsoon seasons remained low throughout (pre-monsoon: 2.2  $\mu\text{g at N l}^{-1}$ ; post-monsoon: 1.9  $\mu\text{g at N l}^{-1}$ ).
- Nitrite was present in measurable concentrations throughout the study (range: 0.03 - 0.8  $\mu\text{g at N l}^{-1}$ ; average: 0.2  $\mu\text{g at N l}^{-1}$ ). Although the spatial variations were statistically significant, concentrations varied in a narrow

range with maximum values at the mouth station ( $0.4 \pm 0.3 \mu\text{g at N l}^{-1}$ ), followed by the middle ( $0.2 \pm 0.1 \mu\text{g at N l}^{-1}$ ) and the reference ( $0.1 \pm 0.1 \mu\text{g at N l}^{-1}$ ) stations. The seasonal changes were significant and showed a monsoon maximum ( $0.4 \mu\text{g at N l}^{-1}$ ) like that of nitrate, while, the lowest concentrations were recorded in the non-monsoon months (pre-monsoon:  $0.1 \mu\text{g at N l}^{-1}$ ; post-monsoon:  $0.3 \mu\text{g at N l}^{-1}$ ).

- Ammonium concentrations ranged from 0.1 to  $1.5 \mu\text{g at N l}^{-1}$  (average:  $0.5 \mu\text{g at N l}^{-1}$ ). The seasonal changes were characterised by high concentrations in the pre-monsoon season (average:  $0.7 \mu\text{g at N l}^{-1}$ ). Low concentrations were recorded in the monsoon ( $0.4 \mu\text{g at N l}^{-1}$ ) and post-monsoon ( $0.3 \mu\text{g at N l}^{-1}$ ) seasons. The spatial variations were significant and identical values were recorded at the reference and middle stations ( $0.6 \pm 0.3 \mu\text{g at N l}^{-1}$ ), but only about half of these at the mouth station ( $0.3 \pm 0.2 \mu\text{g at N l}^{-1}$ ).
- Urea concentrations ranged from 0.1 to  $0.7 \mu\text{g at N l}^{-1}$  throughout the study period (average:  $0.2 \mu\text{g at N l}^{-1}$ ). The spatial differences in urea concentrations were not significant. Although the seasonal variations were statistically insignificant, the monthly changes followed a well defined cycle. High concentrations were recorded in the monsoon season (average:  $0.3 \mu\text{g at N l}^{-1}$ ) with a peak in the month of June at all the stations. The concentrations in the other two seasons remained low (average:  $0.2 \mu\text{g at N l}^{-1}$ ).

- Particulate organic nitrogen (PON) concentrations ranged from 12.1 to 207.9  $\mu\text{g}$  at  $\text{N l}^{-1}$  over the whole study period. The seasonal and spatial variations were significant. Maximum PON concentrations were recorded at the reference station ( $85.8 \pm 43.6 \mu\text{g}$  at  $\text{N l}^{-1}$ ), followed by the middle station ( $72.5 \pm 36.7 \mu\text{g}$  at  $\text{N l}^{-1}$ ) and the lowest at the mouth station ( $48.6 \pm 51.6 \mu\text{g}$  at  $\text{N l}^{-1}$ ). The seasonal maximum of PON concentrations was in the monsoon season (average:  $116.5 \mu\text{g}$  at  $\text{N l}^{-1}$ ). Lower PON concentrations were recorded in the pre-monsoon ( $54.6 \mu\text{g}$  at  $\text{N l}^{-1}$ ) and post-monsoon ( $44.4 \mu\text{g}$  at  $\text{N l}^{-1}$ ) seasons.

### 3.6.2 BIOLOGICAL PARAMETERS

- Chlorophyll-*a* concentrations ranged from 0.1 to 21.6  $\mu\text{g}$  (chl-*a*)  $\text{l}^{-1}$  in the seasonal cycle. Maximum concentrations were recorded in the non-monsoon season (post-monsoon:  $10.2 \mu\text{g}$  (chl-*a*)  $\text{l}^{-1}$ ; pre-monsoon:  $9.9 \mu\text{g}$  (chl-*a*)  $\text{l}^{-1}$ ) and minimum during the monsoon season ( $4.2 \mu\text{g}$  (chl-*a*)  $\text{l}^{-1}$ ).
- Size-fractionated chlorophyll-*a* measurements showed that the nanophytoplankton (20-0.8  $\mu\text{m}$ ) contributed to a major part of the chlorophyll-*a* in the monsoon and pre-monsoon months (76 and 66% respectively), while the microphytoplankton (200-20  $\mu\text{m}$ ) were abundant in the post-monsoon months (81%).
- The large-celled microphytoplankton, dominated by diatoms constituted the major group of phytoplankton and formed 58.7% of the total population. This

was followed by the flagellates and blue green algae which contributed to 20.8% and 20.5% respectively.

- Maximum cell densities were recorded at the middle station ( $525 \pm 367$  cells  $\times 10^3 \text{ l}^{-1}$ ), followed by the reference ( $515 \pm 396$  cells  $\times 10^3 \text{ l}^{-1}$ ) and mouth ( $382 \pm 267$  cells  $\times 10^3 \text{ l}^{-1}$ ) stations.
- The seasonal variations of total phytoplankton revealed higher densities in the non-monsoon months, and lowest densities during the monsoon months.
- Shannon-Weaver diversity indices did not differ to a great extent between the stations, but maximum species diversity was recorded at the reference station. The monthly variations of diversity indices showed higher species diversity in the post-monsoon season.

### 3.6.3 REGENERATION OF NITROGEN

#### 3.6.3.1 Ammonification rates

- The ammonification rates in the present study ranged from 10 to 1500  $\text{ng at N l}^{-1} \text{ h}^{-1}$ . The spatial variations were not significant. Maximum rates were recorded at the middle station ( $410 \pm 500 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ), followed by the reference station ( $360 \pm 430 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and the lowest rates at the mouth station ( $220 \pm 260 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). The seasonal changes of ammonification rates showed significant variations and were marked by a pre-monsoon peak at all stations (average:  $663 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). Rates were moderately high in the post-monsoon season (average:  $130 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and minimum rates were recorded in the monsoon season (average:  $33 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ).

### 3.6.3.2 Nitrification rates

- Nitrification rates ranged from 0.1 to 96.7  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$  (average: 42.6  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$ ).
- Variations in nitrification rates between the stations were not significant. The rates at the reference and middle stations differed only marginally ( $49.9 \pm 28.5 \mu\text{g at N l}^{-1} \text{ h}^{-1}$  and  $47.3 \pm 29.2 \mu\text{g at N l}^{-1} \text{ h}^{-1}$  respectively), while at the mouth station, lower nitrification rates were recorded ( $30.6 \pm 19.3 \mu\text{g at N l}^{-1} \text{ h}^{-1}$ ).
- The seasonal changes were distinct with minimum values in the monsoon season (average: 14.3  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$ ), followed by moderately high values in the post-monsoon season (average: 34.6  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$ ) culminating in peak values in the pre-monsoon season (average: 68.3  $\mu\text{g at N l}^{-1} \text{ h}^{-1}$ ).

## 3.6.4 UPTAKE STUDIES

### 3.6.4.1 Unfractionated uptake

- The  $v\text{NO}_3^-$  showed significant variations between seasons but not between stations. Maximum nitrate uptake was recorded in the post-monsoon months ( $v\text{NO}_3^-$ : 0.004  $\text{h}^{-1}$ ), followed by the pre-monsoon ( $v\text{NO}_3^-$ : 0.0014  $\text{h}^{-1}$ ) and the lowest in the monsoon months ( $v\text{NO}_3^-$ : 0.0009  $\text{h}^{-1}$ ). The  $\rho\text{NO}_3^-$  showed significant spatial variations, with maximum rates at the reference station ( $143.7 \pm 132.5 \mu\text{g at N l}^{-1} \text{ h}^{-1}$ ) and lowest values at the mouth station ( $56.7 \pm 39.1 \mu\text{g at N l}^{-1} \text{ h}^{-1}$ ). High  $\rho\text{NO}_3^-$  was recorded in the post-monsoon season

(192.9  $\eta\text{g at N l}^{-1} \text{ h}^{-1}$ ) and minimum in the pre-monsoon season (56.7  $\eta\text{g at N l}^{-1} \text{ h}^{-1}$ ).

- The  $v\text{NO}_2^-$  and  $\rho\text{NO}_2^-$  were very low but the seasonal changes were similar to those of nitrate uptake. The maximum nitrite uptake was recorded in the post-monsoon (average  $\rho\text{NO}_2^-$ : 20.9  $\eta\text{g at N l}^{-1} \text{ h}^{-1}$ ) months and the lowest in the pre-monsoon months (average  $\rho\text{NO}_2^-$ : 10.6  $\eta\text{g at N l}^{-1} \text{ h}^{-1}$ ). As that of nitrate, the  $v\text{NO}_2^-$  was also high in the post-monsoon months ( $v\text{NO}_2^-$ : 0.0004  $\text{h}^{-1}$ ) and minimum in the monsoon months ( $v\text{NO}_2^-$ :  $1.5 \times 10^{-4} \text{ h}^{-1}$ ).
- The  $v\text{NH}_4^+$  were comparatively higher than  $v\text{NO}_2^-$  and  $v\text{NO}_3^-$  and ranged from  $3.6 \times 10^{-5}$  to  $1.4 \times 10^{-2} \text{ h}^{-1}$ . Both  $v\text{NH}_4^+$  and  $\rho\text{NH}_4^+$  showed significant seasonal and spatial variations. Maximum rates were recorded in the pre-monsoon season (average  $v\text{NH}_4^+$ : 0.006  $\text{h}^{-1}$ ;  $\rho\text{NH}_4^+$ : 354.4  $\eta\text{g at N l}^{-1} \text{ h}^{-1}$ ) and lower rates in the intervening seasons.
- Urea uptake stood third, after ammonium and nitrate. The spatial variations of  $v\text{urea}$  and  $\rho\text{urea}$  were not significant. High uptake rates were recorded in the pre-monsoon (average  $v\text{urea}$ : 0.0006  $\text{h}^{-1}$ ;  $\rho\text{urea}$ : 29.5  $\text{h}^{-1}$ ) and post-monsoon (average  $v\text{urea}$ : 0.0006  $\text{h}^{-1}$ ;  $\rho\text{urea}$ : 25.5  $\text{h}^{-1}$ ) seasons. The minimum was in the monsoon season.

#### 3.6.4.2 Size-fractionated uptake

- The maximum uptake of ammonium was by the smaller fraction (20-0.8  $\mu\text{m}$ ) ( $v\text{NH}_4^+$ : 0.007  $\text{h}^{-1}$ ;  $\rho\text{NH}_4^+$ : 26.4  $\eta\text{g at N l}^{-1} \text{ h}^{-1}$ ) and that of nitrate was by the

larger fraction (200-20  $\mu\text{m}$ ) ( $v\text{NO}_3^-$ :  $0.002 \text{ h}^{-1}$ ;  $p\text{NO}_3^-$ :  $8.27 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) in all the seasons.

- Seasonal variations in the uptake rates were significant for both the fractions. Maximum uptake of nitrate, nitrite, ammonium and urea were recorded in the non-monsoon months, and lowest rates in the monsoon months for both the fractions.

## CHAPTER IV DISCUSSION

### 4.1 AMBIENT NITROGEN

#### 4.1.1 NITRATE ( $\text{NO}_3^-$ )

Nitrate is the thermodynamically stable and most oxidized form of inorganic nitrogen and plays a significant role in the nitrogen cycle. It is the second most abundant species of nitrogen, next to  $\text{N}_2$  gas and is an important nutrient source for phytoplankton. It is also involved in reduction pathways and serves as the terminal electron acceptor in microbially-mediated processes such as denitrification and dissimilatory nitrate reduction.

The importance of nitrate lies in its ability to regulate primary production as a new nitrogen source for primary producers (Eppley and Peterson, 1979; Malone *et al.*, 1983). Nitrate is allochthonously supplied through physically driven transport (upwelling, tidal mixing and vertical diffusion processes), fresh water advection, land run-off, atmospheric and anthropogenic input.

The results showed that nitrate was the most dominant form of nitrogen among the measured nitrogenous nutrients and contributed substantially (72%) to the total measured dissolved nitrogen pool. The concentrations ranged from 0.4 to 19.6  $\mu\text{g at N l}^{-1}$  and are almost similar to those measured in earlier studies (Nair *et al.*, 1984; Nixon *et al.*, 1984). The concentrations of  $\text{NO}_3^-$  measured in other mangrove ecosystems are given in Table 4.1.

Table 4.1 Mean concentrations of dissolved nitrogen ( $\mu\text{M}$ ) in mangrove creeks and estuarine waters.

Location	$\text{NH}_4^+$	$\text{NO}_3^-$	$\text{NO}_2^-$	Reference
Vatuwaga, Fiji				
Unpolluted	0.6	0.65	-	Nedwell, 1975
Polluted	50.94	36.58		
Kerala estuaries, India	5.5-13.54	4.31-6.23	-	Sarala Devi <i>et al.</i> , 1983
West Coast, Malaysia	1.5-24.7	0-13.8	-	Nixon <i>et al.</i> , 1984
Phuket, Thailand	-	4-16	-	Limpsaichol, 1984
Kadinamkulam, India	-	0-15	-	Nair <i>et al.</i> , 1984
Kedah, Malaysia	0-50	0.1-6	0.1-6	Wong, 1984
Porto Novo, India	-	3.16-30.5	-	Kannan and Krishnamurthy, 1985
Hinchinbrook Is., Australia	0.1-0.65	0-0.22	-	Boto and Wellington, 1988
Magdalena Bay, Mexico	-	9-19	-	Guerrero <i>et al.</i> , 1988
Galley Reach, Papua New Guinea	0-5	0-1	-	Liebezeit and Rau, 1988
Fly River, Papua New Guinea	0.1-1.42	1.79-11.75	-	Robertson <i>et al.</i> , 1992
Coral Creek, Northern Australia	0-1.6	0-0.3	0-0.3	Boto and Wellington, 1988
Indus River Delta, Pakistan	2-15	1-8	0.5-2	Harrison <i>et al.</i> , 1997

Although the seasonal variations were well defined at all the stations, the variations between the stations were insignificant. The seasonal maximum was recorded in the monsoon season, and lower concentrations in the pre- and post-monsoon seasons.

The seasonal variations could be explained on the basis of a correlation plot of nitrate concentrations and salinity ( $r = -0.82$ ;  $P < 0.001$ ,  $n = 51$ ) (Fig. 4.1). Two phases can be distinguished: phase I (monsoon season-salinity range: 0-15 PSU), where the nitrate concentrations are controlled by external input through rainfall and freshwater advection and phase II (non-monsoon season- salinity range: 15-36 PSU), by *in situ* biological processes.

*Phase I:* The seasonal maximum in the monsoon season, characterised by high concentrations in June and July, when salinity levels are low, indicates input with freshwater. However, at the end of this season, low salinity does not correspond to high nitrate values. This is because, at the beginning of the monsoon season, the freshwater is highly charged with  $\text{NO}_3^-$ , whilst, in August (average salinity: 6.2 PSU) and September (average salinity: 15.4 PSU), though the salinity is still low, the incoming water was less enriched with  $\text{NO}_3^-$ .

*Phase II:* The lower concentrations in the pre- and post-monsoon seasons can be attributed to biological processes occurring within the system. To understand these influences, a comparison of the observed and calculated nitrate

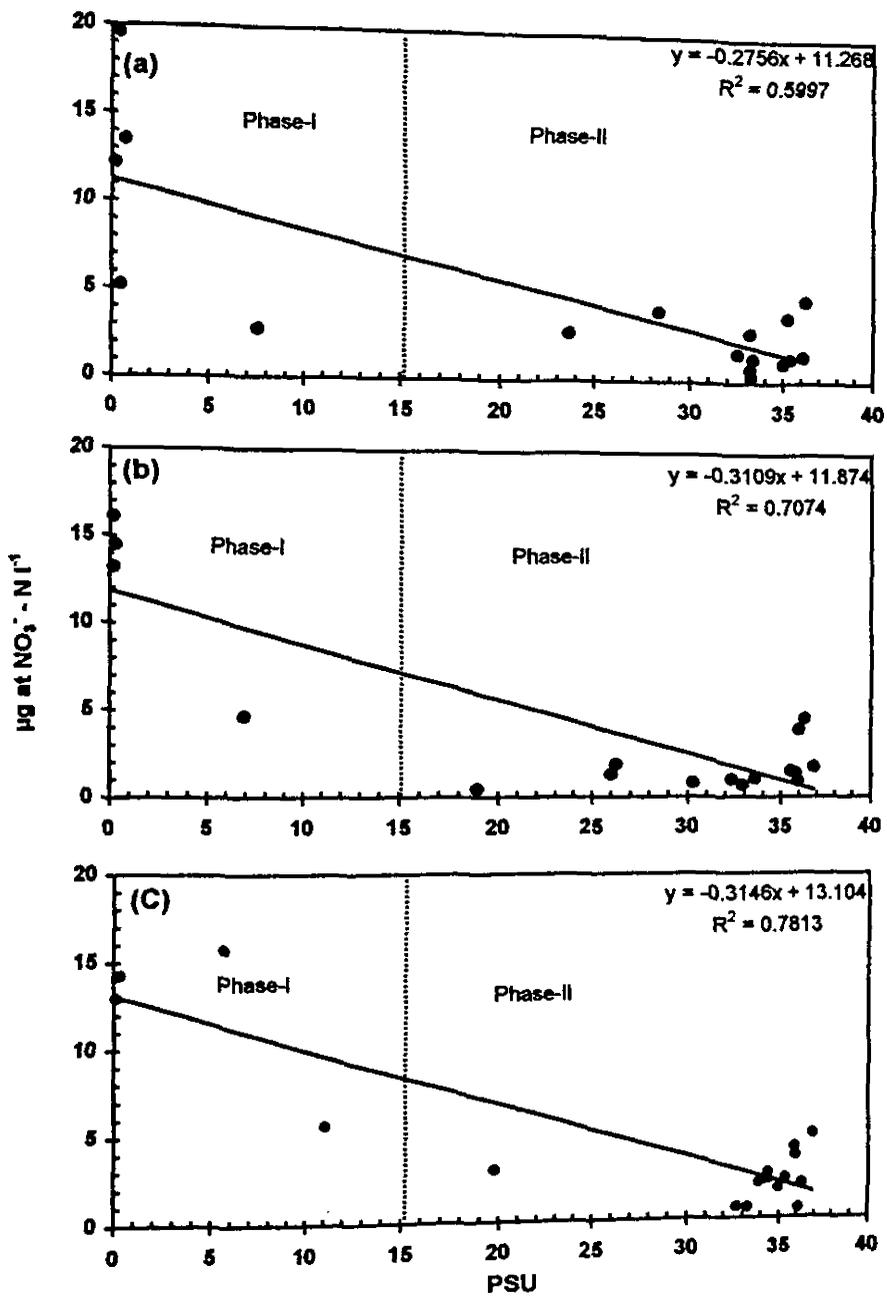


Fig. 4.1 Relation of nitrate concentrations with salinity at the (a) reference, (b) middle and (c) mouth stations

concentrations was made (Fig. 4.2). The calculated nitrate concentrations were estimated using a regression equation derived by plotting observed nitrate concentrations against salinity. During the post-monsoon months, the observed nitrate concentrations were lower than the calculated values which indicated that, during this period, nitrate was taken up more efficiently than in the pre-monsoon and monsoon months (see Fig. 3.13; 3.14). The significant negative correlation of nitrate and chlorophyll-a concentrations supports the above hypothesis ( $r = -0.55$ ;  $P < 0.001$ ,  $n = 51$ ). Nitrate could have also been utilized by mangroves during growth. Boto *et al.* (1985) showed that nitrate was well taken up by *Avicennia marina* seedlings and enhanced root development and above-ground growth.

The low nitrification rates observed in the post-monsoon season may also account for the low nitrate concentrations (see section 4.3.2). At the end of the post-monsoon season, in the months of December and January, the differences between the calculated and observed nitrate concentrations become negligible ( $< 0.3 \mu\text{g at N l}^{-1}$ ). This could be due to the tight coupling between nitrate production and utilization.

The variations in the pre-monsoon season showed a different picture. The gradual increase in  $\text{NO}_3^-$  concentrations was due to the increase in nitrification rates (post-monsoon :  $34.6 \mu\text{g at N l}^{-1} \text{ h}^{-1}$  ▶ pre-monsoon:  $68.3 \mu\text{g at N l}^{-1} \text{ h}^{-1}$ ). The significant correlation of ammonium and nitrate ( $r = 0.33$ ;  $P < 0.04$ ,  $n = 37$ ) in this season

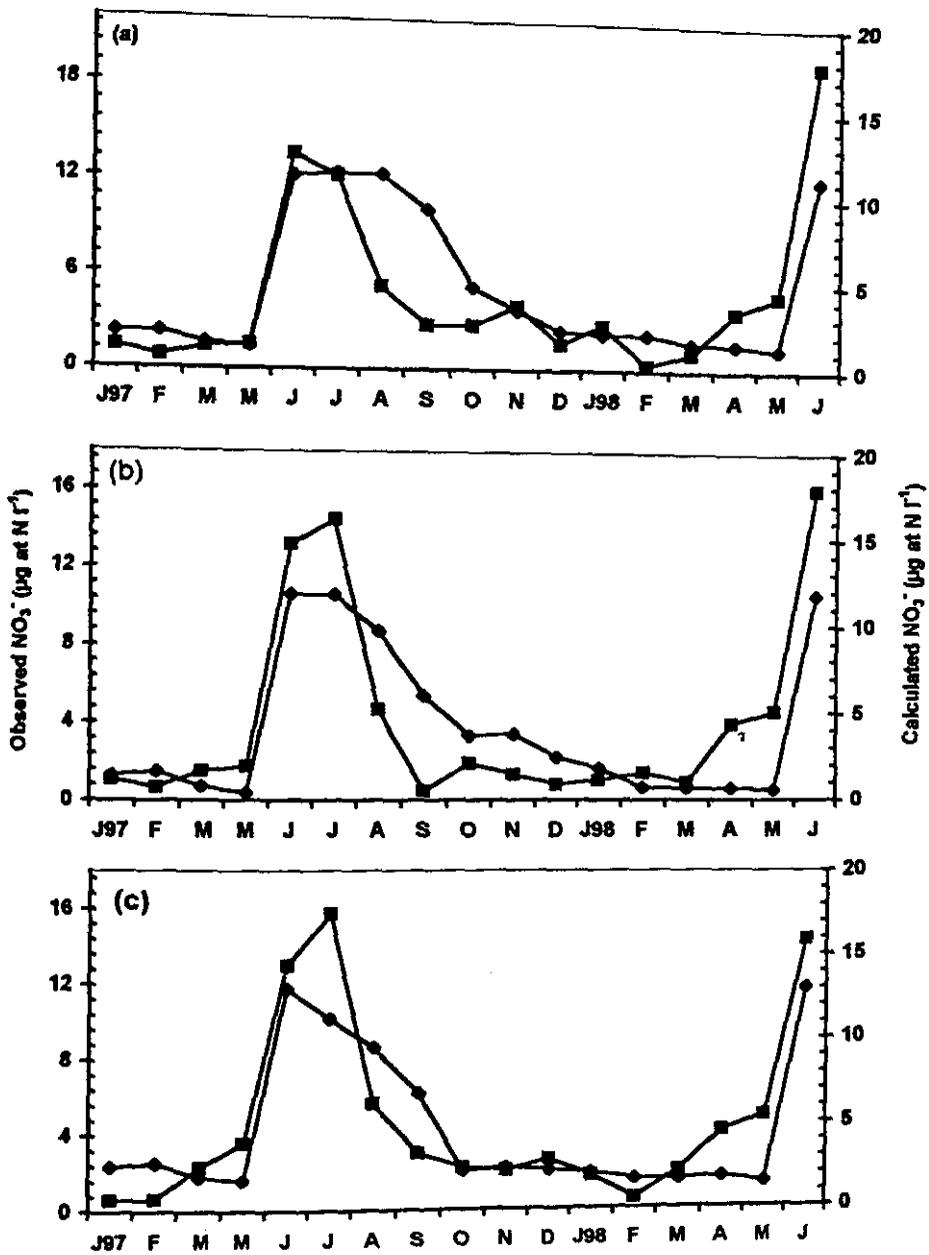


Fig. 4.2 Seasonal changes of observed nitrate ■ and calculated nitrate ◆ concentrations at the (a) reference, (b) middle and (c) mouth stations

suggests that the increased ammonium concentrations could have enhanced the nitrification rates and this ultimately would have resulted in increase in the nitrate concentrations.

Unlike in the case of the post-monsoon season, chlorophyll-*a* and nitrate concentrations showed a significant positive correlation in the pre-monsoon season ( $r=0.47$ ;  $P=0.03$ ,  $n=21$ ). This rather unusual observation could be due to the shift in uptake preference from nitrate to ammonium, which thus resulted in the marginally higher concentrations of nitrate during this season (Fig. 4.3).

The insignificant spatial variations can be explained by the lack of a salinity gradient between the three stations. This would mean that the supply of nitrate, either from upstream or downstream sources, is uniformly distributed along the estuary.

In conclusion, (i) the seasonal variations of nitrate concentrations are influenced by allochthonous sources (freshwater influx) in the monsoon season and by *in situ* processes in the non-monsoon season (ii) the insignificant spatial variations can be attributed to the lack of a salinity gradient between the stations.

#### 4.1.2 NITRITE ( $\text{NO}_2^-$ )

As has been suggested by Rakestraw (1936), nitrite plays an important role in N cycling in the sea because of its intermediate position in the oxidation-reduction processes between ammonium and nitrate. Nitrite appears as an intermediate

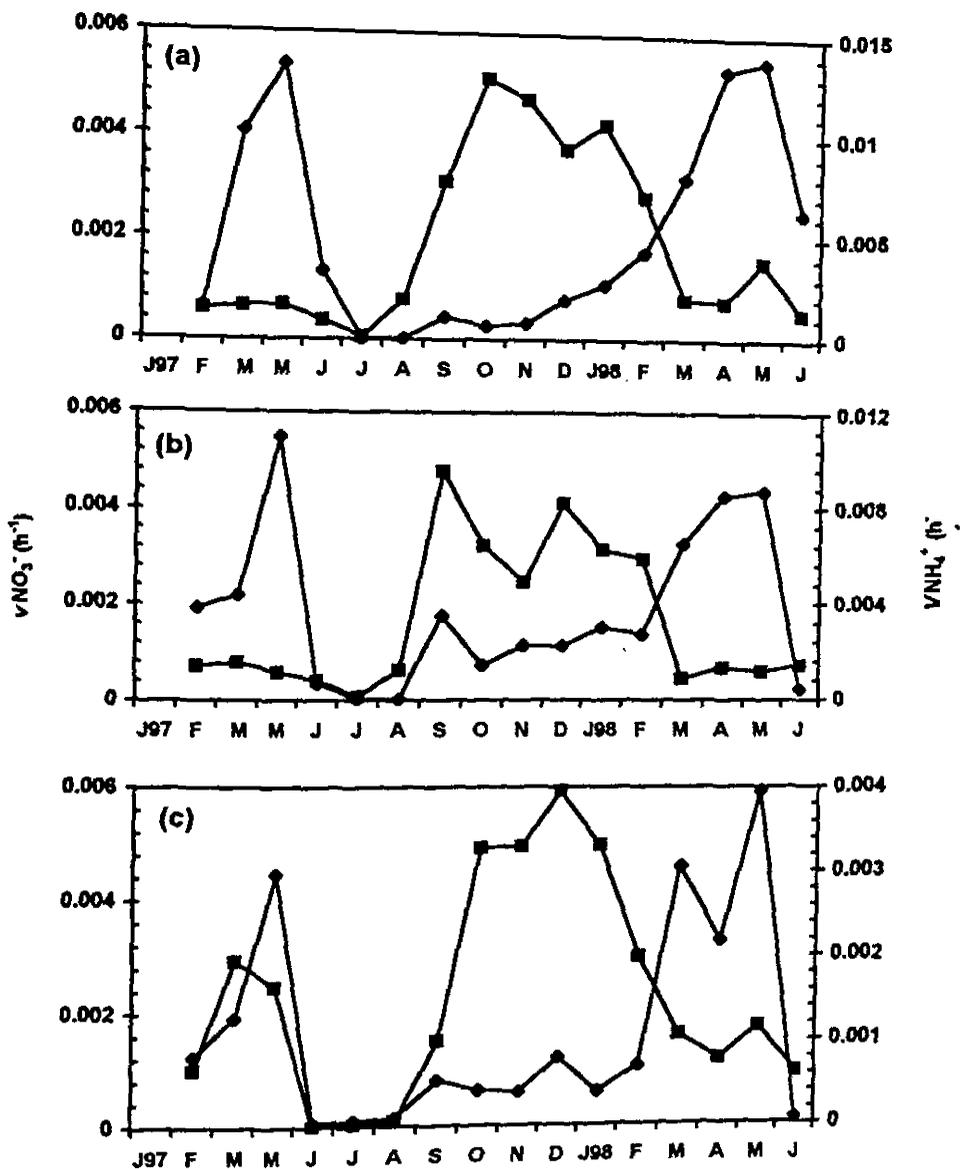


Fig. 4.3 Seasonal changes of specific nitrate uptake rates ■ and ammonium uptake rates ◆ at the (a) reference, (b) middle and (c) mouth stations

product in the following pathways: nitrification ( $\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$ ), denitrification ( $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2\text{O}, \text{N}_2$ ), dissimilatory nitrate reduction ( $\text{NO}_3^- \rightarrow \text{NO}_2^-$ ) and assimilatory nitrate reduction ( $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_4^+$ ). In the first three processes, nitrite is produced from nitrogenous substrates already present in the water column and in the last, the nitrite produced within the cells of autotrophs may be excreted into the water column under certain conditions (high ambient nitrate, low light).

The rate at which these processes contribute to nitrite accumulation in the water column vary as a function of the substrate availability and the rate of its supply through either autochthonous or allochthonous sources. The allochthonous sources may also contribute nitrite directly *i.e.* riverine advection, precipitation.

In coastal waters nitrite concentrations are almost very low and rarely exceed 5% of nitrate levels (McCarthy and Kamykowski, 1972). Reports of nitrite concentrations are not frequent from mangrove ecosystems. In the mangrove forests of Coral Creek, northern Australia, the nitrite concentrations ranged from barely detectable levels up to  $0.3 \mu\text{g at N l}^{-1}$  (Boto and Wellington, 1988). Nitrite concentrations recorded in the present study ranged from 0.03 to  $0.8 \mu\text{g at N l}^{-1}$  and were slightly lower than those of the Indus river delta mangroves of Pakistan (Table 4.1).

The nitrite concentrations were about 10 times lower than those of nitrate and accounted for about 6% of the total measured dissolved assimilable N pool ( $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+ + \text{urea}$ ). Though significant spatial and seasonal variations were observed, the seasonal changes in nitrite concentrations did not follow a pattern similar to that of nitrate, except for the peak values in the monsoon months.

The high nitrite values coincident with low salinity in the monsoon months and the close grouping of the data set around freshwater salinity indicate that nitrite addition to the mangrove waters occurs at this time essentially through riverine advection. Though the curve relating nitrite with salinity was statistically significant ( $r = -0.57$ ;  $n = 51$ ,  $P < 0.001$ ), there was still a certain degree of dispersion of data points at salinities  $> 0$  PSU suggesting that biological processes might be influencing the changes of nitrite concentrations during the non-monsoon months. This is obvious from the comparison of the measured concentrations of nitrite and those calculated from the theoretical dilution line for each of the three stations (Fig. 4.4), which shows an accumulation of nitrite in the post-monsoon season and a consumption in the pre-monsoon season.

As the study site is a zone of active nitrification (annual average rate:  $42.6 \text{ } \mu\text{g at N l}^{-1} \text{ h}^{-1}$ ), it is likely that this process controls the concentrations of nitrite significantly. However, a plot of nitrite changes with nitrification rates in the water column and sediments did not show a good correlation; in fact, the secondary peak in the post-monsoon months coincided with low nitrification rates. It is

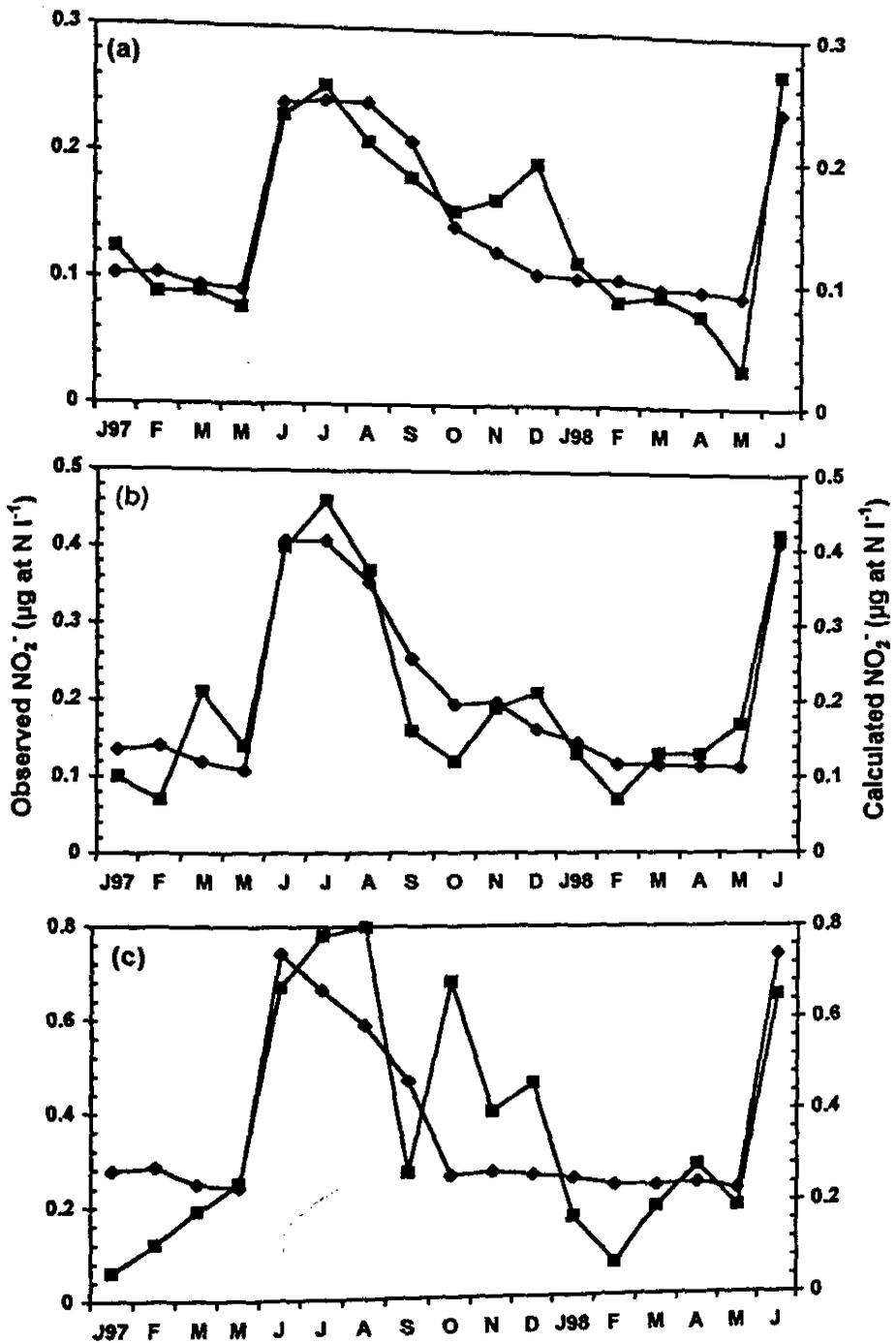


Fig. 4.4 Seasonal changes of observed nitrite ■ and calculated nitrite◆ concentrations at the (a) reference, (b) middle and (c) mouth stations

therefore likely that the nitrite concentrations measured in the post-monsoon months are residuals of what was brought in by advection in the monsoon months. The continuous decrease through the post-monsoon months would therefore be related to an enhanced uptake of nitrite (Fig. 4.5) coupled with low nitrification rates.

Interestingly, nitrite concentrations continued to decrease even when nitrification rates continued to increase (Fig. 4.6). This suggests that the nitrite produced is lost immediately to other pathways. As the uptake rates were more or less constant through the pre-monsoon months, it is likely that the nitrite oxidation to nitrate was probably more, if not equally, rapid (Kaplan, 1983).

The significant variations of nitrite concentrations between the stations may be due to the variations in the absolute uptake rates (see section 4.4.3). Also, the mangrove vegetation could have utilized nitrite during the growth period (Alongi *et al.*, 1992). This may also be one of the reasons for the lower concentrations at the reference and middle stations (mangrove zones) compared to the higher values at the mouth station (non-mangrove zone).

In conclusion, it appears that the seasonal variations in the nitrite concentrations were controlled by the riverine input in the monsoon months, whereas, in the non-monsoon months, *in situ* biological processes were important.

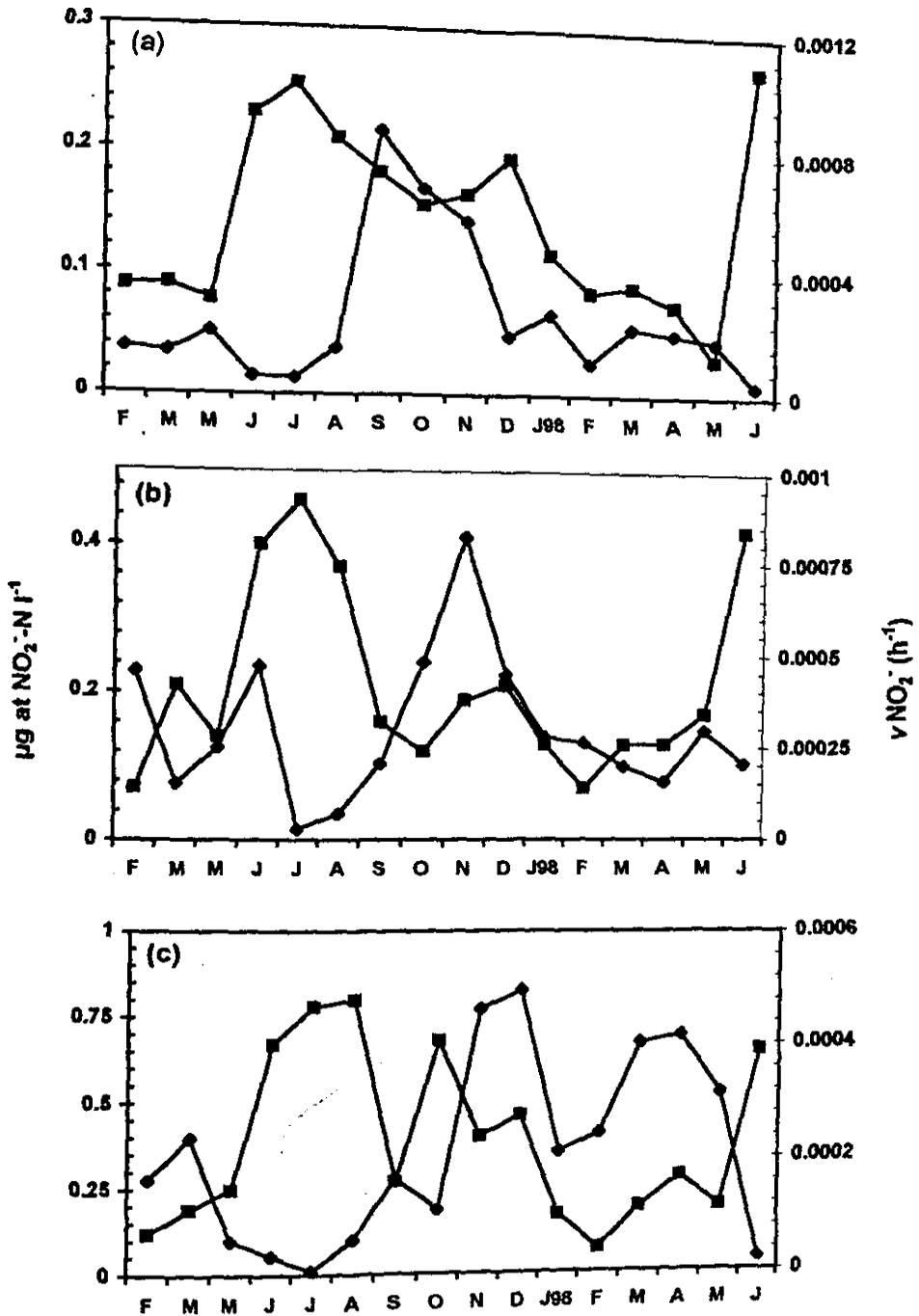


Fig.4.5 Seasonal changes of nitrite concentrations ■ and specific nitrite uptake rates ♦ at the (a) reference, (b) middle and (c) mouth stations

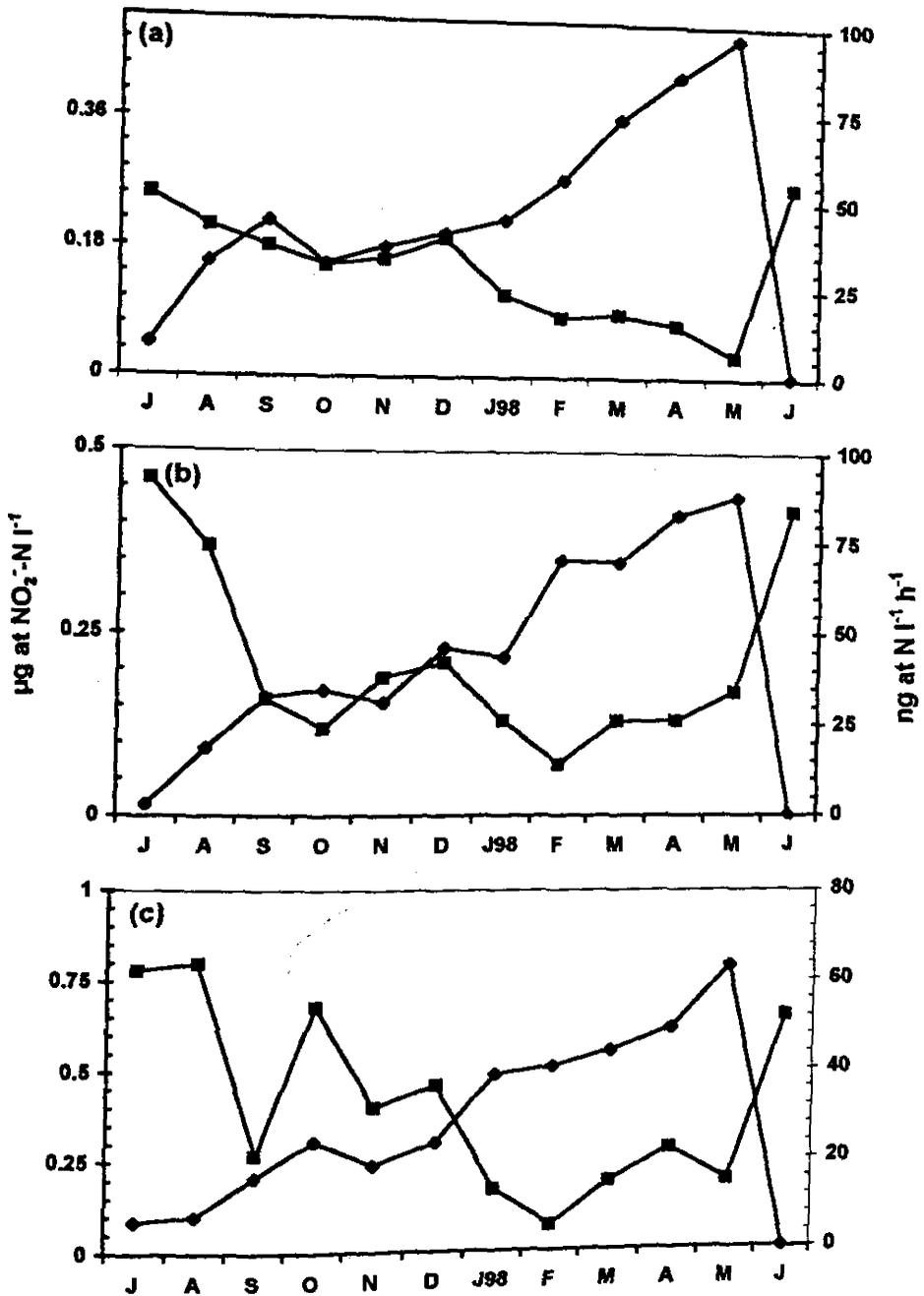


Fig. 4.6 Seasonal changes of nitrite concentrations ■ and nitrification rates ◆ at the (a) reference, (b) middle and (c) mouth stations

### 4.1.3 AMMONIUM ( $\text{NH}_4^+$ )

The significance of ammonium in the marine environment is borne out from the following facts: (i) it is generally the dominant form of combined inorganic nitrogen in the surface waters in the tropics (Menzel and Spaeth, 1962; Thomas, 1966) (ii) the most reduced form of combined nitrogen and an important source for phytoplankton growth (Glibert *et al.*, 1988; Paasche, 1988), (iii) used directly by heterotrophic microorganisms in assimilatory processes (Wheeler and Kirchman, 1986; Tupas and Koike, 1991; Hoch and Kirchman, 1995) and (iv) represents the major form of nitrogen regenerated by microheterotrophs (Harrison, 1980; 1992).

In the water column, numerous factors, both biogenic as well as physical, influence the production and/or removal of ammonium from the dissolved pool.

These include:

*Biogenic processes:* The major process which influences ammonium concentrations in the dissolved pool is the remineralization of particulate matter (Billen, 1978, Boynton *et al.*, 1980) and dissolved organic nitrogen (Pantoja *et al.*, 1997; Berman *et al.*, 1999) by microbial activity. Ammonium is the primary excretory product of nitrogen metabolism of macrozooplankton and microheterotrophs and can be a significant source of regenerated N in estuarine and coastal ecosystems. One of the earliest studies carried out on this aspect was that of Harris (1959) at Long Island Sound, where at least 50% of the regenerated N was due to the excretion by macrozooplankton. More recent

studies on estuarine zooplankton (Harrison *et al.*, 1983; Park *et al.*, 1986; Miller *et al.*, 1995) showed that microzooplankton excretion may fulfil 70-80% of the phytoplankton N requirements. Ammonium in the dissolved pool is also contributed by nitrogen fixation by blue-green algae. Although this is the best studied transformation process of nitrogen in mangroves, low rates have been reported compared to other estuarine environments (Capone, 1983). The turnover of urea could also be a potential source of ammonium in estuarine sediments (Pedersen *et al.*, 1993a). However, turnover rates in the water column are not as significant as in marine sediments and are largely influenced by the availability of organic matter and the abundance of benthic macrofauna (Lomstein and Blackburn, 1992).

*Physical processes:* Estuarine and coastal sediments also add ammonium to the overlying waters and are a significant source of inorganic N made available for pelagic primary production (Boynton *et al.*, 1980; Billen and Lancelot, 1988). These fluxes are largely driven by diffusion (Jorgensen and Revsbech, 1985), advection (Riedle *et al.*, 1972) or sediment resuspension (Sondergaard *et al.* 1992). The three processes are dependent on the hydrodynamics and the local hydrology (Simmons, 1992) and activity of benthic organisms (Aller, 1984). In contrast to temperate ecosystems, direct measurements of fluxes of nitrogen to overlying waters in mangroves are few (Alongi, 1989, 1990). Terrigenous inputs through sewage can also influence ammonium concentrations in coastal waters

(Atwood *et al.*, 1979; Eppley *et al.*, 1979b, Thomas and Carsola, 1980), besides import *via* freshwater (Wong, 1984).

The present study revealed that ammonium was the second most abundant form of inorganic nitrogen (16%), after nitrate. The concentrations ranged from 0.1 to 1.5  $\mu\text{g at N l}^{-1}$  and varied significantly between the stations. The concentrations compare well with earlier estimations of Liebezeit and Rau (1988) and Robertson *et al.* (1998) (Table 4.1). Unlike the case with nitrate and nitrite, where peak concentrations were recorded in the monsoon season, the seasonal maximum of  $\text{NH}_4^+$  was in the pre-monsoon season (May) and the minimum in the monsoon season at the three stations. In the present study the seasonal and spatial variations were well marked.

The significant seasonal variations in ammonium concentrations in the non-monsoon season can be explained by both biological and physical factors. In these months, ammonium concentrations increased gradually, with the moderately high concentrations in the post-monsoon reaching maximum values in the pre-monsoon season.

Ammonium regeneration within the water column appeared to influence the ambient ammonium concentrations. The significant correlation of ammonium regeneration rates with ammonium concentrations ( $r = 0.73$ ;  $P < 0.001$ ,  $n = 48$ ) suggested that regeneration contributed significantly to ammonium in the water column (Fig. 4.7).

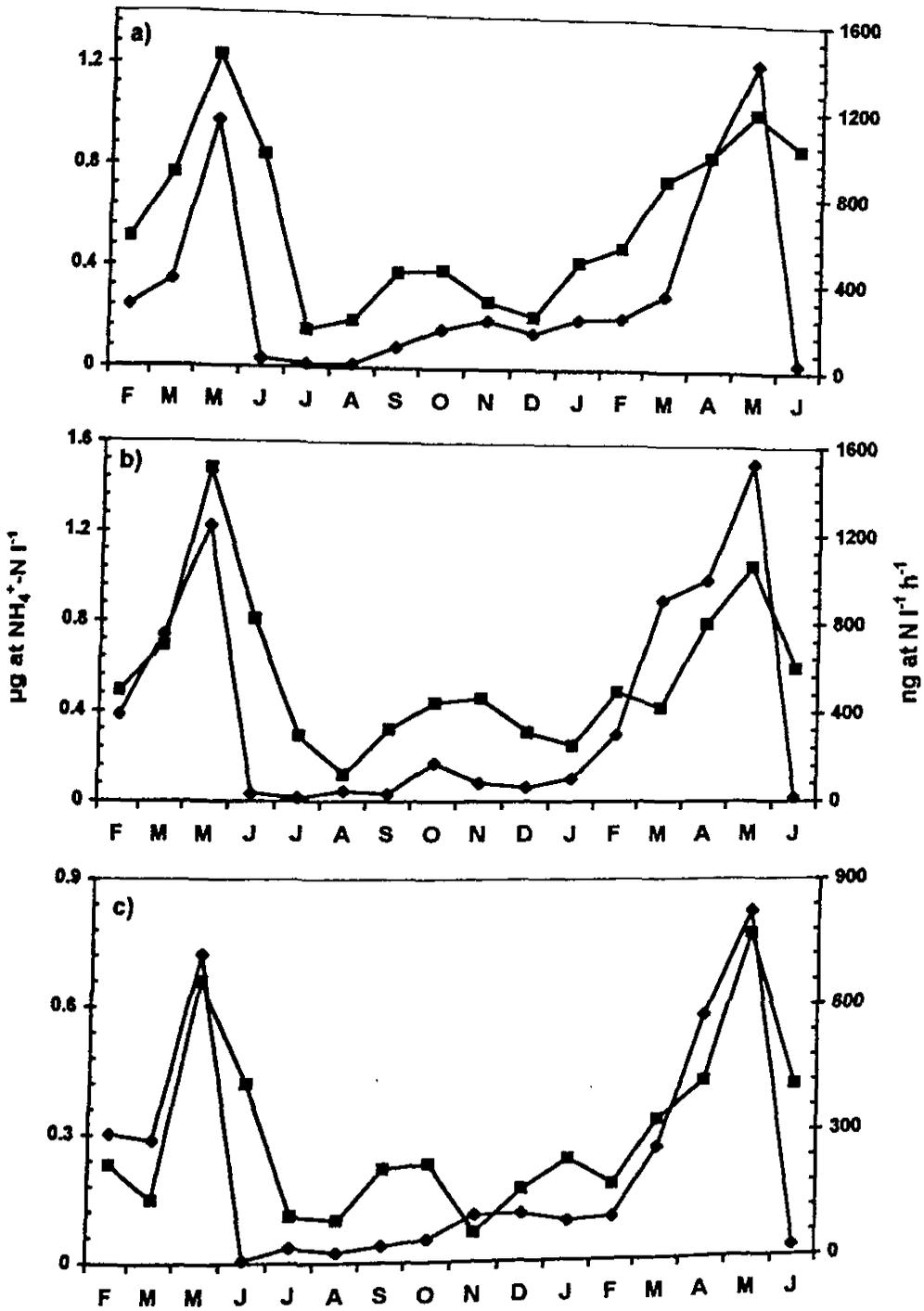


Fig.4.7 Seasonal changes of ammonium concentrations  $\blacksquare$  and ammonification rates  $\blacklozenge$  at the (a) reference, (b) middle and (c) mouth stations.

Since mangrove sediments appear to be rich in organic matter and act as a source of nutrients (Rivera-Monroy *et al.*, 1995a; Alongi, 1996), it was hypothesized that ammonium was also supplied from the sediment by flux processes. Ammonium efflux from pore water occurs *via* diffusion (Falcao and Vale, 1998), bioturbation of the benthic organisms (Kuwaer *et al.*, 1998) and water movement (Asmus *et al.*, 1998). Studies conducted by Henriksen *et al.* (1983) showed that the increase in the pore water ammonium concentrations and ammonium flux were due to the increase in availability and rapid mineralization of labile organic matter.

In the present study, sediment pore waters were found to be enriched with ammonium (range: 0.2 - 13.3  $\mu\text{g at N (l pore water)}^{-1}$ ) compared to that of the water column (Dham, 2000) and hence could have influenced the ammonium concentrations in the overlying waters. The fact that ammonium concentrations in the pore water and water column followed similar trends supports the above conclusion (Fig. 4.8). In the initial monsoon months (June), the increase in the ammonium concentrations in the water column and decrease in the pore waters at the mangrove zone (Fig. 4.8) suggests that fresh rains could have scoured the sediments and caused the release of ammonium to the overlying waters. Alongi (1988), in a mangrove forest in northern Australia, made a similar observation that monsoonal rains scoured surface sediments thus lowering the nitrogen concentrations in the sediments.

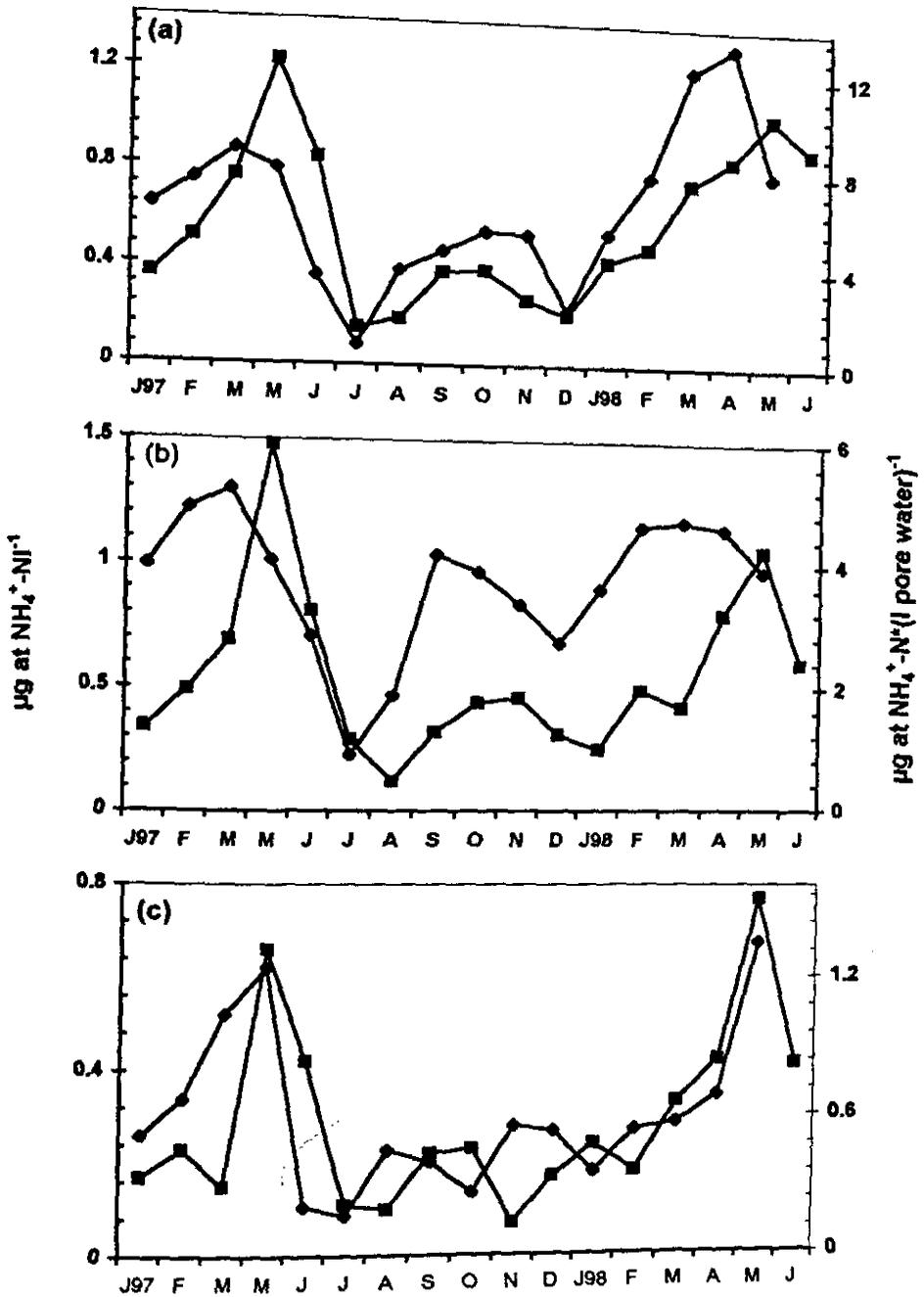


Fig.4.8 Seasonal changes of ammonium concentrations in the water column  $\blacksquare$  and ammonium concentrations in pore water  $\blacklozenge$  at the (a) reference, (b) middle and (c) mouth stations

Also, ammonium regeneration rates in the sediments (Dham, 2000) and ammonium concentrations in the water column showed a significant correlation ( $r = 0.77$ ;  $P < 0.001$ ,  $n = 45$ ) (Fig. 4.9) suggesting that ammonium concentrations are indeed influenced by ammonium production rates in the sediment. The major factors regulating ammonium regeneration rates in sediments include variation in organic matter loading (Nixon, 1981, Kelly and Nixon, 1984; Grebmeier and McRoy, 1989), oxygen concentration (Bartoli *et al.*, 1996) and temperature (Aller and Benninger, 1981). Zooplankton excretion could have also led to high ammonium concentrations (Lehete *et al.*, 1999)

In the present study, the spatial variations in the ammonium concentrations in the water column can be explained as follows:

At the mangrove zone, the high concentrations of ammonium (average:  $0.6 \mu\text{g at N l}^{-1}$ ) were due to high regeneration rates in the water column (360 and 410  $\text{ng at N l}^{-1}\text{h}^{-1}$  at the reference and middle station respectively) and high pore water ammonium concentrations (average:  $4.7 \mu\text{g at N (l pore water)}^{-1}$ ). However, at the non-mangrove zone, the lower regeneration rates (220  $\text{ng at N l}^{-1}\text{h}^{-1}$ ) and low concentrations of porewater ammonium ( $0.5 \mu\text{g at N (l pore water)}^{-1}$ ) were responsible for the low ammonium concentrations.

From the above discussion, the following conclusions can be drawn:

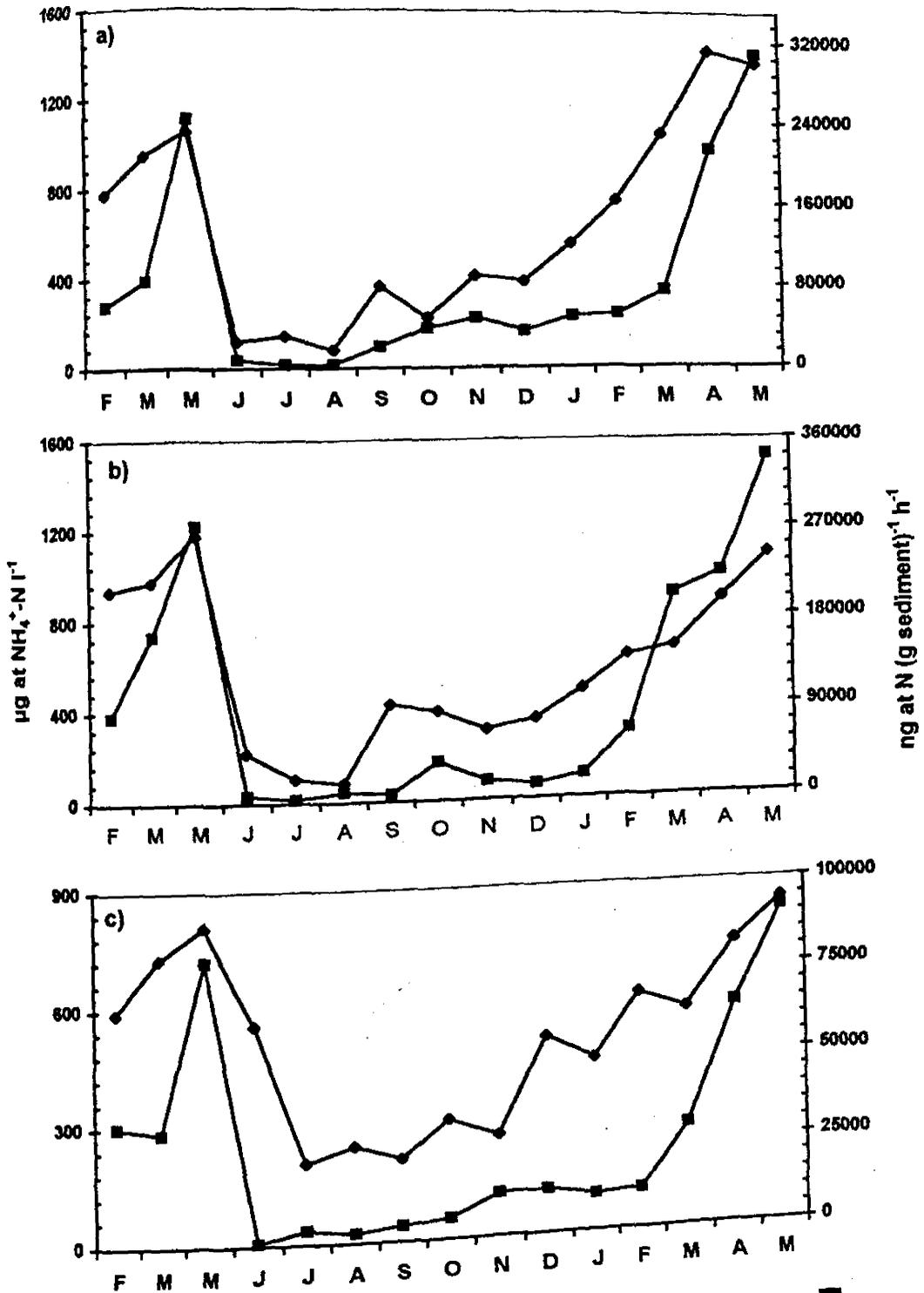


Fig. 4.9 Seasonal changes of ammonium concentrations in the water column  $\blacksquare$  and ammonification rates in the sediments  $\blacklozenge$  at the (a) reference, (b) middle and (c) mouth stations

1. Ambient ammonium concentrations were closely related with ammonium production rates in the water column as well as the sediments.
2. The spatial variations in the ammonium concentrations were due to the variations in the ammonium production rates and efflux of porewater ammonium to the overlying waters, both of which are indirectly influenced by organic load or terrestrial production.

#### 4.1.4 UREA ( $\text{NH}_2\text{-CO- NH}_2$ )

Urea is present in measurable concentrations in most coastal and offshore waters (McCarthy, 1972; Eppley *et al.*, 1973; Remsen, 1971). Among the organic nitrogen compounds, urea serves as an important nitrogen source for phytoplankton in several coastal waters (McCarthy, 1972; Price and Harrison, 1988; Tamminen and Imisch, 1996). However, unlike ammonium, urea is of secondary importance as a nitrogenous excretory product of marine zooplankton (Pandian, 1975; Bidigare 1983).

Urea concentrations in coastal and marine waters are influenced by the following processes:

*Addition:* (i) Urea is added to the dissolved pool as an end product of nitrogen metabolism by marine invertebrates (Wright, 1975; Regnault, 1987; Boucher and Boucher-Rodoni, 1988; Stickle, 1988; Lomstein *et al.* 1989). (ii) Bacteria are also responsible for production of urea through decomposition of organic matter (Sato, 1980; Pedersen *et al.*, 1993 a, b). (iii) Anthropogenic pollution is a

potential source of urea (Paasche and Kristiansen, 1982; Gunkel and Jessen 1986; Gunkel *et al.*, 1990) (iv) Microbial metabolism of amino acids, purines, pyrimidines (Remsen *et al.*, 1974; Vogels and Van der Drift, 1976), (v) Efflux from sediments (Blackburn, 1987; Lomstein *et al.*, 1989; Lomstein and Blackburn, 1992; Therkildsen and Lomstein, 1993) and (vi) input from terrestrial sources (Remsen *et al.*, 1974).

*Removal:* (i) Uptake of urea by phytoplankton (McCarthy, 1972) (ii) Microbial decomposition to yield ammonium (Remsen *et al.*, 1974; Herbland 1976; Kokkinakis and Wheeler, 1988; Therkildsen and Lomstein, 1994).

Urea, as a fraction of the dissolved organic nitrogen pool has not been estimated in mangrove ecosystems, although the total DON has been relatively well studied (Balasubramanian and Venugopalan, 1984; Boto and Wellington, 1988; Nixon *et al.*, 1984). Urea concentrations in the present study were the lowest among the other nitrogenous nutrients, contributing a mere 6% of the total measured dissolved nitrogen. Concentrations ranged from 0.1 to 0.7  $\mu\text{g}$  at  $\text{N l}^{-1}$  and were lower than those generally reported in coastal waters (Remsen, 1971; McCarthy, 1970). Although statistical analysis revealed insignificant variations between the seasons, the monthly changes were well defined, with highest concentrations in the monsoon season (June) at all the stations. The values between the stations varied in a narrow range.

The peak value in the initial stage of the monsoon (June) and the sharp fall during the rest of the monsoon months can be explained mainly by freshwater discharge. During this season, due to the sudden drop in the salinity levels, the *in situ* production of urea by bacteria and marine invertebrates might have been low. In the month of June, the estuary is heavily influenced by freshwater from the upstream region. The freshwater, carrying urea from land derived sources (including sewage) could have given rise to the peak. In the later months, even though the salinity remained low, the urea concentrations dropped sharply and suggested that the concentrations of urea from land derived sources were less significant.

In the post-monsoon season, concentrations of urea were low and did not show much variation, while in the pre-monsoon months, the values showed an increasing trend. It appears that the urea concentrations during these periods are mainly controlled by biological activity. Urea production/removal by the processes mentioned above and the levels at which these processes were active could have influenced the urea concentrations in the dissolved pool during the non-monsoon seasons.

Zooplankton excretion rates have been measured from several coastal ecosystems including estuaries, though not from mangroves. Some of these studies revealed that urea formed a significant portion of the nitrogen excreted by zooplankton. For example, Eppley *et al.* (1973) reported that about 50% of the nitrogen excreted by zooplankton in the sub-tropical waters of the North

Pacific was in the form of urea. Dagg *et al.* (1980) recorded a wide range of urea (6-53%) in nitrogen excreted by copepods in the Peru upwelling system. Although there are no studies carried out on zooplankton excretion in mangroves, studies on zooplankton biomass in estuaries do exist. One such study, in the Mandovi and Zuari estuaries of Goa (Nair, 1979), showed higher biomass in the non-monsoon season compared to the monsoon season. This observation would suggest that the zooplankton population and their excretion rates could have been responsible for the relatively high urea concentrations in the non-monsoon months in the present study.

As mentioned earlier, the microbial degradation of organic nitrogen is also a major source of urea in the dissolved pool. As far as mangroves are concerned, the major supply of the organic matter is from the mangrove vegetation (litter). The significant correlation of PON and urea concentrations in the water column ( $r=0.33$ ;  $P=0.01$ ,  $n= 51$ ) showed that urea may be added by decomposition of this particulate matter in the water column. Also, the trends followed an almost similar pattern (Fig. 4.10).

The heavy load of organic matter on the sediments, its decomposition (therein or at the sediment-water interface) and efflux to the overlying waters could have also been responsible for the urea concentrations in the dissolved pool of the water column. The seasonal changes of sediment PON content and urea concentrations in the water column followed almost similar trends, except in the

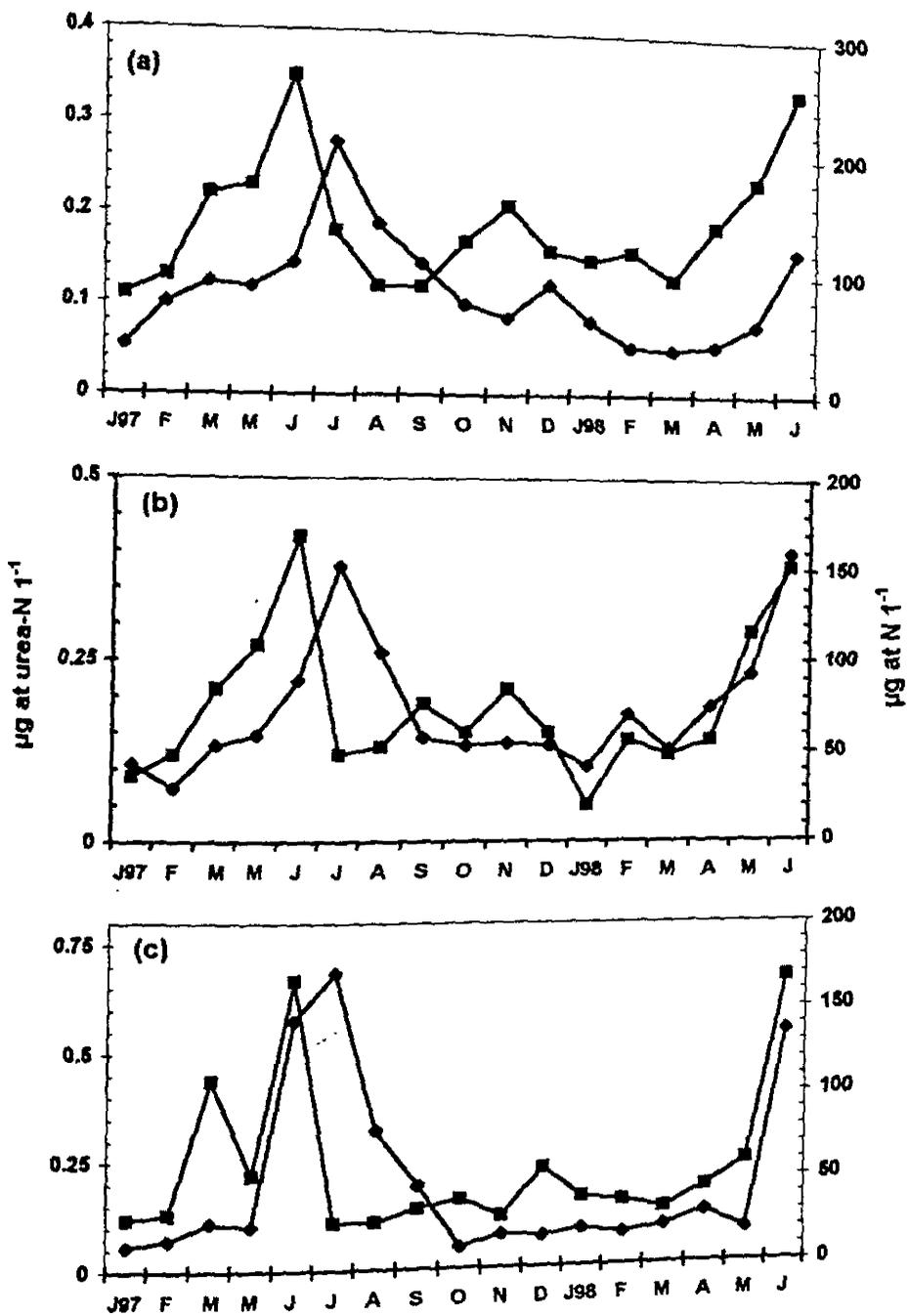


Fig. 4.10 Seasonal changes of urea concentrations ■ and particulate organic nitrogen concentrations ◆ in the water column at the (a) reference, (b) middle and (c) mouth stations

month of June. (Fig. 4.11). From this relationship, it appears that the organic matter of the sediment may have influenced the urea concentrations in the water column. It is however difficult to establish the extent of urea production from this source in the present study because the activity of microorganisms is not known. The microbial degradation of particulate organic matter within the sediment and the water column, together with the increase in zooplankton biomass (Nair, 1979) appear to have influenced the urea concentrations in the non-monsoon months.

Urea is an important nitrogen source for phytoplankton growth (Kaufman *et al.*, 1983; Kristiansen, 1993) and was efficiently utilised in the non-monsoon months. In the present study, urea contributed to 6% of the total estimated dissolved N pool. Nevertheless, even at these low concentrations, urea was a preferred nitrogen source ( $RPI > 1$ ) and the measured uptake rates are sufficient to satisfy ~15% of the urea uptake by phytoplankton. Similar observations were made by Price and Harrison (1988) in the continental shelf waters of the east coast of U.S.A.

The insignificant spatial variations in the urea distribution can be explained by the hydrologic characteristics of the estuary. The estuary is influenced by tidal action and being shallow, the waters are well mixed.

In conclusion:

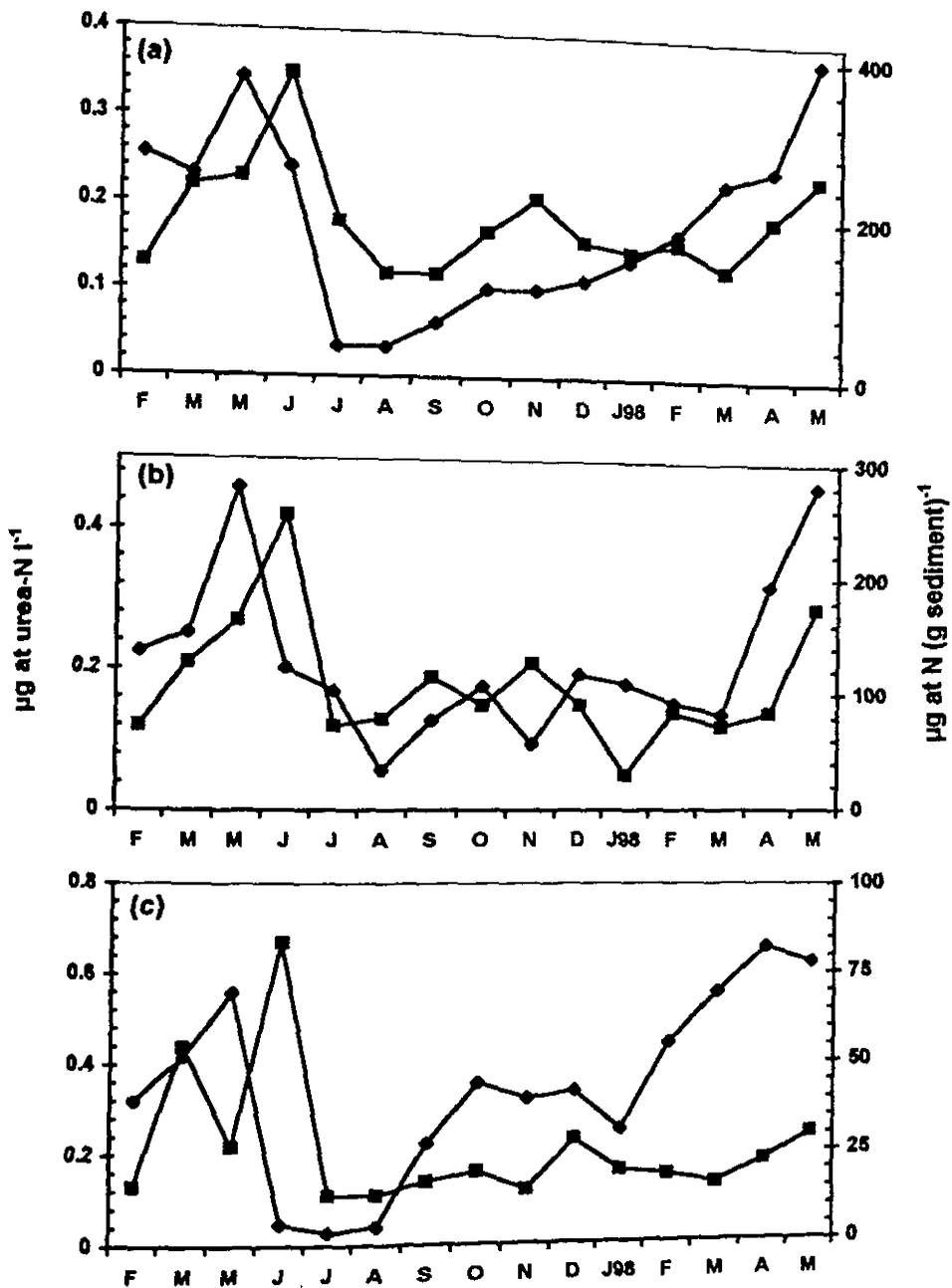


Fig. 4.11 Seasonal changes of urea concentrations ■ and particulate organic nitrogen concentrations in the sediment ◆ at the (a) reference, (b) middle and (c) mouth stations

1. Urea concentrations are influenced by several physical as well as biological factors.
2. The high values at the beginning of the monsoon season (June) are due to the freshwater advection.
3. The close similarity of changes of PON content in the water column and sediments and urea concentrations in the water column suggests that urea concentrations are influenced by decomposition of organic matter.
4. Efficient urea uptake in the non-monsoon months could have resulted in the low urea concentrations.

#### 4.1.5 PARTICULATE ORGANIC NITROGEN (PON)

The distribution of PON in the marine environment reflects the balance between biological production and biological consumption. This can be explained as follows: organic matter produced by biological means (primary production) either serves as a food source to consumers or sinks out of the euphotic zone and is acted upon by microorganisms which convert the organic N (also constituted by dead organisms and their products) to inorganic N. The inorganic N compounds thus produced serve as N sources to primary producers.

In coastal waters, detritus forms an important component of the particulate organic nitrogen, the direct degradation of which forms a major source of dissolved organic and inorganic constituents. Almost all of these compounds serve as nutrient sources for the growth of phytoplankton. Hence the role of

detritus or particulate organic matter is important in maintaining the microbial food chain within the ecosystem.

The detritus in mangrove ecosystems originates from above- and below-ground tree components, including leaves, fallen timber, roots, stipules, reproductive parts, twigs and bark (Alongi *et al.*, 1992). This is also corroborated by the fact that mangrove vegetation accounts for a major portion of the primary production and exceeds that of the aquatic autotrophs (Golley *et al.*, 1962; Mann, 1972). A significant portion of the dissolved and particulate constituents of mangrove litter is exported to nearshore waters or adjacent ecosystems. Tidal exchange of mangrove litter has been well documented (Odum *et al.*, 1982, Boto and Bunt, 1981; 1982; Boto and Wellington, 1988; Robertson *et al.*, 1988; Alongi *et al.*, 1989; Alongi, 1990).

High estuarine PON concentrations in the range of 5 to 100  $\mu\text{g at N l}^{-1}$  have been reported (Postma, 1966; Haines, 1979; Culberson *et al.*, 1982). Few data exist on PON content in mangrove waters as well as on dissolved organic nitrogen compounds (Nixon *et al.*, 1984; Jagtap, 1987; Balasubramanian and Venugopalan, 1984; Boto and Wellington, 1988). In Malaysian creek waters, Nixon *et al.* (1984) found low and variable PON concentrations, ranging from < 10 to 131  $\mu\text{M}$ .

The PON concentrations in the present study ranged widely from 12.1 to 207.9  $\mu\text{g at N l}^{-1}$  and varied significantly between the stations and seasons. The

maximum PON concentration recorded in the present study exceeded that reported by Nixon *et al.* (1984) for the mangrove waters of Malaysia, but were more or less similar to that reported by Jagtap (1987) in the mangrove environment of Goa (242.9  $\mu\text{g at N l}^{-1}$ ). The seasonal variations showed maximum PON concentrations in the monsoon months and lower values in the non-monsoon season at the three stations.

Since the productivity of mangrove ecosystems is partitioned between terrestrial, aquatic and benthic compartments, the sources of PON in the water column are three-fold. These include, (i) input through primary and secondary production in the mangrove waters, (ii) terrestrial production *i.e.* mangrove vegetation and (iii) resuspension of sedimentary PON to overlying waters

In the present study, it was found that terrestrial production and sedimentary resuspension played a major role in the seasonal and spatial variations of PON in the water column. The negative correlation of chlorophyll-*a* and PON ( $r = -0.52$ ;  $P < 0.001$ ;  $n = 51$ ) (Fig. 4.12) suggested that phytoplankton primary production does not have a significant impact on the PON load. Therefore, it appears that the PON was supplied from some other source *i.e.* terrestrial production. In the dry season, the seasonal changes of litterfall and PON followed an almost similar trend (Fig. 4.13). Although litterfall showed a peak in the month of May, this was not exactly followed by that of PON. This was due to the time required for the breakdown of litter into smaller fragments ( $< 200 \mu\text{m}$ ).

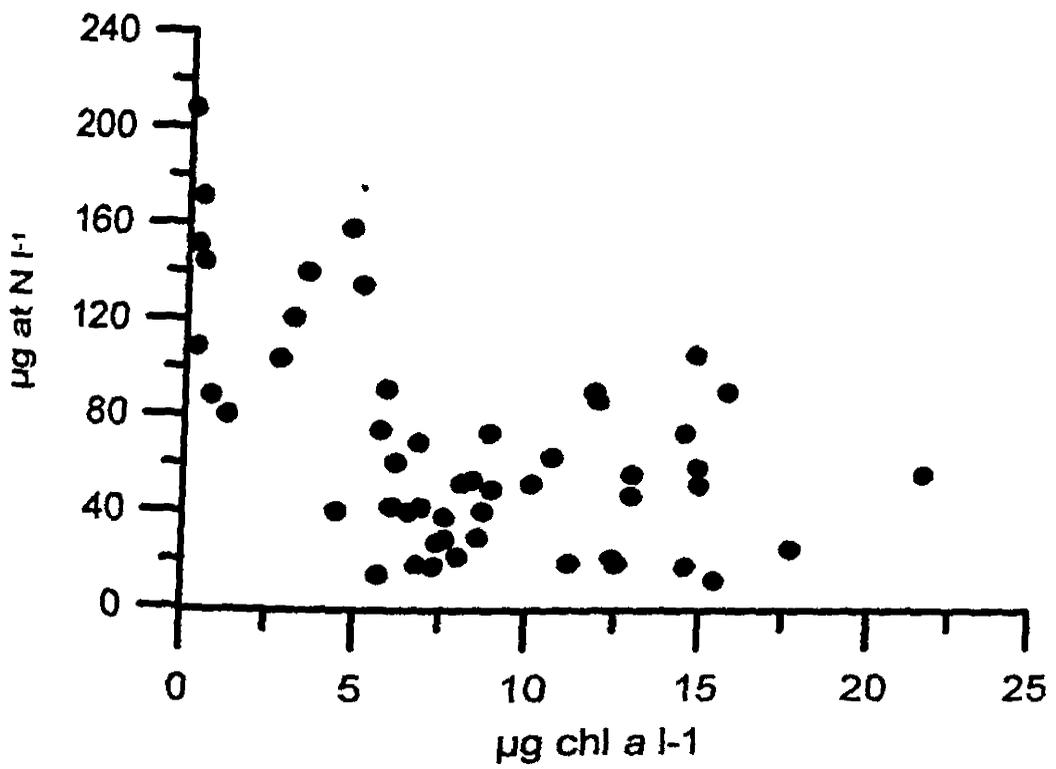


Fig. 4.12 Relation of particulate organic nitrogen concentrations with chlorophyll a  
 $PON = 108.55 - (4.755 * chl\ a)$ ;  $(r=0.52, n=48)$

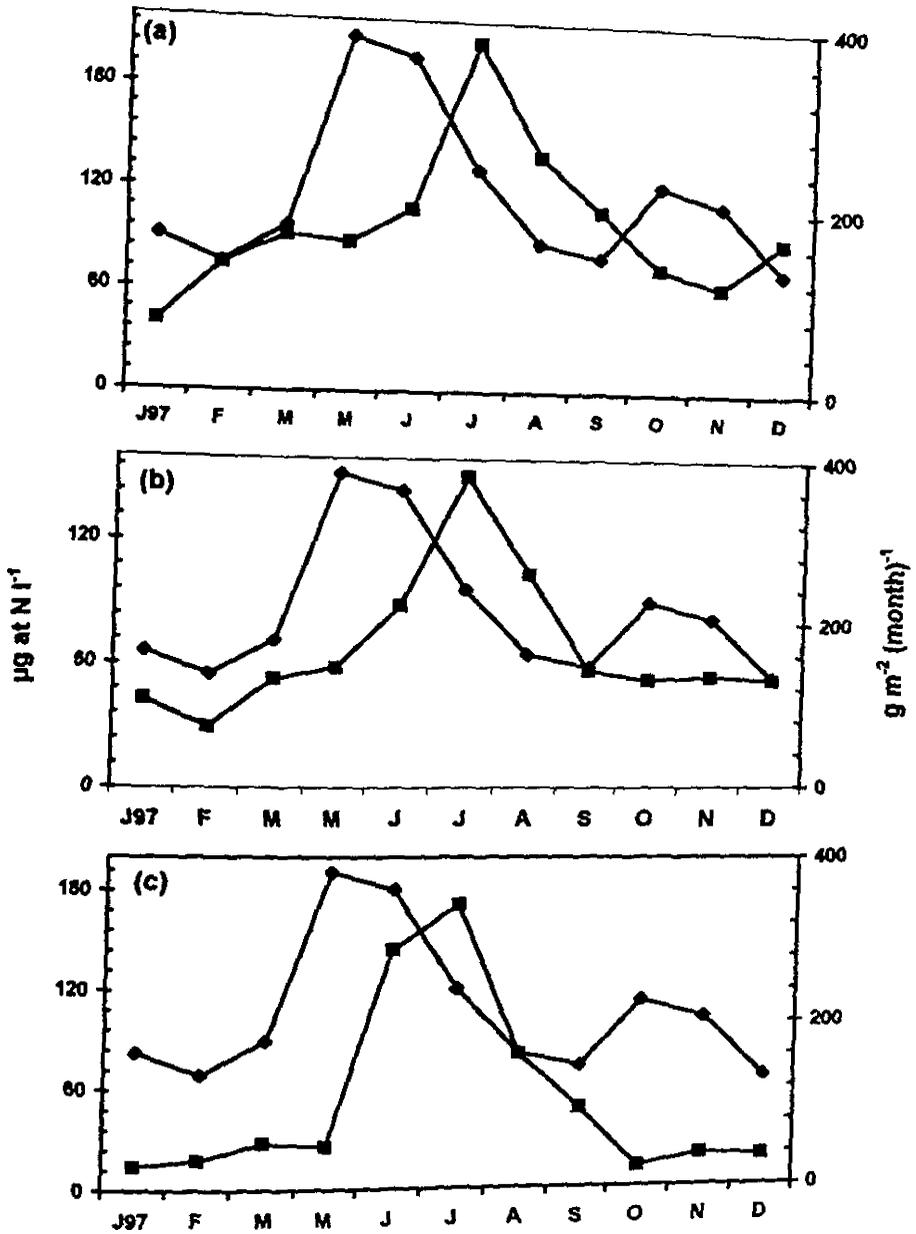


Fig. 4.13 Seasonal changes of particulate organic nitrogen concentrations  $\blacksquare$  and litterfall  $\blacklozenge$  at the (a) reference, (b) middle and (c) mouth stations

Earlier studies showed that litter derived from mangrove vegetation is poor in nitrogen content and degradation time for mangrove litter was 1-2 weeks (Wafar *et al.*, 1997). Also, the percentage nitrogen increases and C:N ratio decreases with decomposition (Tam *et al.*, 1990, Wafar *et al.*, 1997). This is probably associated with the accumulation of N biomass of the microorganisms colonizing the decaying litter. Experiments on decomposition of mangrove litter were also carried out along with this study and it was observed that in submerged litter, %N content increased by 2 to 3 times during decomposition (Fig. 4.14). The PON peak in the monsoon months could be attributed to freshwater advection which carries the organic matter from land derived sources. The relationship between rainfall and PON (Fig. 4.15) supports this statement. Sediment resuspension also played an important role in influencing the PON concentrations of the overlying waters especially in the monsoon months as seen from the inverse relationship (Fig. 4.16). Strong tidal currents during this period could have caused resuspension of sediments and particulate matter resulting in highly turbid waters as has been observed elsewhere (de Jonge and van Beusekom, 1995; de Jonge, 1995).

In the non-monsoon months, the influx of PON with freshwater was less significant and PON concentrations were lower than in the monsoon season but still higher than that of oceanic surface waters (Gordon, 1971; Ichikawa and Nishizawa, 1975). Also, the concentrations remained almost unaltered during this period. The particulate matter in these months is also mainly derived from

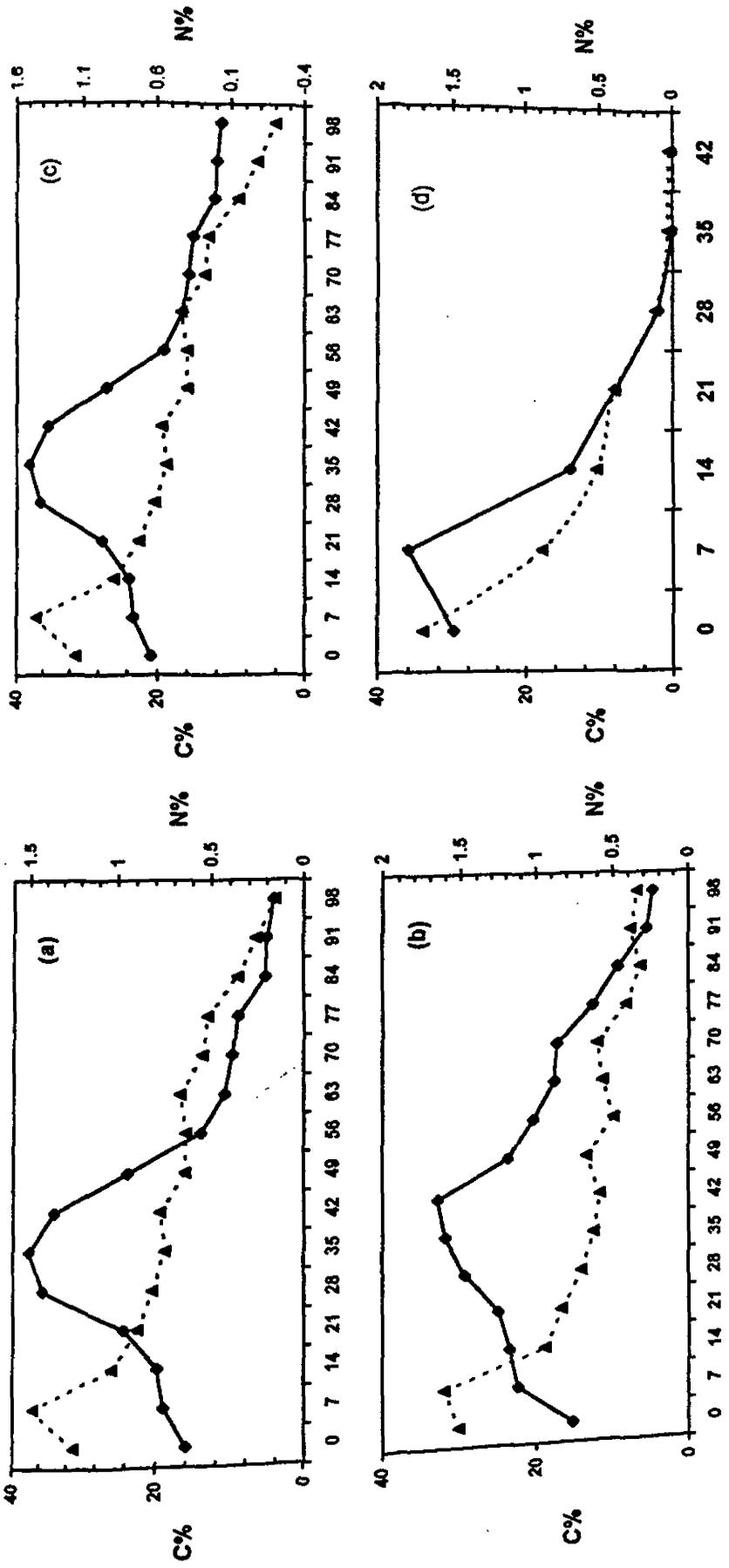


Fig.4.14 Percent change of C ▲ and N ◆ in decomposing leaves of (a) *Sonneratia alba* (b) *Rhizophora mucronata* (c) *Avicennia marina* and (d) *Avicennia officinalis*.

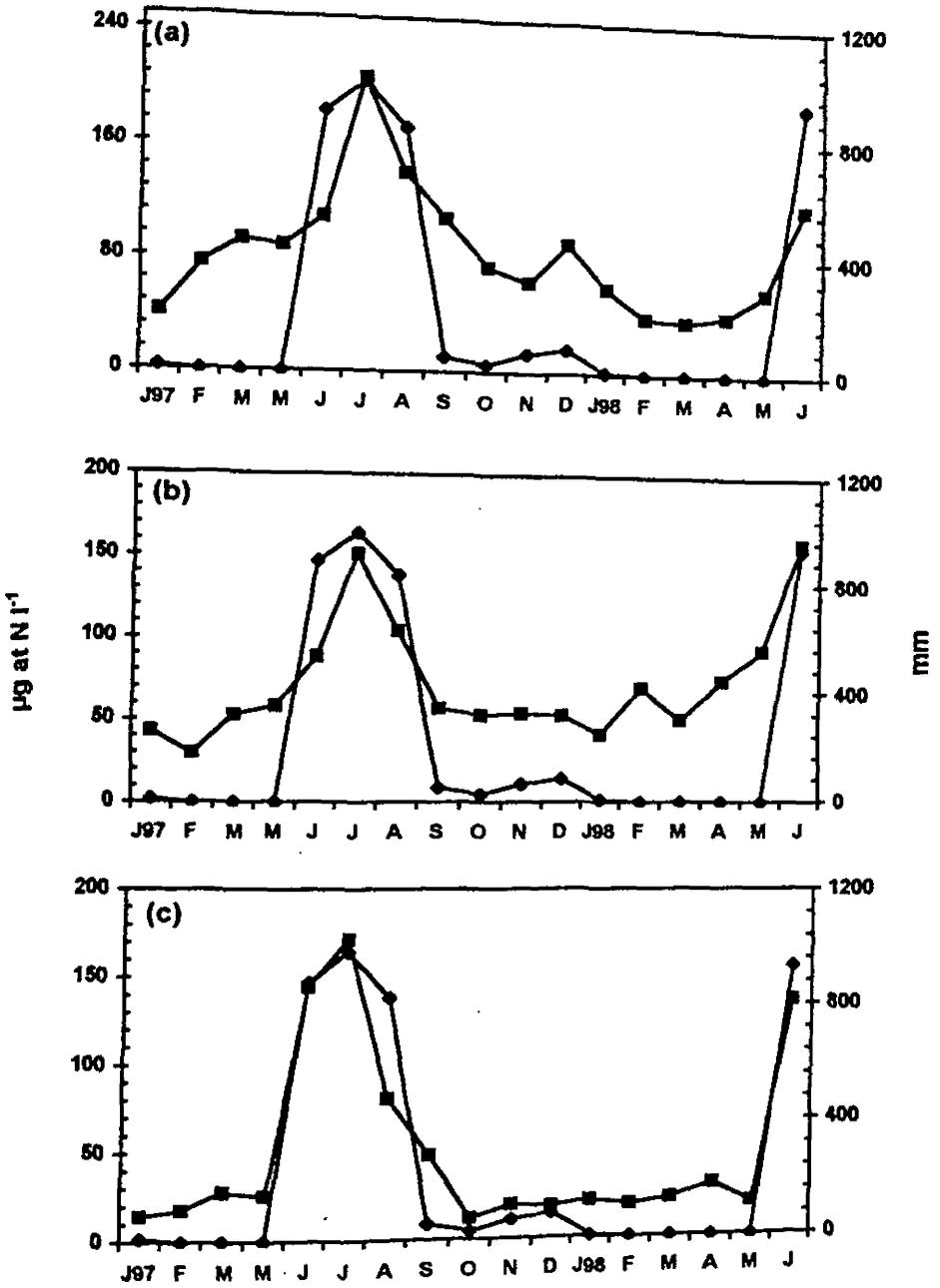


Fig. 4.15 Seasonal changes of particulate organic nitrogen concentrations  $\blacksquare$  and rainfall  $\blacklozenge$  at the (a) reference, (b) middle and (c) mouth stations

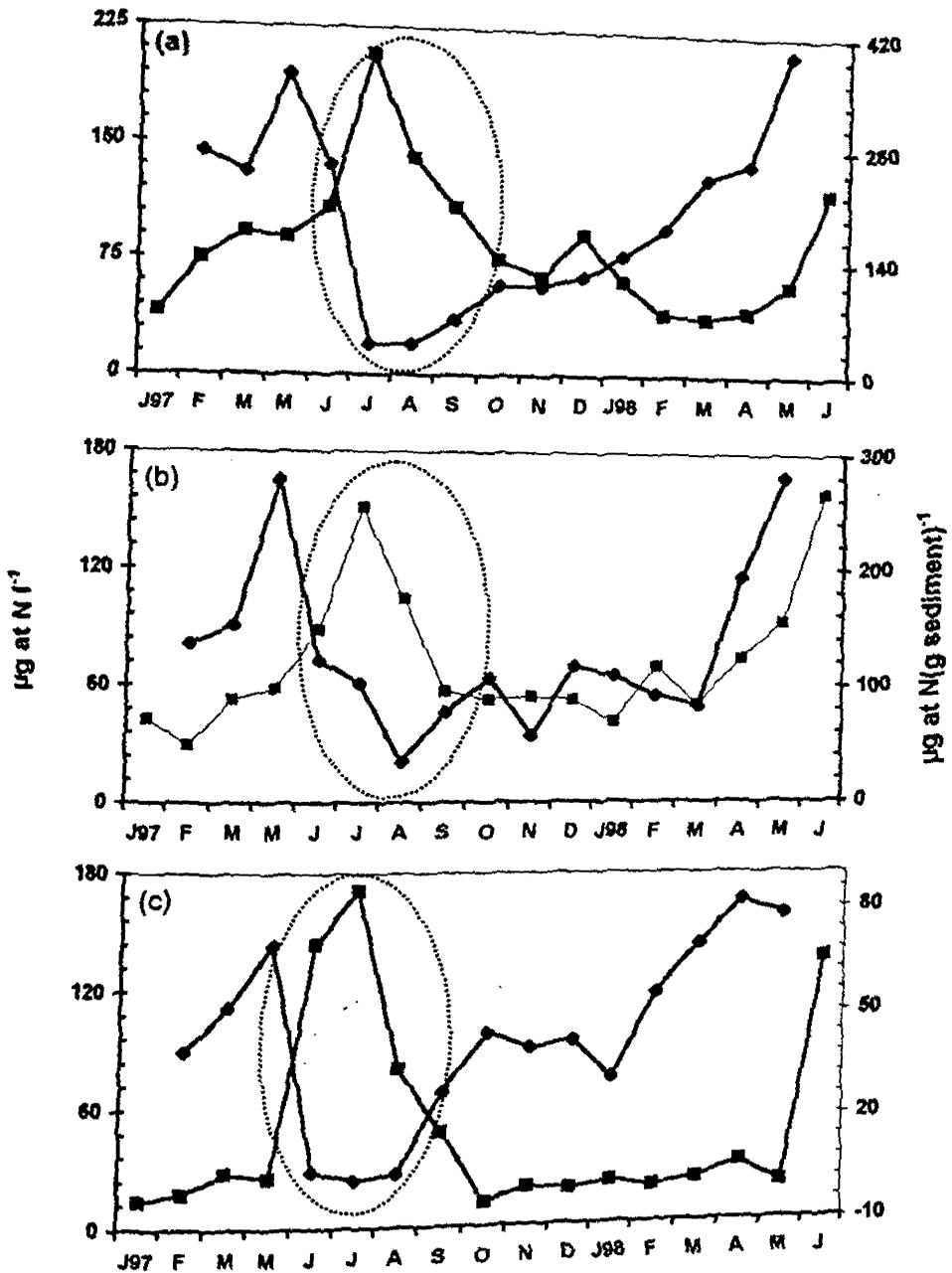


Fig. 4.16. Seasonal changes of particulate organic nitrogen concentrations in the water column  $\blacksquare$  and particulate organic nitrogen concentrations in the sediment  $\blacklozenge$  at the (a) reference, (b) middle and (c) mouth stations

mangrove litter and resuspension from the sediment surface. Input through *in situ* biological production was not a significant source in spite of the high chlorophyll-*a* concentration/phytoplankton and zooplankton abundance (the zooplankton biomass, though not estimated in the present study is known to be generally high during this period, Nair (1979)). The negative correlation of chlorophyll-*a* concentration and PON content supports this argument (see Fig. 4.12).

The influx of PON through physical turbation of the surface sediments by tidal currents appeared to have largely influenced the PON content of the overlying waters in the non-monsoon season. The significant positive correlation of PON concentrations in the water column with those of the sediment ( $r=0.52$ ;  $P<0.001$ ,  $n=34$ ) suggests that the increase in particulate load on the sediment significantly influences the PON concentrations in the overlying waters. Though this was not as significant as in the monsoon season, this process was still responsible for the PON concentrations during these months.

The spatial variations in the PON concentrations are distinct. The PON concentrations at the mangrove zone (85.8 and 72.5  $\mu\text{g at N l}^{-1}$  at the reference and middle stations respectively) were higher than the non-mangrove zone (48.6  $\mu\text{g at N l}^{-1}$  at the mouth station) although the chlorophyll-*a* concentrations did not vary significantly between the stations. These variations can be explained on the basis of location of the stations with respect to mangrove coverage and

resuspension of sediment PON to the overlying waters by tidally induced water movement.

In conclusion:

1. Detritus derived largely through mangrove vegetation formed the major fraction of PON in the water column.
2. The seasonal variations of PON concentrations were due to the variations in the terrestrial input (litterfall), deposition on to the surface sediments and resuspension to the overlying waters.
3. The significant spatial variations of PON concentrations were due to the variations of supply of organic matter and depended on the location of the stations.

## **4.2 BIOLOGICAL PARAMETERS**

### **4.2.1 CHLOROPHYLL-A AND PHYTOPLANKTON TAXONOMIC**

#### **COMPOSITION**

The chlorophyll bearing unicellular algae, collectively called phytoplankton, form the basis of the food chain in the sea. In studies on food web and energy fluxes, it is important to determine the biomass of phytoplankton as accurately as possible because these algae provide food for grazers. Their abundance (biomass) and the rates at which they reproduce (production) determines the biological resources of a given area, and the pattern of their changes is an indication of the impact of the changes on the environment. Phytoplankton also play an important role in influencing the nutrient pools and it is generally

believed that ambient nutrient concentrations are a balance between utilization and supply (Mengesha *et al.*, 1998). The nitrogen supply controls the population size of phytoplankton and possibly its growth rate (Dugdale, 1967).

In the mangrove ecosystems, the major primary producers are the mangrove macrophytes (Heald and Odum, 1970), with phytoplankton playing only a secondary role (Tundusi and Tundusi, 1968). The mangrove waters, being normally very turbid, are generally considered to contain low populations of phytoplankton. Nevertheless, assessments on aquatic primary production are subject to speculation - there are reports of fairly high values for such production. The net phytoplankton production in mangroves is generally from 10 to 100 mg C m<sup>-3</sup> h<sup>-1</sup> (Teixeira *et al.*, 1969; Tundusi, 1969; Untawale *et al.*, 1977; Pant *et al.*, 1980). However, Krishnamurthy and Sundararaj (1973) reported very high rates, up to 525 mg C m<sup>-3</sup> h<sup>-1</sup>, in the Pichavaram mangroves of India.

The factors that influence chlorophyll-*a* concentrations in mangrove and estuarine waters are several, and they can act at different levels according to whether they have an inhibitory or stimulatory effect on the phytoplankton. These include: illumination and turbidity (Cloern, 1987), nutrients (D'Elia *et al.*, 1986), zooplankton grazing (Verity, 1986), salinity (Kinne, 1971), temperature (Platt and Jassby, 1976; Harding *et al.*, 1986), and water column stability (Mengesha *et al.*, 1998). In some systems, among the nutrients, nitrogen (Harrison and Platt, 1980; Rudek *et al.*, 1991; Mallin *et al.*, 1993) may regulate phytoplankton production throughout the year, while in other systems, the

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limiting factor undergoes seasonal shifts where one factor limits production at a particular season and another in the following season (Malone *et al.*, 1988; 1996).

Early studies along the west coast of India showed maximum levels of phytoplankton production in the post-monsoon season (Nair *et al.*, 1973; Tiwari and Nair, 1998) associated with the large influx of nutrient-rich waters in the monsoon season. Other studies revealed that phytoplankton peaks frequently occurred when nitrate concentrations were high (Sankaranarayanan and Qasim, 1969; Qasim *et al.*, 1969). In several studies carried out on aquatic production in mangrove ecosystems, it has been shown that riverine input played a major role in influencing phytoplankton biomass - the low salinity and high nutrient concentrations accelerated phytoplankton growth (Qasim, 1973; Kutner, 1974). Large phytoplankton blooms were reported by Bhattathiri *et al.* (1976) during the monsoon season because of the increased availability of nutrients and the ability of several phytoplankton species to optimize photosynthesis at low salinities. In the Cananea mangroves of Brazil, freshwater influenced the occurrence of phytoplankton blooms such as *Skeletonema costatum* (Kutner, 1974) and production was limited by nitrate (Tundusi *et al.*, 1973). In the Cochin harbour zone, high chlorophyll-*a* concentrations (10 - 14 mg chl-*a* m<sup>-3</sup>) were recorded during the monsoon period, when nutrient levels were high (Qasim, 1973). However, in the Guadeloupe mangal, the periods of heavy freshwater flow coincided with decreased phytoplankton biomass (Ricard, 1979). In the

Pichavaram mangroves, along the east coast of India, the low biomass during the north east monsoon season (October- December) was due to the lowering of salinity, high turbidity and seaward flushing (Mani, 1994).

Chlorophyll-*a* concentrations ranged from 0.1 to 21.6  $\mu\text{g}$  (chl-*a*)  $\Gamma^{-1}$ . Throughout the seasonal cycle, the phytoplankton population was dominated by pennate and centric diatoms which formed 58.7% of the total population. This was followed by the flagellates and blue-green algae which contributed to 20.8 % and 20.5% respectively to the total population. Phytoplankton cell counts showed significant seasonal variations and corresponded to that of chlorophyll-*a*. The chlorophyll-*a* concentrations were high in the post-monsoon months, moderate in the pre-monsoon months and low in the monsoon months. A secondary peak in the month of May was observed at all the stations. There were no significant variations in chlorophyll-*a* distribution between the stations.

In the present study, total nitrogen nutrient availability does not appear to be a significant factor limiting biomass as the nutrient concentrations were high (pre-monsoon:  $\Sigma\text{N}=3.2 \mu\text{g}$  at  $\text{N } \Gamma^{-1}$ ; post-monsoon:  $\Sigma\text{N}=2.5 \mu\text{g}$  at  $\text{N } \Gamma^{-1}$  and monsoon:  $\Sigma\text{N}=10.1 \mu\text{g}$  at  $\text{N } \Gamma^{-1}$ ) in all the seasons. The availability of a particular form of N, however does influence the chlorophyll-*a* concentrations. Among the estimated nutrients, nitrate is the most dominant form of nitrogen contributing 72% to the total estimated dissolved nutrient pool. In the post-monsoon months, nitrate appears to be the major nutrient influencing the phytoplankton growth rates.

During this period, the rapidly growing phytoplankton community largely utilized nitrate as the main N source ( $p\text{NO}_3^- = 192.9 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). Studies conducted by Sankaranarayanan and Qasim (1969) and Qasim *et al.* (1969) revealed high phytoplankton biomass when nitrate concentrations were high.

The significant positive correlation of ammonium and chlorophyll-*a* ( $r=0.29$ ;  $P=0.04$ ,  $n=48$ ) and urea and chlorophyll-*a* ( $r=0.67$ ;  $P=0.00001$ ,  $n=34$ ) suggest that ammonium and urea were limiting nutrients (Fig. 4.17a and b). These relationships showed that chlorophyll-*a* concentrations increased with increase in the ammonium and urea concentrations. In the month of May, the chlorophyll-*a* concentrations showed a secondary peak, which coincided with that of ammonium.

The change in the salinity levels could also be an important factor for the seasonal distribution of chlorophyll-*a* in coastal waters (Qasim *et al.*, 1972; Tundusi *et al.*, 1973). In the present study, the chlorophyll-*a* concentrations decreased remarkably at low salinity (0 - 1 PSU), whereas, at the salinity range of 10 - 23 PSU, maximum phytoplankton growth was recorded. Similar observations were made by Qasim *et al.* (1972) in the Cochin backwaters, where phytoplankton biomass was high at salinities ranging from 10 to 20 PSU. At high salinity (30 - 36 PSU), growth was moderate. The ability of phytoplankton to tolerate varying salinities could have thus resulted in the variation in the distribution of chlorophyll-*a*.

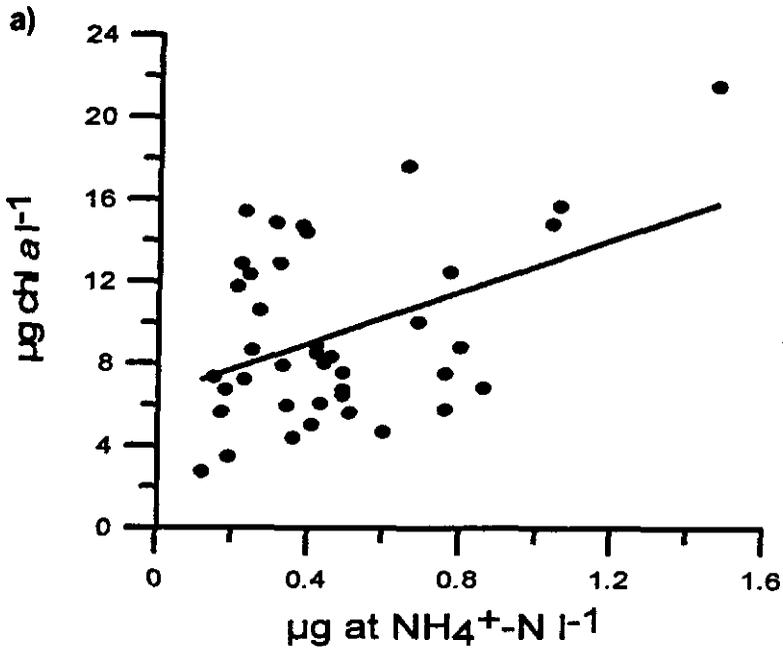


Fig. 4.17a Relation of chlorophyll a with ammonium concentrations  
 $\text{chl a} = 6.1492 + (4.6107 * \text{ammonium concentrations})$ ; ( $r=0.29$ ,  $n=51$ )

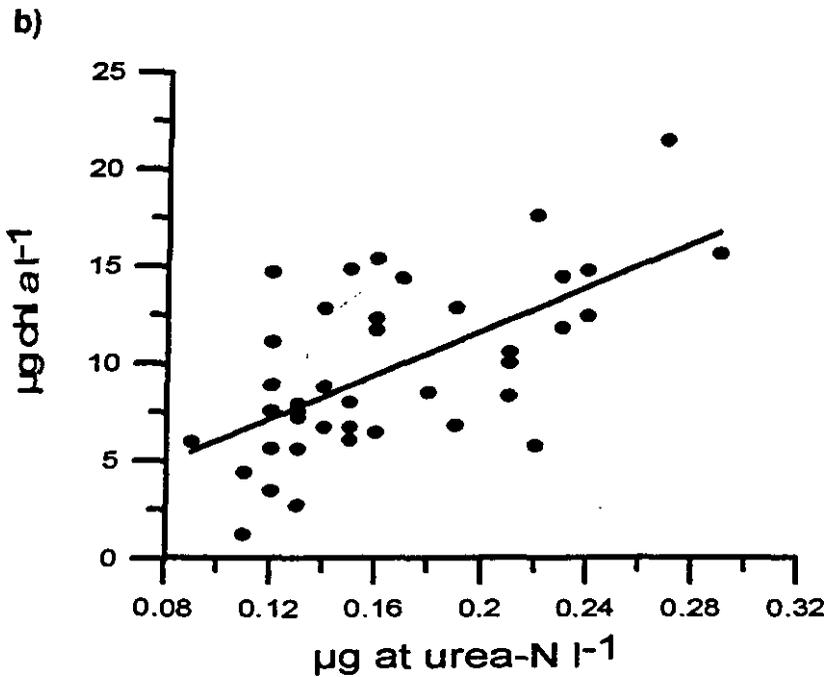


Fig. 4.17b Relation of chlorophyll a with urea concentrations  
 $\text{chl a} = 1.66 + (51.38 * \text{urea concentrations})$ ; ( $r=0.67$ ,  $n=34$ )

The chlorophyll-*a* concentrations in the non-monsoon months were higher than in the monsoon months (non-monsoon: 10.1  $\mu\text{g at N l}^{-1}$  and monsoon: 4.2  $\mu\text{g at N l}^{-1}$ ). The variations in the chlorophyll-*a* concentrations were due to the change in the climatic conditions. In the monsoon months, due to freshwater advection and sudden change in the salinity levels (34  $\rightarrow$  0 PSU), the autotrophic biomass decreased significantly (Fig. 4.18). During the monsoon months, the high turbidity due to the physical forcing of freshwater and tidal currents caused resuspension or erosion of the sediments. The low light intensity induced by the highly turbid waters significantly affects the phytoplankton population (Devassy, 1983; Mani, 1994; Huszar and Caraco, 1998). As pointed out by Teixeira *et al.* (1969), the discoloration of the water by dissolved substances may prevent light penetration. In this period, as the nutrient concentrations are high enough ( $\Sigma\text{N}=10.1\mu\text{g at N l}^{-1}$ ) to sustain the productivity, nutrient availability does not appear to be the limiting factor. Therefore, it appears that during the monsoon months, the chlorophyll-*a* concentrations were controlled by hydrological and climatic conditions.

The significant correlation between the cell number and chlorophyll-*a* concentrations suggests that the estimated phytoplankton biomass significantly contributes to the chlorophyll-*a* concentrations ( $r=0.71$ ;  $P<0.001$ ,  $n=48$ ) (Fig. 4.19). The variations in phytoplankton cell counts were enormous (0 to  $1405 \times 10^3$  cells  $\text{l}^{-1}$ ) and the average cell count of the entire estuary was  $474 \times 10^3$  cells  $\text{l}^{-1}$ .

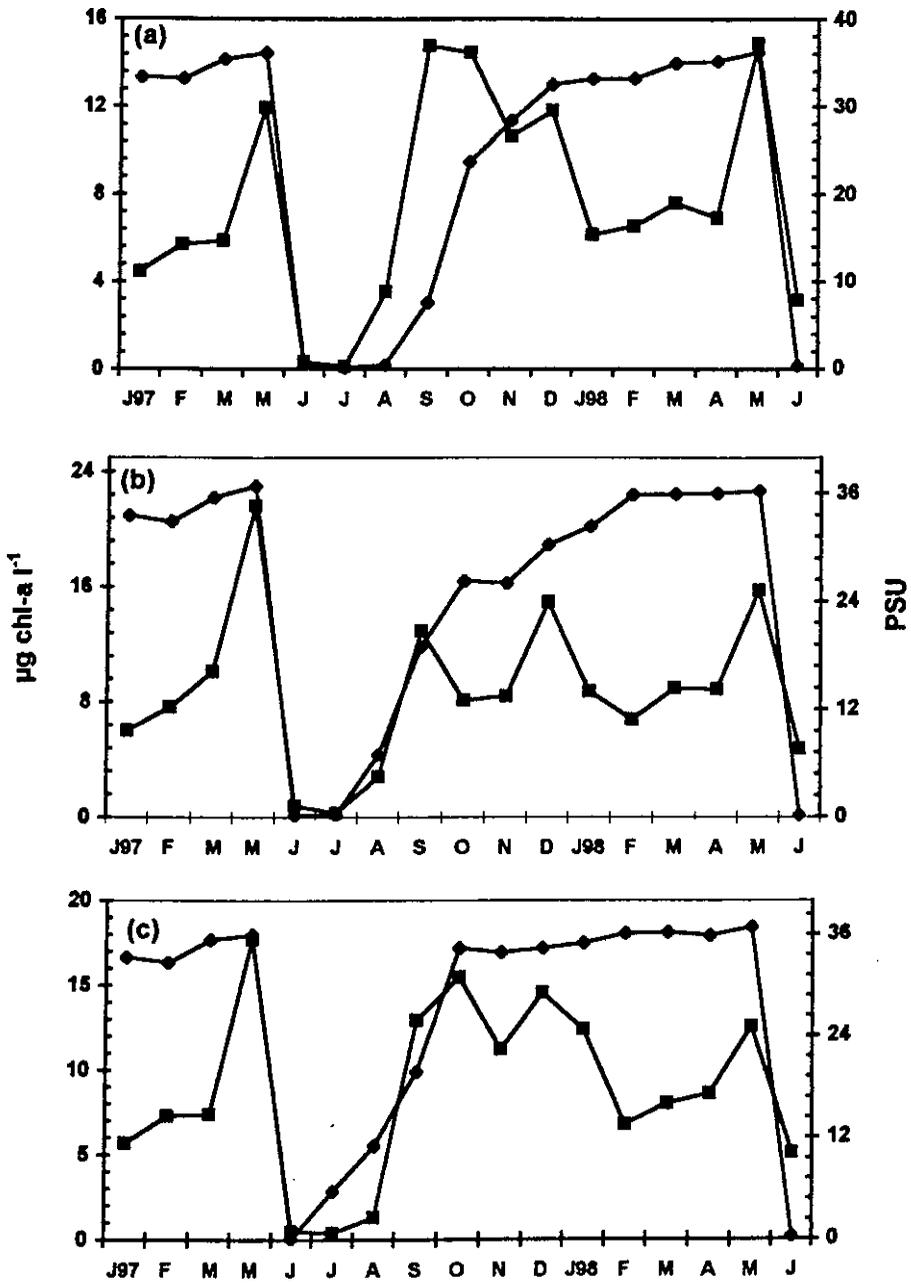


Fig.4.18 Seasonal changes of chlorophyll-a concentrations ■ and salinity levels ◆ at the (a) reference, (b) middle and (c) mouth stations

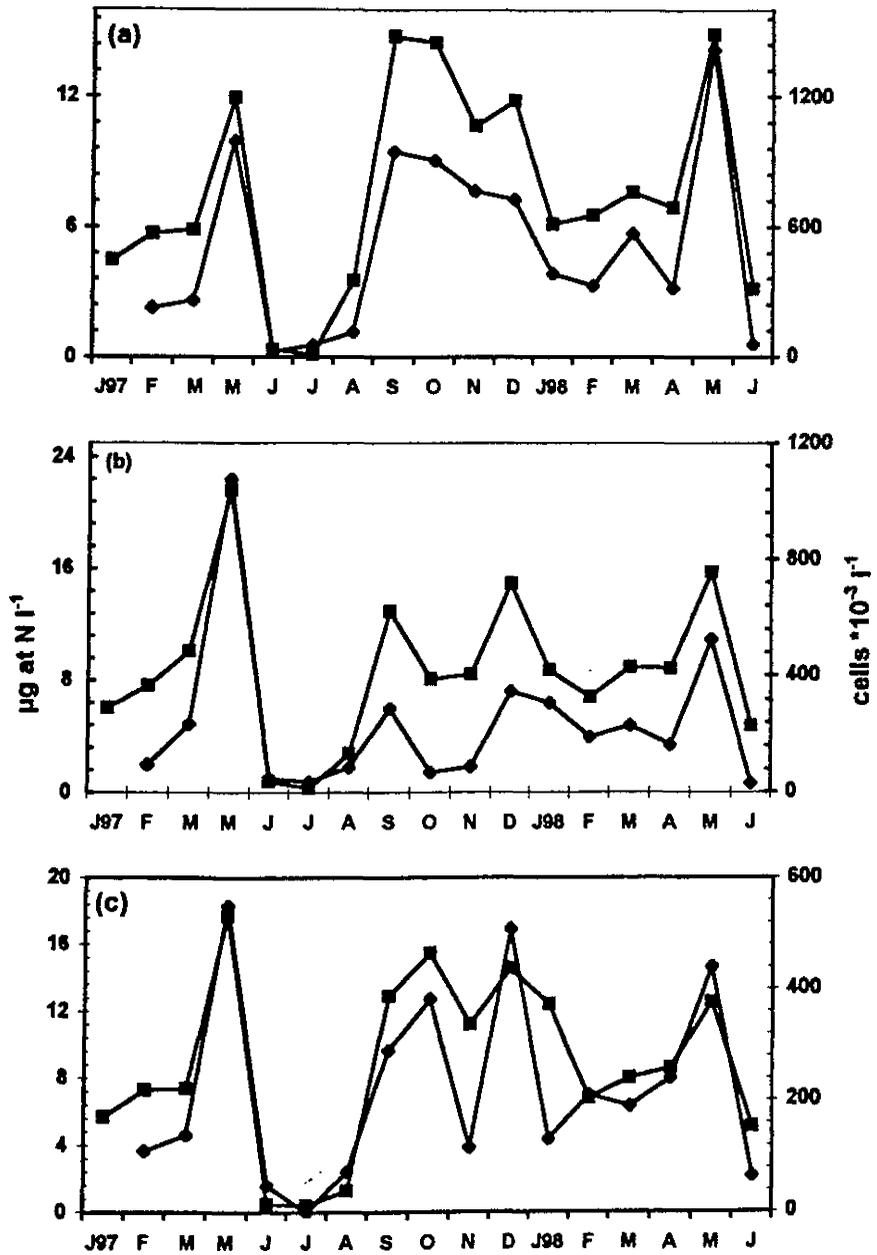


Fig. 4.19 Seasonal changes of chlorophyll-a concentrations ■ and cell counts ◆ at the (a) reference, (b) middle and (c) mouth stations

The overall distribution of phytoplankton indicated a bimodal pattern with a population maximum during September to December, followed by a secondary peak in May (Fig. 3.7). The phytoplankton population showed a clear seasonal succession pattern at all the stations (Fig. 3.9 a-c). The population maximum generally coincided with the peak values of chlorophyll-a (Fig. 4.19).

The phytoplankton size structure also changes significantly with the season (Fig. 3.8b). In the post-monsoon months, the microphytoplankton (200-20  $\mu\text{m}$ ) dominated the population whereas in the pre-monsoon and monsoon months, the nanophytoplankton (20-0.8  $\mu\text{m}$ ) formed the major group.

The seasonal nutrient distribution is the major gradient affecting both the taxonomic composition and size structure of the phytoplankton. This agrees with the findings of Mengesha *et al.* (1998) in the Indian sector of the Southern Ocean. In the present study, it appears that the phytoplankton community depended on the availability of ammonium and nitrate. Earlier studies have shown that larger cells (microphytoplankton) utilize nitrate while smaller cells efficiently utilize ammonium as the nitrogen source (Glibert, 1982; Probyn, 1985). In the post-monsoon months, the microphytoplankton formed the major group and contributed to 81% of the total chlorophyll-a. The larger fraction solely utilized nitrate as the nitrogen source ( $\rho = 24.4 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) (see section 4.4.2) to sustain the high productivity. However, in the pre-monsoon months, the microphytoplankton population was replaced by the nanophytoplankton

population, which utilized ammonium as the major source of nitrogen ( $\rho = 45 \text{ ng at N } \Gamma^{-1} \text{ h}^{-1}$ ) (see section 4.4.4). These uptake rates suggested that the phytoplankton productivity in the post-monsoon months was based on nitrate, while in the pre-monsoon months, the phytoplankton community relied on ammonium. Therefore, the change in the taxonomic composition and size structure in the non-monsoon months is due to the variations in the ambient concentrations of the nitrogen forms, ammonium and nitrate. At low ammonium concentrations ( $<0.3 \mu\text{g at N } \Gamma^{-1}$ ), microphytoplankton dominated the population, and with the gradual increase in ammonium concentrations (from  $<0.3$  to  $>0.5 \mu\text{g at N } \Gamma^{-1}$ ), the community changed from micro- to nanophytoplankton.

Change in salinity levels also appeared to be one of the reasons for the change in the taxonomic composition and size structure of the phytoplankton community (Andersson *et al.*, 1996). At low salinity (10 to 28 PSU), the microphytoplankton were dominant, while at higher salinity (30 to 36 PSU), a shift in the population was observed, with the nanophytoplankton dominating. At low salinity (0 to 8 PSU), the nanophytoplankton was the major group. This change in the size structure and taxonomic composition could have been due to the variation in the salinity tolerance levels of phytoplankton species. Admiraal and Peletier (1980) showed that *Navicula salinarum* can become dominant only at the lower limit of its salt tolerance (ca 1‰). Their experimental results on different phytoplankton species in culture provides the explanation of variations in the species composition with changes in salinity.

The spatial variations in chlorophyll-*a* distribution and cell number were not significant and could be due to the lack of variations in salinity and nutrient contents between the stations.

Shannon's diversity index ( $H'$ ) varied from 0 to 5.3 bits. Considering mean diversity indices (2.7 bits), it can be concluded that Achara estuary sustains a fairly healthy and diverse phytoplankton community.

From this discussion, the following conclusions can be drawn: (i) The change in the nutrient composition and salinity levels significantly influenced the chlorophyll-*a* concentrations (ii) The change in the taxonomic composition or species succession could have been due to the variation in the ability of a particular species to utilize a particular nitrogen source and its salinity tolerance.

### **4.3. REGENERATION OF NITROGEN**

#### **4.3.1 AMMONIFICATION RATES**

Nutrient regulation of marine primary production is determined by regenerative fluxes except in areas where there are significant nutrient inputs from external sources such as upwelling areas and river mouths. Ammonium is the main form of regenerated nitrogen in marine environments and is the central component in the regeneration pathway (Boucher *et al.*, 1994). Numerous studies on nitrogen cycling in nearshore (McCarthy, 1972; Haines, 1975) and estuarine (Harrison

and Hobbie, 1974; McCarthy *et al.*, 1975; Stanley and Hobbie, 1977) waters have shown that the major fraction of nitrogen required for maintenance of observed high phytoplankton standing stocks and productivity rates comes from rapid *in situ* remineralization. Eppley and Peterson (1979), examining a large number of studies from various waters concluded that 50-90% of autotrophic production in oceanic and coastal ecosystems is supported by regenerated nitrogen. Therefore, the remineralization of ammonium-N is a key process towards regulating regenerated production, and by consequence, the overall biological production.

Ammonification or ammonium regeneration is the process in which ammonium is the end product of mineralization of organic nitrogen. The breakdown of nearly all organic nitrogen occurs *via* this pathway in organisms ranging in size from bacteria to vertebrates where ammonium is the end product, which is subsequently excreted. The remineralization of ammonium by heterotrophic organisms can take place in two ways, either directly by excretion as in the case of all metazoans or by bacterial decomposition of organic matter (McCarthy and Carpenter, 1983; Glibert, 1988a, b).

The role of microheterotrophs (organisms <200  $\mu\text{m}$  in size, mainly represented by protozoans (tintinids, flagellates, radiolarians *etc.*), metazoans (naupliar and post-naupliar stages of copepods, pteropods *etc.*) and bacteria in the recycling of nutrients has long been recognized (Johannes, 1964, 1965). Early

estimations of Harrison (1978) showed that zooplankton accounted for >90% of the ammonium regenerated in coastal waters. Further studies by Caperon *et al.* (1979), Glibert (1982), Probyn (1987) and Harrison (1980, 1992) have also demonstrated that microheterotrophs are responsible for most of the ammonium regeneration in marine ecosystems.

In the water column, zooplankton are considered to be primarily ammonotelic, since ammonium makes up more than half of the total nitrogen excreted by them (Comer *et al.*, 1976; Dagg *et al.*, 1980). The importance of excretion of nitrogen by microzooplankton in estuarine waters was documented by Harrison *et al.* (1983), Park *et al.* (1986) and Miller *et al.* (1995). They showed that this could fulfill 70-80% of the phytoplankton N requirement. Experiments carried out on excretion of the copepod *Calanus helgolandicus*, revealed that ammonium comprises 60-100% of the total nitrogen excreted by this species (Comer and Newell, 1967; Comer *et al.*, 1972, 1976). Jawed (1969) found that ammonium comprised 82-85% of the total nitrogen excreted by the euphausiid, *Euphausia pacifica*. Zooplankton excretion is controlled by several factors such as temperature and/or body size (Biggs, 1977; Ikeda *et al.*, 1982; Ross, 1982; Vidal and Whitlegde, 1982; Bidigare, 1983), food availability (Harrison, 1980; Gardner and Paffenhofer, 1982) and variations in salinity (Raymont *et al.*, 1968; Corkett and McLaren, 1978).

It is generally accepted that bacteria less than 1  $\mu\text{m}$  in size are associated with heterotrophic activity (Azam and Hodson, 1977; Harrison *et al.*, 1977). The bacteria regenerate nitrogen from dissolved organic matter or from particulate organic detritus. The microbial decomposition of detritus (organically bound particulate N) and DOM is an important pathway of release of dissolved inorganic N. In mangrove ecosystems, much of the detritus is derived from terrestrial sources (litter) and is unavailable to consumers (Benner and Hodson, 1985). As such, microorganisms (bacteria and fungi) hydrolyze the detritus to DOM, which is finally remineralized to ammonium (Vacaro, 1965). Thus, the ultimate formation of ammonium by recycling processes is controlled by the activity of bacteria and zooplankton excretion rates.

In mangrove and estuarine waters, direct measurements of ammonium remineralization have not been carried out until now. Thus the results presented here provide the first comprehensive study of ammonium regeneration measured directly.

In the present study, ammonium regeneration by microheterotrophs ( $<200 \mu\text{m}$ ) ranged from 10 to 1500  $\eta\text{g at N l}^{-1} \text{ h}^{-1}$ . The rates measured are among the highest reported so far for nearshore waters (Glibert, 1982; Lipschultz *et al.*, 1986; Bode and Dortch, 1996) and are probably characteristic of high detritus ecosystems. The regeneration rates showed significant seasonal variations with a peak in the pre-monsoon months at all the stations and the lowest rates in the

monsoon months. Although the regeneration rates did not show statistical spatial variations, there was a marked gradient in the regeneration rates. Highest rates were recorded at the mangrove zone and decreased significantly towards the seaward station. The spatial variations in the regeneration rates are mainly due to the variations in the particulate organic load between the stations.

The strong positive correlation between ambient ammonium concentrations with ammonification rates ( $r=0.85$ ;  $P<0.001$ ,  $n=48$ ) confirms that regeneration processes regulate the concentrations of ammonium in the dissolved pool. Particulate organic nitrogen serves as the major substrate for bacterial growth (Probyn, 1987). In the present study, PON concentrations were substantially high (annual average:  $68.9 \mu\text{g at N l}^{-1}$ ) and the correlation of PON concentrations and ammonium regeneration rates was significant (Fig. 4.20) in the dry months suggesting that bacterial remineralization of PON was an important process maintaining the nutrient pool in the dry season.

Ammonium regeneration rates showed a significant correlation with water temperature ( $r=0.66$ ;  $P<0.001$ ,  $n=48$ ) (Fig. 4.21). Increase in salinity also appeared to enhance the regeneration rates ( $r=0.56$ ;  $P<0.001$ ,  $n=48$ ). These relationships suggest that the high temperature and salinity in the non-monsoon months may have favoured higher metabolic activity of the microorganisms. Although the microzooplankton biomass was not estimated in the present study, it is generally accepted that their abundance is high during these months (Nair,

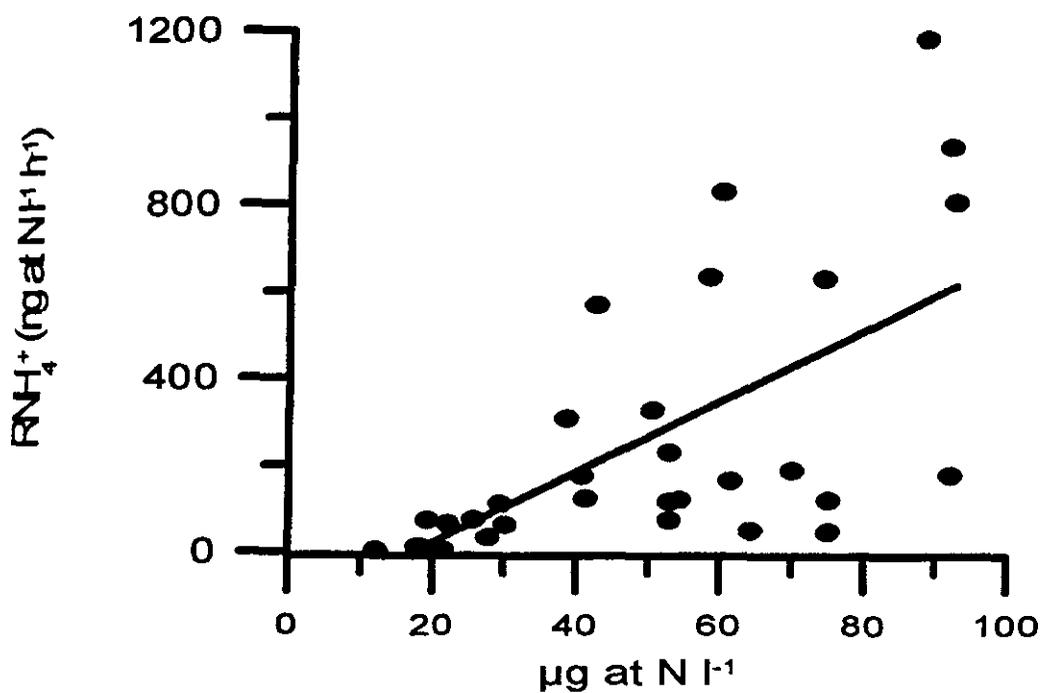


Fig. 4.20 Relation of ammonification rates with PON

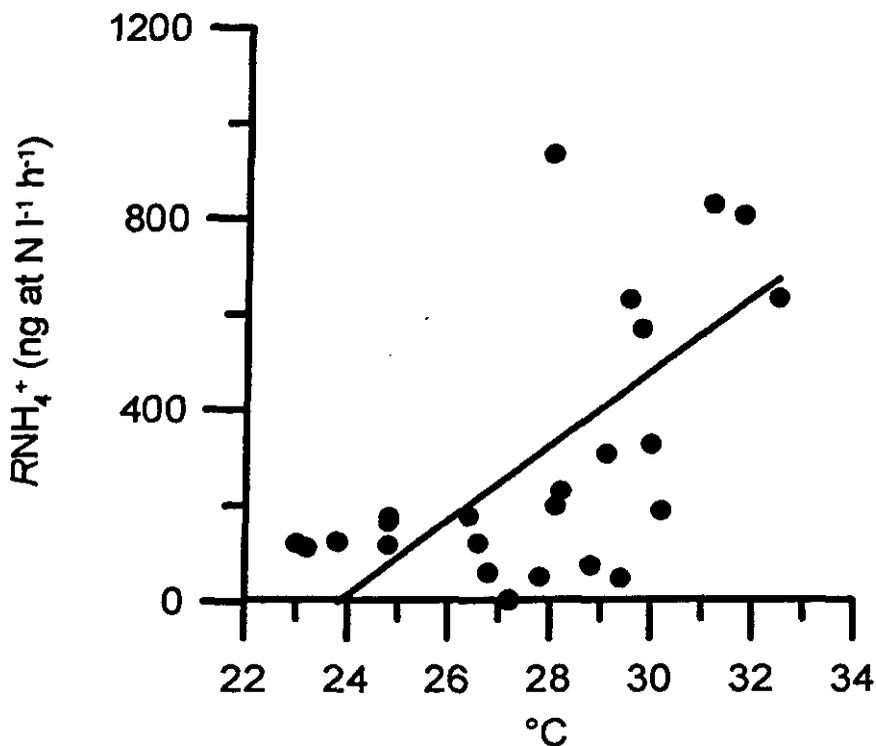


Fig. 4.21 Relation of ammonification rates with the water temperature  
 Ammonification rates =  $-2.73 + (0.1103 \cdot \text{water temp.})$ ; ( $r=0.65$ ;  $n=48$ )

1979). The rates of ammonium excretion by marine zooplankton are thought to be tightly coupled with food availability (Harrison, 1980). In an earlier study by Corner *et al.* (1976), a progressive increase in the rates of ammonium excretion by *C. helgolandicus* occurred when exposed to high food levels. The high chlorophyll-*a* and substantially high particulate organic matter could have supported high microzooplankton biomass. The microzooplankton are known to be omnivorous, consuming bacteria, phytoplankton and other heterotrophs, such as protists (Coffin and Sharp, 1987; McManus and Fuhrman, 1988; McManus, 1991; Verity, 1991; Sherr and Sherr, 1994). Hence, they play a central role in both the transfer of nitrogen to higher trophic levels and its remineralization and return to the microbial autotrophs and heterotrophs (Lehrter *et al.*, 1999). The release of ammonium by this pathway (*i.e.* microzooplankton excretion) could probably have significantly contributed ammonium to the dissolved nutrient pool. Therefore, high particulate organic load in these waters was a major factor significantly enhancing the ammonium regeneration activity during the dry seasons.

In the monsoon months, although the particulate organic matter was high, a sharp fall in the ammonium regeneration rates was observed ( $10 \text{ } \mu\text{g at N l}^{-1} \text{ h}^{-1}$ ). The sudden change in the environmental conditions during this period could have influenced metabolic activity of the microorganisms. Generally, during the monsoon months, it has been observed that zooplankton biomass is low (Nair, 1979). The sharp decrease in the ambient water temperature and salinity due to

excessive precipitation and freshwater advection could have resulted in decreased microzooplankton biomass. This therefore suggests that the low regeneration rates during the monsoon months may have been due to the sudden change in the environmental conditions rather than substrate availability.

In conclusion, it can be said that (i) mangrove waters are active sites of microbial ammonium regeneration (ii) ammonium regeneration rates are significantly dependent on the particulate organic load in the dry season (ii) the variations in temperature and salinity could have influence the seasonal variations of regeneration rates in the water column.

#### 4.3.2 NITRIFICATION RATES

Nitrification plays a central role in the marine nitrogen cycle and influences the distribution of nitrogen compounds in the sea. The major inorganic species of fixed nitrogen found in the dissolved pool is nitrate, the end product of complete nitrification. In the deep ocean, nitrification is generally accepted as the main source of nitrate and the upwelling of nitrate from deep waters to the photic zone supplies nitrogen for new production (Dugdale and Goering, 1967; Eppley and Peterson, 1979). Nitrate produced through nitrification also serves as the terminal electron acceptor for denitrification (Falkowski, 1997). The importance of nitrification is also evident in the formation of the primary nitrite maximum at the bottom of the euphotic zone in most oceanic environments (Brandhorst, 1959; Wada and Hattori, 1971; Olson, 1981). Nitrification in the water column in

the marine environment is highly variable but most productive estuarine and coastal areas have higher rates than the oceanic waters (Kaplan, 1983).

The process of nitrification involves the oxidation of ammonium to nitrite and its subsequent oxidation to nitrate. All marine nitrifying bacteria are gram-negative, *obligate chemolithotrophs* (Kelly, 1971) and can obtain the energy necessary for growth and carbon assimilation from the aerobic oxidation of ammonium to nitrite or nitrite to nitrate. In the marine environment, two genera of bacteria, *Nitrosomonas* and *Nitrosococcus* mediate the first step of nitrification, while a second set, the *Nitrobacter*, *Nitrospira* and *Nitrococcus* group, oxidize  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (see Watson *et al.* (1980) for a detailed description of nitrifying bacteria).

Saturation uptake kinetics is exhibited by nitrifiers in cultures in response to increasing concentrations of ammonium (Carlucci and Strickland, 1968; Sharma and Ahlert, 1977). However, in natural environments, marine nitrifiers utilize their substrates at much lower levels than those observed in culture studies. Kinetic experiments conducted by Ward *et al.* (1982) in the coastal waters of California showed that  $\text{NH}_4^+$  oxidation rates did not increase consistently when  $\text{NH}_4^+$  levels ranged from 0.1 to 20  $\mu\text{M}$ . Berounsky and Nixon (1993) observed a strong relationship between ammonium concentrations and nitrification rates along an estuarine gradient in Narragansett Bay.

In pelagic systems, light intensity is an important factor influencing the magnitude of nitrification (Kaplan, 1983). The photic zone is a region of minimum nitrification activity and this is attributed to the fact that marine nitrifiers are inhibited by light (Muller-Neugluck and Engel, 1961; Schon and Engel, 1962; Bock, 1965). For the Southern California Bight, Olson (1981) and Ward *et al.* (1982) reported negligible rates of ammonium oxidation in surface waters, but an increase with depth. Subsequent studies of Ward (1986) supported the above findings. Ward *et al.* (1982) showed that nitrite oxidizers show greater sensitivity to light than the ammonium oxidizers and explained that the occurrence of the primary nitrite maximum was due to differences in their responses to light inhibition.

Oxygen serves as the terminal electron acceptor in the oxidation of ammonium and is incorporated directly into the substrate (Rees and Nason, 1966). However, marine nitrifiers can grow and oxidize their substrates at very low oxygen levels (Carlucci and McNally, 1969; Gundersen, 1966). Also, the production of nitrous oxide (Goreau *et al.*, 1980) and nitric oxide (Lipschultz *et al.*, 1981) is enhanced at low oxygen concentrations. In the oxygen minimum zone off Peru, high nitrite concentrations upto 23  $\mu\text{M}$  were recorded (Codispoti *et al.*, 1986). In the present study, oxygen may not be a limiting factor for nitrification, as these waters are well mixed and highly oxygenated (4 to 5 ml /l).

It has also been observed that temperature influences the growth of the nitrifiers (Focht and Verstraete, 1977; Kaplan, 1983; Horrigan, 1981). Based on temperature, a seasonal selection of the different heterotrophic bacteria may be likely (Sieburth, 1967). At high temperatures (25 - 35 °C), optimal activity of ammonium oxidizers was observed (Focht and Verstraete, 1977), whereas at lower temperatures (< 4-5°C), nitrifiers do not grow (Kaplan, 1983) However, Horrigan (1981) found that nitrifying activity could even occur at temperatures down to - 2°C.

In the present study, nitrification rates ranged from 0.1 to 96.7  $\mu\text{g N l}^{-1}\text{h}^{-1}$  and were more or less similar to Scheldt estuary (77.4  $\mu\text{g N l}^{-1}\text{h}^{-1}$ ) reported by Somyille (1978). The seasonal variation in the nitrification rates could probably be related to variation in suspended load or particulate organic matter, since it is the remineralization of organic matter that ultimately forms the substrate for nitrification (Kaplan, 1983). Nitrifiers are closely associated with particulate surfaces (Keen and Prosser, 1988) and the high biomass of nitrifiers could have led to the high nitrification rates. Nitrification rates showed well-defined seasonal changes with maximum rates in the pre-monsoon season. The spatial variations were significant. The results presented here show a strong correlation of ammonium concentrations and nitrification rates ( $r= 0.63$ ;  $P<0.001$ ,  $n=36$ ) (Figs. 4.22; 4.23). Hence, it is clear that the nitrification rates in the water column are dependent on the availability of the substrate,  $\text{NH}_4^+$ .

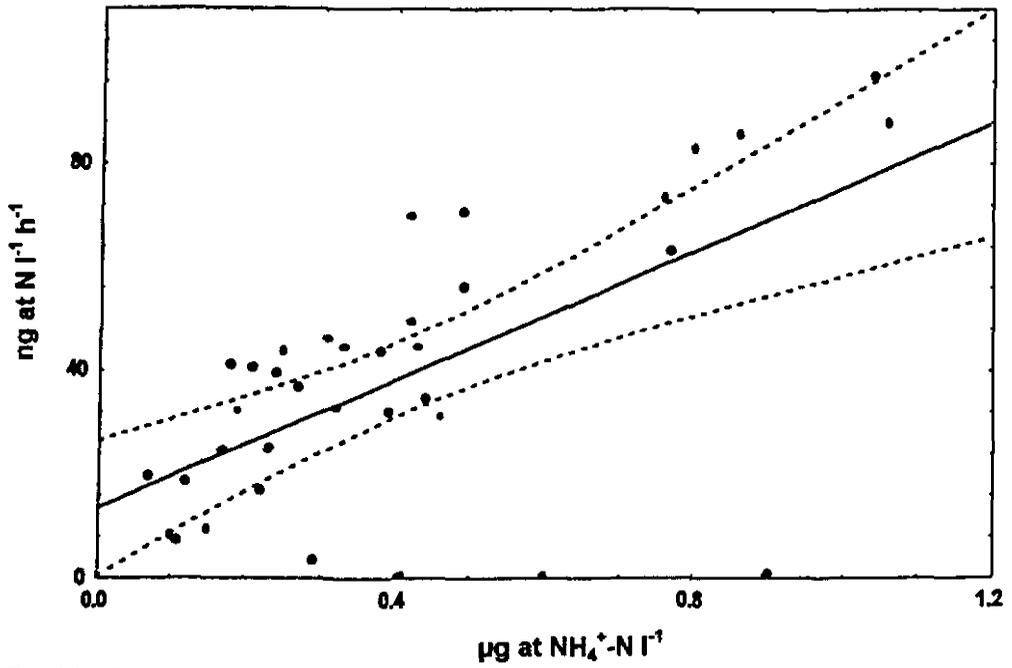


Fig. 4.22 Relation of nitrification rates with ammonium concentrations  
 Nitrification rates =  $13.410 + (62.04 \cdot \text{ammonium concentrations})$  ( $r = 0.67$ ;  $n = 36$ )

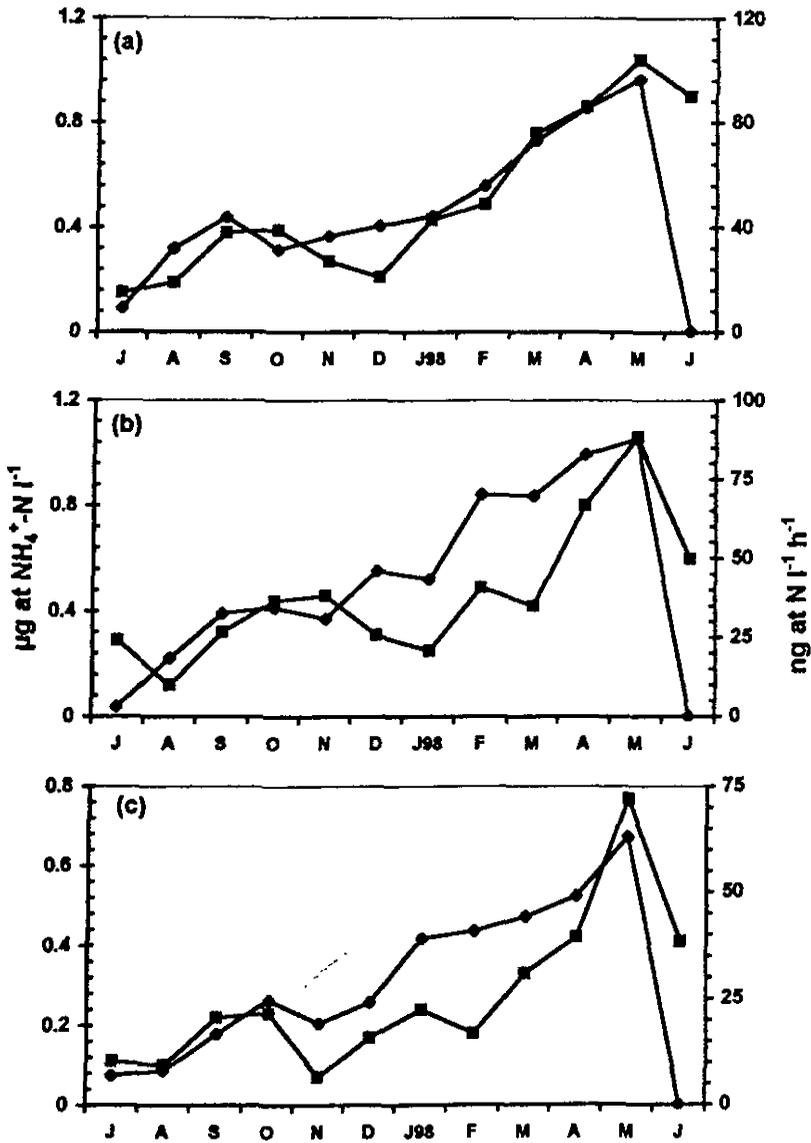


Fig. 4.23 Seasonal changes of nitrification rates  $\blacklozenge$  and ammonium concentrations  $\blacksquare$  at the (a) reference, (b) middle and (c) mouth stations

The maximum nitrification rates in the pre-monsoon months can be explained by taking the above correlation into consideration. The high substrate availability (average:  $0.7 \mu\text{g at N l}^{-1}$ ) led to the peak in nitrification rates. During these months, the temperature was also optimum and this also could have enhanced the nitrification activity. The positive significant correlation of water temperature and nitrification rates supports this ( $r=0.51$ ;  $P=0.001$ ,  $n=36$ ) (Fig. 4.24).

The low nitrification activity in the post-monsoon months can be attributed to the low substrate availability during this period (average:  $0.3 \mu\text{g at N l}^{-1}$ ). As the ammonium concentrations gradually increased towards the pre-monsoon, corresponding gradual increases in the nitrification rates were recorded. The lower water temperature may have also been responsible for the low activity compared to that of the post-monsoon season.

In the monsoon months, a sharp fall in the nitrification rates was recorded (pre-monsoon:  $68.3 \eta\text{g at N l}^{-1}\text{h}^{-1}$   $\blacktriangleright$  monsoon:  $14.3 \eta\text{g at N l}^{-1}\text{h}^{-1}$ ). The sudden change in the environmental conditions (low salinity and temperature) may have inhibited the growth of the nitrifiers. Also, the low substrate availability could have resulted in the low nitrification rates. Finstein and Bitzky (1972) showed that at low salinity levels marine nitrifiers did not grow. The significant correlation of salinity and nitrification rates supports the above hypothesis ( $r=0.74$ ;  $P<0.05$ ,  $n=36$ ) (Fig. 4.25).

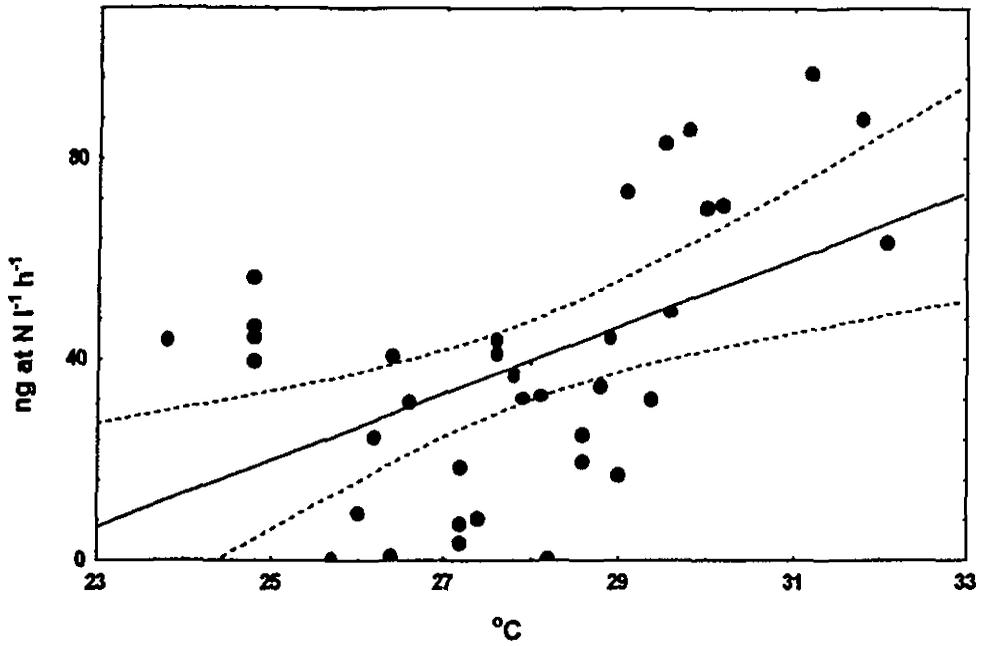


Fig. 4.24 Relation of nitrification rates with water temperature  
 nitrification rates =  $-146.4 + (6.6569 \cdot \text{water temperature})$  ( $r=0.51$  ;  $n=36$ )

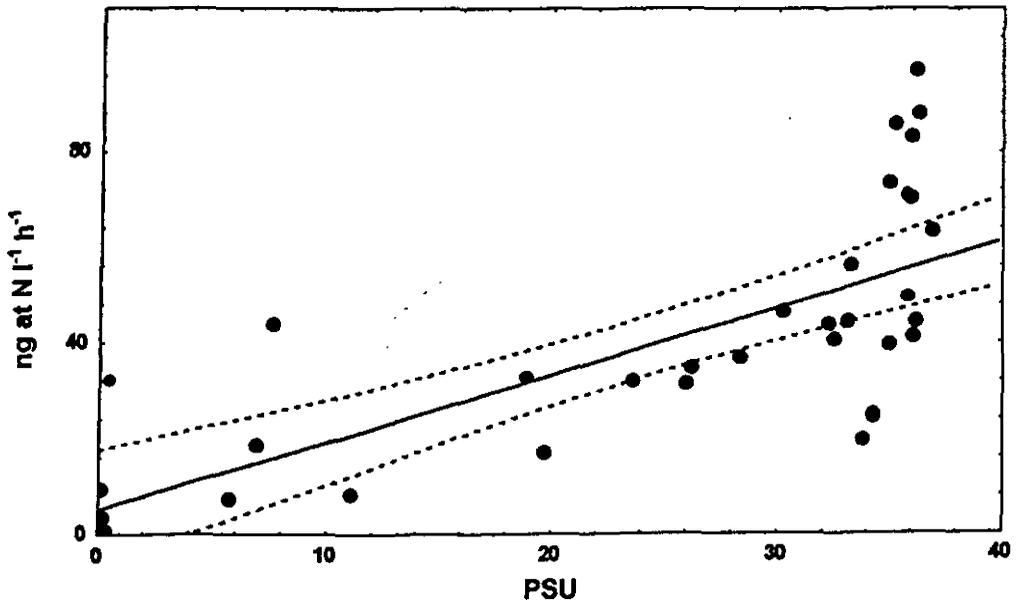


Fig. 4.25 Relation of nitrification rates with salinity  
 Nitrification rates =  $5.1229 + (1.4020 \cdot \text{salinity})$  ( $r=0.51$ ,  $n=36$ )

Although the spatial variations were statistically insignificant, there was a distinct gradient in the averages between the first two and the third (49.9, 47.3 and 30.6  $\text{ng at N l}^{-1} \text{ h}^{-1}$ ). This was proportional to the gradient in average PON (85.8, 72.5 and 48.6  $\mu\text{g at N l}^{-1}$ ) and substrate (0.6, 0.6 and 0.3  $\mu\text{g at N l}^{-1}$ ) concentrations.

In conclusion, (i) the high nitrification rates are due to the adequate supply of organic matter, the remineralization of which forms the substrate ( $\text{NH}_4^+$ ) for the growth of nitrifiers (ii) The variations in the water temperature and salinity levels could have lead to the variations in the nitrification rates (iii) The spatial variations in nitrification rates are due to the variations in the PON concentrations and ambient ammonium concentrations at the different stations.

## 4.4 NITROGEN UPTAKE STUDIES

### 4.4.1 INTRODUCTION

#### *Historical background:*

The role of nutrients in regulating primary production in the sea has been recognized since the beginning of the 19<sup>th</sup> century, when it was realized that nitrate and phosphate are removed from seawater (by phytoplankton activity) in the same ratio in which they occur. This concept eventually led to the classical derivation of 'Redfield ratio' (Redfield *et al.*, 1963). The process of removal of nutrients (uptake) is usually measured by the disappearance of the substrate from the medium or by the estimation of accumulation of total labeled elemental material in the cell.

At first, studies on nutrient uptake by phytoplankton were undertaken by simple estimations of depletion in nutrient concentrations in the medium during growth of the culture over time (Eppley *et al.*, 1969a,b; Carpenter and Guillard, 1970; Caperon and Meyer, 1972). This approach however, had applications only with phytoplankton cultures of high biomass and at high nutrient concentrations and not to marine systems with low phytoplankton standing stocks. Further drawbacks of this approach are listed below.

1. The sensitivity of the spectrophotometer (in the range of 0.05-45  $\mu\text{M}$  for nitrate) is often not significantly different from the rate of nutrient removal by natural phytoplankton populations at low ambient concentrations over several days of incubation.
2. The cause of depletion may not be due to phytoplankton alone, but may also be due to the bacterial processes occurring, which, in natural systems are not differentiated.
3. Metabolic pathways cannot be traced.

The present knowledge of the rates of marine primary production and nutrient uptake and cycling are largely based on the application of isotopic tracer techniques. Steemann and Nielsen (1952) introduced the use of  $^{14}\text{C}$  in primary production estimations. While this was rated an elegant method of measuring primary production and has been applied extensively in the last five decades, it

was not without drawbacks. One among them is that it does not provide any means of differentiating the pattern of utilization of individual nutrients. This was especially true with nitrogen which occurs in more than one assimilable form.

Neess *et al.* (1962) developed the method of using the stable isotope of nitrogen ( $^{15}\text{N}$ ) to estimate nitrogen utilization by phytoplankton. Dugdale and Goering (1967) later on used this technique to study the uptake patterns of ammonium and nitrate, which gave new insights into the mechanisms of control of primary productivity in the marine environment. Ever since, data on nitrogen uptake by phytoplankton have been accumulating and are now available for many oceanic areas. It has also been realized, mostly from work on algal cultures, that phenomena such as non-linearities in uptake, feedback inhibition and interactions with limiting and non-limiting nutrients have complicated the interpretation of such data (Dugdale, 1977). This may be the reason why conclusions concerning nutrient limitation, are sometimes radically different for nitrogen (D'Elia *et al.*, 1977) and carbon uptake (Menzel and Ryther, 1964).

#### *Rates and Ratios:*

Generally, studies on nutrient uptake and primary productivity in marine systems are made in order to understand how nutrient availability, either through physical transport processes or through biologically regulated regeneration processes, control the growth and primary production rates of marine phytoplankton populations (Goldman and Glibert, 1983). Dugdale (1967), in his pioneering

studies, postulated the relationship between phytoplankton nitrogen uptake and nitrogen availability using the modified equation of Monod (1942), which is defined as:

$$V = V_{max} \frac{N}{K_s + N} \quad \text{————— (equation 1)}$$

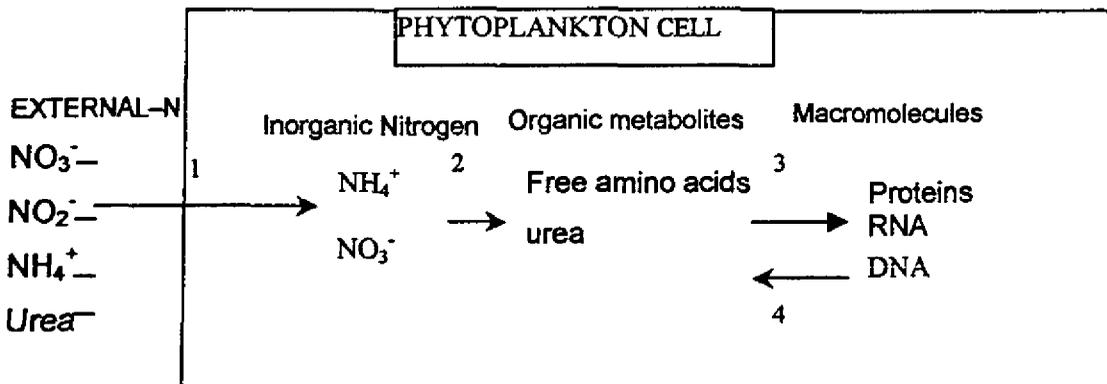
Where,  $V$  is the specific growth rate ( $\text{time}^{-1}$ ) in terms of limiting nitrogen concentration  $N$  ( $\text{mass volume}^{-1}$ ),  $V_{max}$  is the maximum uptake velocity (maximum growth rate) ( $\text{time}^{-1}$ ) in terms of  $N$ , and  $K_s$  is the concentration of  $N$  at which  $V = 0.5 V_{max}$  ( $\text{mass volume}^{-1}$ ).

Goldman and Glibert (1983) argued that these rectangular hyperbolic equations are valid when substrate concentration or active surface area remains constant over the period during which the rate of the particular process is being measured. Under non-steady-state situations, these equations are not applicable and are valid only for describing the instantaneous rate reactions (Powell, 1967). Nevertheless, the above mentioned equation found widespread applications in earlier studies on the uptake kinetics of inorganic nitrogen. These studies, however, considered only the summation of the several metabolic processes involved in the uptake. Individual pathways were identified only later and have been reviewed by Wheeler (1983).

The four major pathways described were:

1. *Membrane transport*: the movement of a particular form of nitrogen across the plasmalemma into the cell.
2. *Assimilation*: the metabolic conversion of inorganic nitrogen to small organic metabolites
3. *Incorporation*: the synthesis of macromolecules from small metabolites.
4. *Catabolism*: the breakdown of macromolecules into small metabolites.

The following diagram depicts these pathways.



Rates of nitrogen metabolism have been estimated based on the above grouping and are further discussed. The rates commonly estimated are specific and absolute uptake rates and the ratios commonly used are the ratio of  $\text{NO}_3^-$  uptake against total uptake (*f*-ratio) and relative preference indices. Also fairly studied is the kinetics of uptake.

#### *Specific uptake (V) and absolute uptake (ρ) rates:*

The process of membrane transport is categorized as specific uptake (*v*) and assimilation, as absolute uptake (Dugdale, 1967). Specific uptake rate is defined as the transfer of N from the dissolved to the particulate form per unit time,

whereas absolute uptake rate is the increase in N in the cell organic metabolite per unit time. Dugdale and Wilkerson (1986) and Collos (1987) recommended two different equations to calculate N assimilation (absolute uptake) rates. One is to be used when the concentration of particulate organic nitrogen (PON) is measured at the end of the incubation, and the other, for the situation when the PON is determined at the beginning. Since algae use several sources of nitrogen, as is generally the case with natural populations, they recommend the use of the first equation, where the PON is measured at the end of the incubation.

*Uptake kinetics ( $V_{max}$  and  $K_s$ ):*

The kinetics of uptake mainly represents the physiology of the cell. The physiology of phytoplankton uptake can be expressed as a function of substrate concentration, in the form of the rectangular hyperbolic equation of Monod (1942) and this was adopted for the first time for uptake studies by Dugdale (1967) (equation 1). The two constants in this equation are  $V_{max}$  and  $K_s$ . The concentration at which the uptake rate reaches its maximum is denoted as  $V_{max}$  and  $K_s$  represents the substrate concentration at which V is half of maximum uptake (Dugdale, 1967; Eppley and Coatsworth, 1968). The hypotheses underlying much of this work was that, (i) for a specific habitat, the indigenous species of phytoplankton would have been selected on the basis of their ability to compete for a nutrient at concentrations characteristic of the habitat, and (ii) this could be a contributing factor in the determination of the temporal succession of dominant species in environments that undergo seasonal

changes in nutrient availability (Mengesha *et al.*, 1998; Collos and Slawyk, 1986; Probyn and Painting, 1985; Hattori and Fukuchi, 1989).

Maclsaac and Dugdale (1969) observed that the half saturation constant ( $K_s$ ) appears to be related to the nutrient and productivity regime of an area. They found that the average  $K_s$  values for natural mixed populations from oligotrophic oceanic waters and eutrophic neritic waters differed significantly. They reported  $K_s$  values of  $< 0.2 \mu\text{mol l}^{-1}$  for nitrate uptake in the tropical oligotrophic region of the northeastern Pacific Ocean and higher  $K_s$  values ( $>1.0 \mu\text{M l}^{-1}$ ) in the eutrophic Alaskan coastal waters. These values suggested that the phytoplankton populations in oligotrophic regions are adapted to the low ambient nutrient concentrations and are able to take up nutrients at a higher rate under those conditions than would phytoplankton species characteristic of eutrophic regions. Eppley *et al.* (1969b) further confirmed this concept with their experiments on 16 species of marine phytoplankton both from oceanic and neritic waters. They demonstrated that the half-saturation constants ( $K_s$ ) of nitrate and ammonium uptake were low for oceanic than for the neritic species and there was a direct correlation between the cell size and the  $K_s$  value. Carpenter and Guillard (1971) added further support to this notion with their observation that the pattern in  $K_s$  values for clones of a single species isolated from oceanic and neritic waters represented the nutrient regime of the waters of origin of each clone. The half saturation constants ( $K_s$ ) estimated in different waters and for different species of phytoplankton are listed in Table 4.2a,b.

Table 4.2 a. Summary of reported values of Ks for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in various waters.

Location	Incubation	Ks ( $\mu\text{g. atom N liter}^{-1}$ ) $\text{NO}_3^-$	$\text{NH}_4^+$	References
<i>Oligotrophic waters:</i>				
Tropical pacific	6-24	0.01-0.21	0.10-0.55	MacIsaac and Dugdale (1969)
Mediterranean	24	0.1-0.3	<0.1	MacIsaac and Dugdale (1972)
North Pacific Central gyre	24	-	0.15	Eppley et al. (1973)
	24	0.00-0.91	0.00-0.13	Eppley et al. (1977)
<i>Eutrophic oceanic waters:</i>				
Tropical pacific	6-24	0.98	1.30	MacIsaac and Dugdale (1969)
Subarctic Pacific	6-24	4.21	1.30	
Peru coast	24	-	1.11	MacIsaac and Dugdale (1972)
<i>Coastal waters:</i>				
Vineyard sound, Massachusetts	0.50		0.62	Gilbert et al. (1982b)
	0.50		0.72	
	0.50		0.58	
	0.50		0.73	
	0.25		0.27-0.46	Wheeler et al. (1982)
Chesapeake Bay	0.50		0.71	
Station 744-1	0.017		0.27	
Station 707-0	0.25		0.40	
	0.083		0.07	
Station 744-2	0.50		0.26	
	0.017		0.46	
	0.25		0.56	

Table 4.2 b. Summary of reported values of K<sub>s</sub> from laboratory experiments on various cultures of marine phytoplankton species.

Species	Incubation	half-saturation coefficient ( $\mu\text{g. at. l}^{-1}$ )			References
		NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup> urea	
<b>Diatoms:</b>					
<i>Asterionella japonica</i>	0.25-2	0.7			Eppley and Thomas (1969)
<i>Chaetoceros gracilis</i>		0.3			Harrison <i>et al.</i> (1976)
<i>Skeletonema costatum</i>	3-4		0.5		"
<i>Chaetoceros debilis</i>	6-7		0.5		"
<i>Thalassiosira grayii</i>	6.7		0.4		McCarthy (1981)
<i>Thalassiosira pseudonana</i>	0.08		1.1	4.0	Eppley and Rogers (1970)
<i>Ditylum brightwellii</i>		0.6			Cresswell and Syrett (1981)
<i>Phaeodactylum tricornutum</i>		10.0			Rees and Syrett (1979)
<i>Phaeodactylum tricornutum</i>				0.6	Caperon and Meyer (1972)
<i>Coccolithus</i>		0.31			"
<i>Dunaliella</i>		0.21	0.17		"
<i>Monochrysis</i>		0.42	0.29		"
<i>Cyclotella</i>		0.35			"

These values could be used in the interpretation of uptake rates of populations in different nutrient regimes, as they demonstrate that the variation in the uptake rates and preference for a particular nutrient to achieve maximal rate of uptake is dependent on the availability of the nutrient and species composition (Caperon and Meyer, 1972).

*f-ratio:*

The *f-ratio* (Eppley and Peterson, 1979) is defined as the ratio of new to total production (Dugdale and Goering, 1967). The *f-ratio* finds its most straightforward application in conjunction with the physical model of a two-layer ocean where the pycnocline coincides with the bottom of the euphotic zone (Dugdale and Goering, 1967). It is generally considered that the supply of dissolved nitrogenous nutrients to the euphotic zone is of major importance in regulating primary production in the ocean. In turn, production rate is controlled by the supply of organic matter to the deep ocean. Dugdale and Goering (1967) partitioned oceanic primary production according to its nitrogen source: new production is fuelled by allochthonous sources of N, principally nitrate mixed into surface waters from the deep ocean, and secondarily, nitrogen fixation, riverine and rainfall inputs; regenerated production is fuelled by autochthonous sources, principally ammonium, derived from *in situ* biological processes. Dugdale and Goering (1967) further suggested that under steady-state conditions, the new production should be balanced by the export (in the form of sinking particles) of organic matter from the photic zone.

Following Eppley and Peterson (1979), the relative contribution of new and regenerated nitrogen to primary production can be estimated as:

$$f = \frac{\rho\text{NO}_3^-}{\rho\text{NO}_3^- + \rho\text{NO}_2^- + \rho\text{NH}_4^+ + \rho\text{urea}}$$

Where  $\rho\text{NO}_3^-$ ,  $\rho\text{NO}_2^-$ ,  $\rho\text{NH}_4^+$  and  $\rho\text{urea}$  are the rates of uptake of nitrate, nitrite, ammonium and urea respectively. They pointed out that the  $f$ -ratio and new production estimates using  $^{15}\text{N}$  tracer measurements may not be reliable in coastal waters of less than 200 m depth. This is because the new nitrogen entering the water column from the land, sediments and benthos, as well as through sewage or agricultural runoff may be in the form of reduced nitrogen, *i.e.* ammonium and urea. In addition to this, a certain amount of nitrate could also be produced through regeneration *i.e.*, from organic matter of external origin (mangrove detritus in the case of mangrove systems) remineralized into ammonium, which in turn acts as the substrate for nitrification. The application of the  $f$ -ratio is also based on the observation that, although phytoplankton preferentially utilize the reduced nitrogen forms (Dugdale and Goering, 1967; Eppley *et al.*, 1973; McCarthy *et al.*, 1977, 1982; Glibert *et al.*, 1982b,c), simultaneous utilization of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  may occur when insufficient ammonium is available to meet the phytoplankton nitrogen requirement. Further, Eppley and Peterson (1979) showed regional differences in  $f$ -ratios *i.e.* lower  $f$ -ratios (on the order of 0.05-0.2) in oligotrophic, nutrient poor regions and high  $f$ -ratios (on the order of 0.5-0.8) in eutrophic, nutrient rich regions. Pioneering

studies by McCarthy *et al.* (1975, 1977) in Chesapeake Bay, demonstrated that inshore primary production is largely fuelled by reduced nitrogen compounds, as shown by the low *f*-values. Exceptions to this generalization have also been reported. For example, Carpenter and Dunham (1985) reported high *f*-values (> 0.95) in the Carmans estuary, Long Island, where the depletion of ammonium upstream results in the utilization of nitrate as the main nitrogen source further downstream.

*Relative Preference Index (RPI):*

McCarthy *et al.* (1977) introduced a relative preference index (RPI) to describe in a more comprehensive way, nitrogen utilization relative to nitrogen availability. They defined RPI for a given substrate,  $N_1$ , as

$$RPI_{N_1} = \frac{\rho_{N_1}}{\rho_{N_1} + \rho_{N_2} + \dots + \rho_{N_i}} \bigg/ \frac{(N_1)}{(N_1) + (N_2) + \dots + (N_i)}$$

where  $\rho_{N_i}$  is the uptake rate and  $(N_i)$ , the concentration of the *i*th substrate.

According to them, all nitrogen sources would be used simultaneously and in proportion to their concentrations at ambient ammonium concentrations less than 0.5  $\mu\text{M}$ . Conversely, the demand for nitrogen by the plankton would be fully satisfied by ammonium, or by ammonium and urea, at ambient ammonium concentrations above this value. Useful as this might seem, it is difficult to reconcile the application of the RPI as a test for nitrogen limitation with the extremely high affinity of algal cells for ammonium that has been demonstrated

in the laboratory experiments (Goldman and Glibert, 1983). Stolte and Reigman (1996) further pointed out that this index could be easily misused when it is applied as a physiological preference indicator. Their study showed that RPI may be more dependent on nutrient concentrations than on the algal preference for ammonium under certain circumstances.

In the present study, the interpretation of absolute uptake rates, the *f*-ratio and RPI was made with caution, as their calculation could incur errors that are expected of specialized ecosystems. The problems encountered in using each of the equations are discussed separately in the following paragraphs.

*Absolute uptake rates ( $\rho$ ):*

The high estimation of absolute uptake, for example, is discussed below. The particulate nitrogen estimated/trapped on the filters includes detritus, bacteria, microzooplankton, and mesozooplankton, in addition to phytoplankton (Dugdale and Goering, 1967; Dugdale and Wilkerson, 1991) and often, phytoplankton make up only a small percentage of the particulate nitrogen/planktonic biomass (Holligan *et al.*, 1984; Kokkinakis and Wheeler, 1987). Generally, the living biomass in eutrophic waters is mainly contributed by phytoplankton, whereas, in oligotrophic regions, bacteria and zooplankton dominate (Dortch and Packard, 1989). It is usually difficult to separate heterotrophic bacteria and phytoplankton as there is overlap in their particle sizes (Wheeler and Kirchman, 1986). In the present study, however, there could be an error of a slightly different sort.

Litterfall is the major source of particulate organic nitrogen at any time of the year and as the calculation of absolute uptake rate includes the multiplication by PON (Dugdale and Goering, 1967), it could be assumed that the high values are imposed by the detrital PON. Therefore, though the specific uptake rates were low, the absolute uptake rates were high. For example, in the month of June, very low specific uptake rates were recorded, while the absolute uptake rates were very high due to the high particulate organic nitrogen concentrations.

#### *f*-ratio

The *f*-ratio is another concept that could be inappropriately applied within the context of the present study site. In the present study, the *f*-ratios averaged to 0.4 (Table 4.3). This value is close to the generalized value (0.5) reported by Eppley and Peterson (1979) in highly productive upwelling areas where there is sufficient input of new nitrogen. Therefore, it would be easy to conclude that the high *f*-ratio in the present system represents high productivity due to new nitrogen input. However, the absolute uptake rates present a different picture.

In the present study, the high nitrate concentrations in the monsoon months were due to freshwater influx. The productivity in this season was low mainly due to unfavorable environmental conditions for phytoplankton uptake. In spite of this high new nitrogen input, the *f*-ratio did not increase; the *f*-ratio in the monsoon months was low (average *f*-ratio: 0.4). However, in the post-monsoon

Table 4.3 Seasonal variation in the Relative Preference Indices (RPI) of four nutrients and f-ratio at the three stations

Months	Reference				Middle				Mouth						
	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	urea	f-ratio	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	urea	f-ratio	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	urea	f-ratio
F	0.9122	0.3974	1.7201	1.9427	0.20807	1.6131	0.2553	1.9451	1.065	0.1286	0.6163	0.7115	1.5957	1.6986	0.4038
M	0.33	0.1003	2.8604	0.535	0.05671	0.3298	0.2357	2.8879	0.9626	0.13585	0.7464	0.7578	5.0878	0.9821	0.56587
M	0.5463	0.0897	2.2437	0.6358	0.04503	0.5375	0.0984	2.2312	0.3685	0.04743	0.1732	0.5032	3.3005	3.1683	0.38295
J	0.9755	0.1085	15.562	0.4793	0.09816	10.468	0.2741	7.6673	1.9367	0.24401	2.9744	0.0578	6.706	13.717	0.05087
J	14.988	0.3867	28.258	0	0.36902	4.5401	0.3527	10.314	42.971	0.33272	0.7419	0.274	54.64	52.876	0.25761
A	4.0651	0.8408	1.588	1.6024	0.76439	1.385	0.9638	2.1902	0.1087	0.85106	1.9296	0.4666	24.855	0	0.39593
S	2.9124	0.68	1.7721	3.1211	0.54681	0.1622	1.1958	1.3781	0.5534	0.51104	1.0183	0.8133	4.0039	0.2727	0.67288
O	2.2222	0.9383	0.871	1.1907	0.74722	1.81	0.7608	1.477	1.9828	0.55383	0.0878	1.1862	0.9585	2.3535	0.80038
N	2.4644	0.8289	2.173	1.6578	0.71549	1.5886	0.6829	1.818	0.669	0.41347	0.4701	0.9698	2.3021	2.5272	0.75471
D	0.3537	0.7771	3.2753	1.1693	0.58327	0.4315	1.0088	1.4673	0.782	0.55519	0.4775	0.9979	2.0878	1.265	0.752
J	1.0676	0.6811	2.9423	1.5242	0.5489	0.4324	0.6124	2.4401	3.2603	0.43202	0.4876	1.1046	0.6432	0.9291	0.83436
F	0.1727	1.087	1.23	0.5396	0.35736	1.2454	0.6956	1.8368	0.9924	0.46373	0.7235	1.1437	0.807	0.8433	0.65701
M	0.5467	0.1607	2.3114	0.8134	0.08534	0.3196	0.101	3.2307	0.8968	0.05872	0.9633	0.3675	4.2122	2.5335	0.27664
A	0.9182	0.0677	5.2045	0.5492	0.05217	0.622	0.0875	5.4922	1.2966	0.06878	1.7226	0.3285	6.0099	3.4804	0.27049
M	2.2634	0.1236	5.084	0.6676	0.09708	1.0352	0.0769	4.887	1.3509	0.05771	1.4785	0.3191	4.604	2.9319	0.25622
J	0.4024	0.0811	21.304	0.7003	0.07534	5.6893	0.5217	8.5556	4.177	0.48002	0.3359	0.6409	1.6328	8.9162	0.57168
Average	2.1963	0.4593	6.15	1.0705	1.07051	2.0131	0.4952	3.7387	3.9609	3.96087	0.9342	0.6651	7.7154	6.1559	6.15593

season, the nitrate concentrations were low compared with other seasons and surprisingly the  $f$ -ratio was high (average: 0.6). Nevertheless, it is difficult to label this nitrate uptake as new nitrogen, since this could be the product of nitrification of the remineralized nitrogen, derived from the input of organic matter of mangrove origin. Moreover, according to Eppley and Peterson (1979), in shallow systems, the new nitrogen could also be in reduced forms, which again does not agree with the conventional interpretation of the  $f$ -ratio.

*Relative Preference Index:*

The RPI estimations may not be applicable in the present study. Paasche (1988) noted that it may or may not reflect physiologically important characteristics such as ammonium inhibition of nitrate uptake or nitrogen limitation, depending on circumstances. He suggested that it should not be used uncritically, since its numerical value is very sensitive to variations in the ambient concentrations of the respective substrates.

This inadequacy of RPI in context with the present study is discussed below. The RPI values are presented in Table 4.3. It was observed that ammonium was the most preferred nutrient and nitrate, the least preferred. It is in fact surprising to see from the values that nitrite and urea are also significantly preferred though their absolute uptake rates are lower than those of nitrate. Considering the total uptake, nitrate uptake accounted for 39%. Though nitrate stood second in the uptake rate, interestingly, the RPI values were low thus indicating a low

preference for this form (nitrate). These values do not agree with the high nitrate uptake ( $\rho\text{NO}_3^-$ : 39%). Stolte and Reigman (1996) showed that RPI may be more dependent on nutrient concentrations than on the algal preference for ammonium. For example, given the constant and equal rates of (saturated) nitrate and ammonium uptake and a low (non-inhibiting) ammonium concentration of  $0.2 \mu\text{M}$ , the RPI for ammonium can be made to increase 25-fold merely by increasing the ambient nitrate concentrations from  $0.2$  to  $10 \mu\text{M}$  (McCarthy *et al.*, 1977). This is true with the present observation where the nitrate concentrations vary widely, which thus might alter the RPI drastically. The high nitrate concentrations might have lowered the RPI value of nitrate. In this regard, the RPI values are considered with care in the present discussion.

#### *Mangrove systems and phytoplankton:*

Although there are several reports on nitrogen uptake in various environments (Table 4.4a), this aspect has not yet been addressed to in mangrove ecosystems. The highest reported rates of nitrogen uptake by coastal phytoplankton are shown in Table 4.4b. This study is the first attempt concerning nitrogen uptake by phytoplankton in a mangrove ecosystem and aims at understanding the role played by these autotrophs in the nitrogen cycle. Measurements of uptake rates of the four major nitrogenous nutrients (nitrate, nitrite, ammonium and urea) were carried out at three stations. The objectives of the uptake study were:

1. To study the seasonal and spatial variations in nutrient uptake rates.

Table 4.4a Uptake values from earlier estimations, specific uptake- $v$  ( $h^{-1}$ ) and absolute uptake- $p$  ( $ng$  at  $N l^{-1} h^{-1}$ )

Region	NO <sub>3</sub>		NH <sub>4</sub>		Urea		Reference
	V	P	V	P	V	P	
	<b>Shelf and coastal</b>						
Southern California	2.033	170.9	1.817	106	1.46	85	McCarthy, 1972
Narragansett Bay, Rhode Island	0.005	na	0.012	na	0.0045	na	Fumas, 1983
Barner Island Estuary Great south Bay	na	na	na	165	na	na	Kaufman <i>et al.</i> , 1983
Cap Blanc, NW Africa	0.075	350	0.05	390	na	na	MacIsaac <i>et al.</i> , 1985; Wilkerson <i>et al.</i> , 1987
	0.019	33	na	na	na	na	Codispoti <i>et al.</i> , 1982; Dugdale, 1985
Pt. Conception, CA	0.023	46	na	na	na	na	Wilkerson <i>et al.</i> , 1987
Baja, California	0.023	83	na	na	na	na	Wilkerson <i>et al.</i> , 1987
Laholm Bay, South eastern Kattegat, Sweden	na	17.2	na	23.2	na	17.2	Sahlsten <i>et al.</i> , 1988
Baltic Sea	na	16.1	na	44.1	na	294.1	Sahlsten and Sorenson, 1989
<b>Oligotrophic</b>							
Central North Pacific Ocean.	na	na	na	0.5	na	0.4	Eppley <i>et al.</i> , 1977

Region	NO <sub>3</sub>		NH <sub>4</sub>		Urea		Reference
	V	P	V		V	P	
Fiberling, Guyot	0.0009	0.35	-	-	-	-	Kopezak <i>et al.</i> , 1990; Wilkerson <i>et al.</i> , 1990
Mediterranean Sea	0.0029	0.58	na	na	na	na	MacIsaac and Dugdale, 1972
Sargasso Sea	0.0018	0.2	0.0052	2.6	0.004	2.1	Gilbert <i>et al.</i> , 1988
Gulf Stream	0.0005	0.1	0.0024	0.9	0.0009	0.4	Gilbert <i>et al.</i> , 1988
<i>High nutrient and low productivity</i>							
Antarctic: Scotia Sea	0.0026	2.7	0.0024	1.9	na	na	Gilbert <i>et al.</i> , 1982a
Ross Sea	0.0036	2.7	0.0025	1.9	na	na	Olson, 1980
Equatorial Pacific	0.0032	7.1	0.0034	7.3	na	na	Olson, 1980
North east Pacific	0.0022	1.8	0.0025	1.9	na	na	Wheeler and Kokkinakis, 1990
<i>Upwelling</i>	0.0047	7	0.0018	5.2	0.0137	na	Miller <i>et al.</i> , 1988
North east Pacific	na	402	na	89.7	na	na	Kokkinakis and Wheeler, 1987
Peru	na	121.5	na	129	na	na	Kokkinakis and Wheeler, 1987
Benguela Namibian	na 0.0032	278 163.5	na 0.0044	55.7 232.7	na 0.0019	na 936	Probyn, 1988
<i>Polar</i> Eastern Canadian Arctic	na	161.6	na	62.7	na	78.8	Hamison <i>et al.</i> , 1985

Table 4.4 b. Highest reported rates of nitrogen uptake by coastal phytoplankton

Area	Incubation Time (h)	Isotope enrichment	Max. rate ( $\mu\text{g at N l}^{-1} \text{h}^{-1}$ )		References
			NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> -urea	
Southern California Bight					
Offshore	24	trace	5	3	Eppley <i>et al.</i> (1979b)
Inshore	24	trace	7	31	Eppley <i>et al.</i> (1979b)
Inshore	24	trace	10	16	McCarthy (1972)
Near sewer outfall	24	trace	29	4	Eppley <i>et al.</i> (1979b)
Near sewer outfall	24	trace	9	22	McCarthy (1972)
Middle Atlantic Bight, inshore	6	trace	236	33	Harrison <i>et al.</i> (1983)
Vineyard sound, Mass.	1-2	trace	100	22	Glibert <i>et al.</i> (1982a).
Vineyard sound, Mass.	1-2	saturated	180	22	Glibert <i>et al.</i> (1982a)
North Carolina estuaries	-	-	1000	700	Fisher <i>et al.</i> (1982)
New York Bight, apex	2-8	saturated	850	480	Garside (1981)
Great South Bay, N.Y.	2-3	trace	1400	120	Kaufman <i>et al.</i> (1983)
Carmans Estuary, N.Y.	2	trace	7590	7240	Carpenter and Dunham (1985)
Narragansett Bay, R.I.					
Winter	3-9	trace	745	127	Furnas (1983)
Summer	3-9	trace	360	380	Furnas (1983)
Bedford Basin, Nova Scotia	4	trace	420	280	La Roche (1983)
Oslofjord, Norway	3-5	trace	403	265	Paasche and Kristiansen (1982)
Oslofjord, Norway	3-5	trace		229	Kristiansen (1983)
Oslofjord, Norway	3-5	saturated	840	375	Paasche and Kristiansen (unpub.)

2. To understand the dependence of primary productivity on the nutrient concentrations.

The following chapters discuss the seasonal uptake pattern of each of these N compounds.

#### 4.4.2 NITRATE UPTAKE

The importance of nitrate in marine phytoplankton production has long been recognized. It is the second most abundant form of dissolved nitrogen, next to dinitrogen ( $N_2$ ), in the sea. In spite of the abundance of  $N_2$  in the marine environment, its usefulness as a nitrogen source is restricted, since only few organisms can fix free nitrogen. Nitrate, on the other hand, supplied by terrestrial and atmospheric inputs and through vertical advection from below the euphotic zone, is rapidly taken up by phytoplankton. Consequently, uptake of nitrogen through this form represents most of the new production in many productive regions of the ocean. In a steady state, the nitrate uptake is also a proxy for export of organic matter to the deep sea (Dugdale and Goering, 1967; Eppley and Peterson, 1979).

The uptake rate of nitrate, as with most nutrients, bears a hyperbolic relationship with the substrate concentrations, in which  $V_{max}$  is the maximum rate of uptake and  $K_s$  is the half saturation constant, a substrate concentration at which uptake rate is half the  $V_{max}$ . (Dugdale, 1967; Eppley and Coatsworth, 1968; MacIsaac

and Dugdale, 1969). The two coefficients,  $V_{max}$  and  $K_s$  define the affinity of phytoplankton towards a particular nitrogenous nutrient. For example, a low  $K_s$  and high  $V_{max}$  indicates greater nutrient affinity, while a high  $K_s$  and low  $V_{max}$ , a low nutrient affinity. Eppley and Thomas (1969) demonstrated that the  $K_s$  values for nitrate and ammonium uptake were lower for oceanic than for the neritic clones and that there was a direct correlation between the cell size and the  $K_s$  value. MacIsaac and Dugdale (1969) reported  $K_s$  values of  $\geq 1\mu\text{M l}^{-1}$  for nitrate uptake by phytoplankton in eutrophic waters. The  $K_s$  values are considered to be important characteristics of organisms living in nitrogen-limited environments and hence reflects the ability of phytoplankton to utilize low levels of nutrients.

It is generally believed that the rate of nitrate uptake is reduced in the presence of ammonium. This effect has been the subject of many laboratory and field studies (Syrett and Morris, 1963; Eppley *et al.*, 1969a; Conway *et al.*, 1976; Cresswell and Syrett, 1982; Harrison *et al.*, 1987; Collos *et al.*, 1989; Dortch, 1990; Dortch *et al.*, 1991) and these suggest that  $\text{NH}_4^+$  is preferentially utilized when both forms are present in the medium. Other studies have documented the simultaneous uptake of both nitrate and ammonium (Eppley and Renger, 1974; Caperon and Ziemann, 1976; Price *et al.*, 1985; Collos *et al.*, 1989). These findings reflect the degree to which a given concentration of ammonium would suppress  $\text{NO}_3^-$  utilization. The mechanism of inhibition of nitrate uptake by ammonium is however not well understood but it could be due to the inhibition of the activity of the enzyme, nitrate reductase (which reduces  $\text{NO}_3^-$  to  $\text{NO}_2^-$ ).

Dortch (1990) divided the negative effect of ammonium on nitrate uptake as (i) preference for ammonium and (ii) inhibition of nitrate uptake by ammonium, and described a threshold ammonium concentration of 1  $\mu\text{M}$ , above which no nitrate uptake occurs. There have also been reports on inhibition of nitrate uptake by nitrite (Bilbao *et al.*, 1981).

Light is an important factor influencing nitrate uptake by phytoplankton (Martinez *et al.*, 1987; Kanda *et al.*, 1989; Berges and Harrison, 1993, 1995). Reduction in uptake rates has been reported to occur at high light intensities, mainly in oligotrophic regions. In the permanently well-mixed waters of the Western English Channel, light controlled nitrate uptake throughout the seasonal cycle (L'Helguen *et al.*, 1996). Other studies pointed out that light dependence of nitrate uptake may vary with the phytoplankton species (Conway and Whittedge, 1979). Temperature effects on nitrate uptake by phytoplankton have been studied to a small extent. Early studies found that the half saturation constant for nitrate uptake was related to temperature in *Skeletonema costatum* (Eppley *et al.*, 1969b). Higher temperatures enhanced nitrate utilization by phytoplankton populations of the East China Sea (Lee-cheng and Yuh-ling, 1994). They found that during the warm season, nitrate was the significant contributor in maintaining high surface chlorophyll-*a* concentrations.

Although it is generally accepted that phytoplankton preferentially utilize the reduced forms of nitrogen (Dugdale and Goering, 1967; McCarthy and Eppley, 1972; Eppley *et al.*, 1973; McCarthy *et al.*, 1977, 1982; Glibert *et al.*, 1982a, b; Smith and Nelson, 1990; Owens *et al.*, 1991; Rees *et al.*, 1995) even when reduced nitrogen constitutes a small fraction of the total dissolved nitrogenous pool (Glibert *et al.*, 1982a), cells with different sizes may exhibit different preferences for a nitrogen form *i.e.* small cells prefer reduced forms and larger cells, the oxidized forms of nitrogen (Malone, 1980; Glibert *et al.*, 1982b; Probyn and Painting, 1985; Koike *et al.*, 1986; Probyn *et al.*, 1990; Owens *et al.*, 1991).

In the present study, estimations of uptake rates have been carried out for the first time in mangrove-dominated estuaries. The specific nitrate uptake rates ( $v\text{NO}_3^-$ ) and absolute uptake rates ( $p\text{NO}_3^-$ ) showed significant seasonal variations at all the stations (Fig. 3.13; Fig. 3.14). The  $v\text{NO}_3^-$  ranged from  $1.25 \times 10^{-5}$  to  $593 \times 10^{-5} \text{ h}^{-1}$  which are comparatively lower than earlier estimations in shelf and coastal waters (Table 4.4a). The highest specific nitrate uptake is still two orders of magnitude lower when compared with the mean rate ( $0.005 \text{ h}^{-1}$ ) recorded at Narragansett Bay (Fumas, 1983). The absolute uptake rates in the present study ranged from 1.8 to  $385.1 \text{ ng at N l}^{-1}\text{h}^{-1}$  and were more or less comparable to those obtained in shelf and coastal waters (MacIsaac *et al.*, 1985; Wilkerson *et al.*, 1987; Fumas, 1983) (Tables 4.4a,b). Both  $v\text{NO}_3^-$  and  $p\text{NO}_3^-$  showed a pronounced peak in the post-monsoon months while, in the pre-monsoon months, the uptake rates were moderate. Lowest uptake was recorded

in the monsoon months. There were differences between  $v\text{NO}_3^-$  and  $\rho\text{NO}_3^-$  with respect to spatial variations -  $\rho\text{NO}_3^-$  showed significant spatial variations whereas this was not true in the case of  $v\text{NO}_3^-$ .

Both  $v\text{NO}_3^-$  and  $\rho\text{NO}_3^-$  showed insignificant negative correlations with ambient nitrate concentrations which suggest that nitrate uptake is not substrate limited. The insignificant correlation was due to the high availability of nitrate in the dissolved pool (annual average:  $4.5 \mu\text{g at N l}^{-1}$ ) where nitrate accounted for 72% of the total nitrogen estimated.

The autotrophic biomass plays an important role in influencing the uptake rates. In the present study, chlorophyll-*a* and  $v\text{NO}_3^-$  showed a significant positive correlation ( $0.53$ ,  $P < 0.001$ ,  $n = 48$ ) (Fig. 4.26) and a positive relation with  $\rho\text{NO}_3^-$ . These positive correlations suggest that the uptake rates are significantly dependent on the autotrophic biomass rather than the ambient levels of nitrate.

The seasonal variations in the uptake rates can be explained taking the above correlations into consideration. In the post-monsoon months, of all the nutrients, nitrate is taken up significantly (average  $v\text{NO}_3^- = 0.004 \text{ h}^{-1}$ ; average  $\rho\text{NO}_3^- = 192.9 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and contributes ~62% to the total nutrient uptake rate ( $\Sigma v\text{N} = 0.0067 \text{ h}^{-1}$ ). This is reflected in the pronounced peak in chlorophyll-*a* concentrations during this period (Fig. 3.7). The high chlorophyll-*a* concentrations and high nitrate uptake rates suggest that phytoplankton utilized

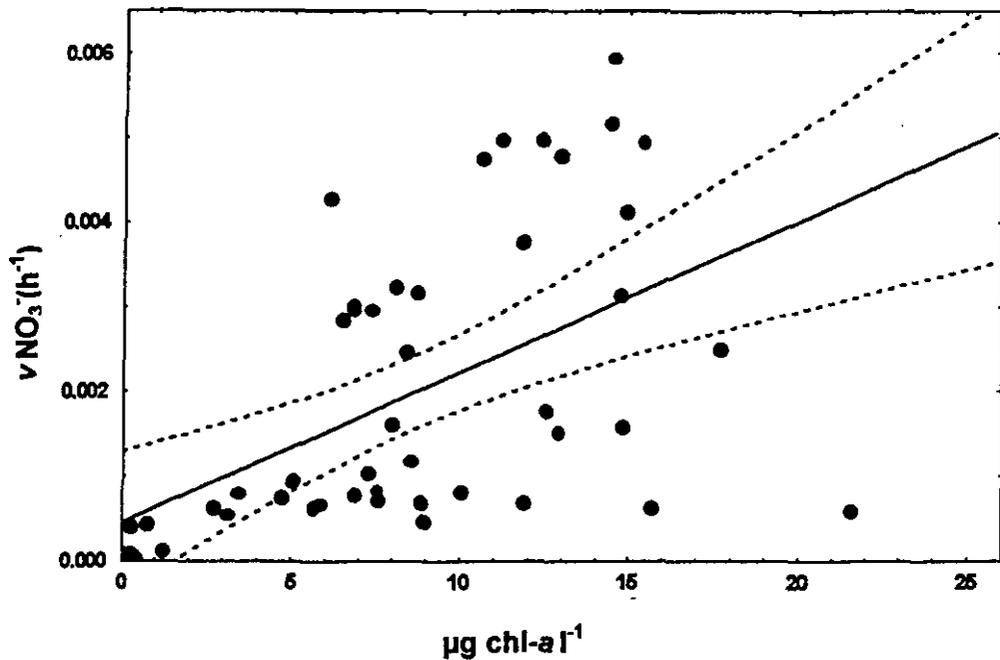


Fig. 4.26 Relation of  $v\text{NO}_3^-$  with chlorophyll-a concentrations  
 $v\text{NO}_3^- = 0.00045 + (0.000018 * \text{chl-a})$  ( $r=0.53, n=48$ )

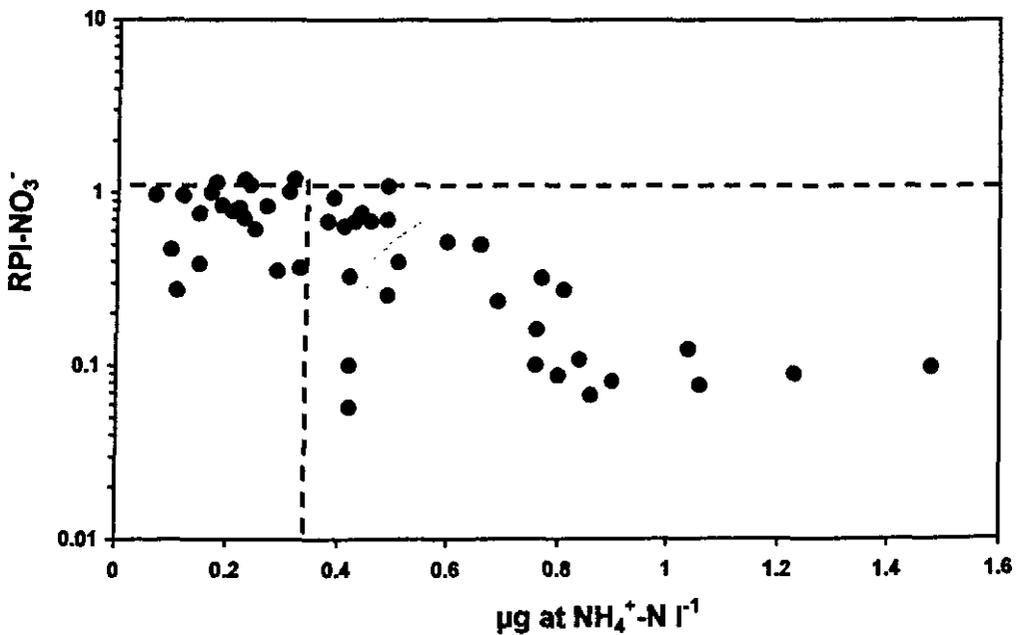


Fig. 4.27 Relation of  $\text{RPI-NO}_3^-$  with ammonium concentrations

nitrate as the major source of N during this period. This could be due to the low concentrations of other nutrients ( $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and urea) and high availability of nitrate in this season. As the ammonium concentrations are low or not adequately available in these months ( $0.3 \mu\text{g at N l}^{-1}$ ), the rapidly growing phytoplankton community must have been utilizing nitrate to sustain the high primary productivity.

However, in the pre-monsoon months, the nitrate uptake decreased by ~69% (post-monsoon:  $0.004 \mu\text{g N l}^{-1} \text{ h}^{-1}$  ▶ pre-monsoon:  $0.001 \mu\text{g N l}^{-1} \text{ h}^{-1}$ ). Interestingly, the chlorophyll-a concentrations did not change significantly (post-monsoon:  $10.2 \mu\text{g chl-a l}^{-1}$  ▶ pre-monsoon:  $9.9 \mu\text{g chl-a l}^{-1}$ ) and nitrate concentrations increased from  $1.9 \mu\text{g at N l}^{-1}$  ▶  $2.2 \mu\text{g at N l}^{-1}$ . The variations in the autotrophic population was also responsible for the changes in the uptake rates. In the present study, a seasonal succession of phytoplankton species and change in the cell size structure was observed (Fig. 3.9a-c and Fig. 3.8). The phytoplankton community changed from microphytoplankton in the post-monsoon months to nanophytoplankton species in the pre-monsoon months.

The microphytoplankton appeared to be the major group utilizing nitrate efficiently in the post-monsoon months. In this period, the microphytoplankton dominated the total biomass (81%) and contributed 98% (average  $v\text{NO}_3^- = 0.006 \text{ h}^{-1}$ , average  $\rho\text{NO}_3^- = 24.3 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) to the total nitrate uptake whereas, in the pre-monsoon months, the microphytoplankton community was replaced by

the nanophytoplankton, which contributed 66% to the total chlorophyll-*a* and utilized nitrate at lower rates (average  $v\text{NO}_3^- = 8.6 \times 10^{-5} \text{ h}^{-1}$ , average  $\rho\text{NO}_3^- = 0.3 \text{ } \mu\text{g at N l}^{-1} \text{ h}^{-1}$ ). Therefore, it appears that the microphytoplankton utilize nitrate at a greater extent than the nanophytoplankton. Therefore, the change in the taxonomic composition and cell size structure reduces the nitrate uptake significantly in the pre-monsoon months. Numerous studies have shown that new production is associated with the larger phytoplankton and regenerated production with small phytoplankton (Glibert, 1982; Probyn, 1985) and thus support the above conclusion.

It is interesting to note the significant negative correlation of nitrate uptake rates with ammonium concentrations ( $r=-0.39$ ,  $P<0.05$ ,  $n=48$ ). This negative correlation suggests that increase in ammonium concentrations inhibits nitrate uptake. Fig. 4.27 clearly demonstrates that ammonium concentrations  $>0.5 \text{ } \mu\text{g at N l}^{-1}$  significantly affect nitrate uptake rates. In the post-monsoon months, the ammonium concentrations were  $\sim 0.3 \text{ } \mu\text{g at N l}^{-1}$ . These were probably not sufficient to satisfy the nitrogen demand for the rapidly growing phytoplankton community and therefore nitrate was predominately utilized. In the pre-monsoon months, the ammonium concentrations increased from  $0.3 \text{ } \mu\text{g at N l}^{-1}$  to  $>0.5 \text{ } \mu\text{g at N l}^{-1}$ , which significantly reduced the nitrate uptake rates. It thus becomes evident that the increase in ammonium concentrations significantly inhibited nitrate uptake. Several studies report the inhibition of nitrate uptake by ammonium (Conway,

1977; McCarthy and Eppley, 1972; McCarthy *et al.*, 1977; Serra *et al.*, 1978; Dortch, 1990; L'Helguen *et al.*, 1993).

In the monsoon months, nitrate uptake rates were low. During this period, utilization of nitrate and ammonium was negligible. This suggests that ammonium concentrations may not be inhibitory, as ammonium uptake also reduced significantly. This was probably due to the changes in the climatic and hydrological conditions. The low chlorophyll-*a* concentrations during this period ( $4.2 \mu\text{g chl-}a \text{ l}^{-1}$ ), and the combined effect of low light intensity ( $443.3 \mu\text{E s}^{-1}\text{M}^{-2}$ ) and high water current may not have been favourable conditions to initiate uptake. This ultimately resulted in the significant decrease in nitrate uptake rates during this period.

The significant spatial variations in the  $\rho\text{NO}_3^-$ , but not  $v\text{NO}_3^-$ , can be explained by the variations in the PON concentrations at the three stations. Since absolute uptake rate is derived by multiplying the specific uptake rate and PON, and if there happens to be too much PON not associated with chlorophyll-*a*, then  $\rho\text{NO}_3^-$  uptake would be high, although the actual uptake by the phytoplankton may not be that much significant. Hence, the variations in the PON between the three stations leads to the significant spatial variations in the  $\rho\text{NO}_3^-$  rates. The insignificant spatial variations in the specific uptake rates are mainly due the even distribution of nitrate and chlorophyll-*a*/phytoplankton biomass at the three stations.

In conclusion :

1. Nitrate uptake was not substrate dependent
2. Nitrate uptake rates were significantly dependent on the phytoplankton biomass -. the change in the taxonomic composition and cell size structure were responsible for the seasonal variations in the uptake rates.
3. In the post-monsoon months, the microphytoplankton mainly utilized nitrate, while in the pre-monsoon months, the nanophytoplankton efficiently took up ammonium.
4. High ammonium concentrations inhibit nitrate uptake rates and hence the variations in the ammonium concentrations lead to the variations in the nitrate uptake rates.

#### 4.4.3 NITRITE UPTAKE

One of the earliest studies on nitrite uptake was that of Harris (1959). He showed that nitrite was a more important source of nitrogen than nitrate for certain species of marine phytoplankton, especially flagellates such as *Peridinium*. However, studies on nitrite uptake are still few, thanks mainly to its low concentrations in most surface waters. Table 4.5 however shows that the role of nitrite in phytoplankton nutrition should not be neglected. It has been shown that uptake of nitrite usually occurs when nitrate concentrations are close to exhaustion (Collos, 1998). Collos (1998) studied nitrite uptake following nitrite excretion by laboratory cultures of marine microalgae. He expressed nitrite

Table 4.5 Nitrate and nitrite uptake by natural populations of marine phytoplankton. *U*: uptake; L: light; D: dark;  $I_0$ : surface irradiance

Study area	$U_{NO_2}/U_{NO_3}$	Conditions	Source
Chesapeake Bay	0.01-8.0	Near surface, L	McCarthy <i>et al.</i> (1977)
	0.2-1.0	Near surface, L	McCarthy <i>et al.</i> (1984)
	0.15-3.3	Near surface, L + D	Gilbert & Garside (1992)
	1.0-2.0	Near surface, L + D	Bronk & Gilbert (1993)
	0.12-0.53	Surface, L	Gilbert <i>et al.</i> (1995)
NW Africa	0.1-2.0	NO <sub>3</sub> added	Harrison & Davis (1977)
Tropical Atlantic	0.6	11h D	Slawyk & Collos (1982)
	3.5	6h L	Slawyk & Collos (1982)
North Pacific	0.13-0.30	24 h profiles	Olson (1981)
	0.5-10	12 h L profiles	Hattori & Wada (1972)
	0.5-2.4	12 h D profiles	Hattori & Wada (1972)
Coastal	1.9	5 m, 5.5 h L	Miyazaki <i>et al.</i> (1973)
Equator. Pacific	0.05-0.16	Mixed layer	Price <i>et al.</i> (1991)
	0.05-0.85	El Nino	McCarthy <i>et al.</i> (1996)
	0.14-1.79	Post El Nino	McCarthy <i>et al.</i> (1996)
S. California	0.01-08	24 h profiles	Olson (1981)
Scotia Sea	0.004-0.01	24 h profiles	Olson (1981)
	0.09-0.38	Surface, L	Olson <i>et al.</i> (1980)
	0.02-0.28	10% $I_0$ , L	Olson <i>et al.</i> (1980)

uptake as a percentage of nitrate uptake, and suggested that nitrite uptake rates can reach higher values than nitrate uptake (up to 10 times). Nitrite and Nitrate uptake can be considered as being competitive processes, as has been shown for laboratory cultures of phytoplankton (Eppley and Coatsworth, 1968; Olson *et al.*, 1980; Bilbao *et al.*, 1981). The results of Olson (1981) showed that the potential for nitrite uptake in the upper waters was generally greater than the total nitrite production, while at the depth of the nitrite maximum, uptake was less than 5% of the total nitrite produced. Thus, nitrite accumulation at this depth was unlikely to be much affected by phytoplankton uptake.

Throughout the study period, nitrite uptake rates were consistently lower than those of nitrate, ammonium and urea. On an average, nitrite uptake accounted for only about 4% of the total N uptake, indicating clearly that it is not a preferred nutrient source. This agrees with the general pattern found in other marine waters (McCarthy *et al.*, 1977; Slawyk and Collos, 1982; Pennock, 1987; L'Helguen *et al.*, 1996).

In the field conditions (with natural populations of marine phytoplankton), the presence of bacteria can also influence the uptake rates, as bacteria may take up nitrite (Hattori and Wada, 1972; Olson, 1981; Slawyk and Collos, 1977; Price *et al.*, 1991). Hattori and Wada (1972), in the central northern North Pacific, estimated that bacterial processes contributed to a negligible portion of the observed nitrite uptake. Data from Olson (1981) for the Scotia Sea, Slawyk and

Collos (1977) for the tropical Atlantic and Price *et al.* (1991) for the Equatorial Pacific indicate a significant relationship between nitrite uptake and chlorophyll-*a*. Nevertheless, these studies did not preclude uptake by bacteria although it was mainly by phytoplankton.

So far, there have been very few reports on uptake rates of nitrite from estuarine and mangrove waters and the low attention paid for this may be due to the very low nitrite concentrations in these waters.

The seasonal variations in the  $v\text{NO}_2^-$  and  $\rho\text{NO}_2^-$  in the present study were well marked and were similar to those of  $v\text{NO}_3^-$  and  $\rho\text{NO}_3^-$  respectively. The  $v\text{NO}_2^-$  was 10 times lower than the  $v\text{NO}_3^-$  and  $\rho\text{NO}_2^-$  was 6 times lower than the  $\rho\text{NO}_3^-$ . But as was the case with nitrate, nitrite uptake rates also did not show any spatial variations. The maximum  $v\text{NO}_2^-$  was recorded in the post-monsoon months (average  $v\text{NO}_2^- = 0.0004 \text{ h}^{-1}$ ), followed by the pre-monsoon ( $0.0002 \text{ h}^{-1}$ ) and the lowest  $v\text{NO}_2^-$  was in the monsoon months ( $0.0001 \text{ h}^{-1}$ ). The  $\rho\text{NO}_2^-$  also showed maximum values in the post-monsoon months ( $20.9 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). This was followed by the monsoon ( $17.1 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and the lowest rates were recorded in the pre-monsoon months ( $10.6 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ).

The significant negative correlation of  $v\text{NO}_2^-$  with nitrite concentrations ( $r = -0.30$ ,  $P < 0.04$ ,  $n = 48$ ) suggests that the uptake of nitrite is not substrate-dependent (Fig. 4.28). However, from this negative correlation, it appears that at low

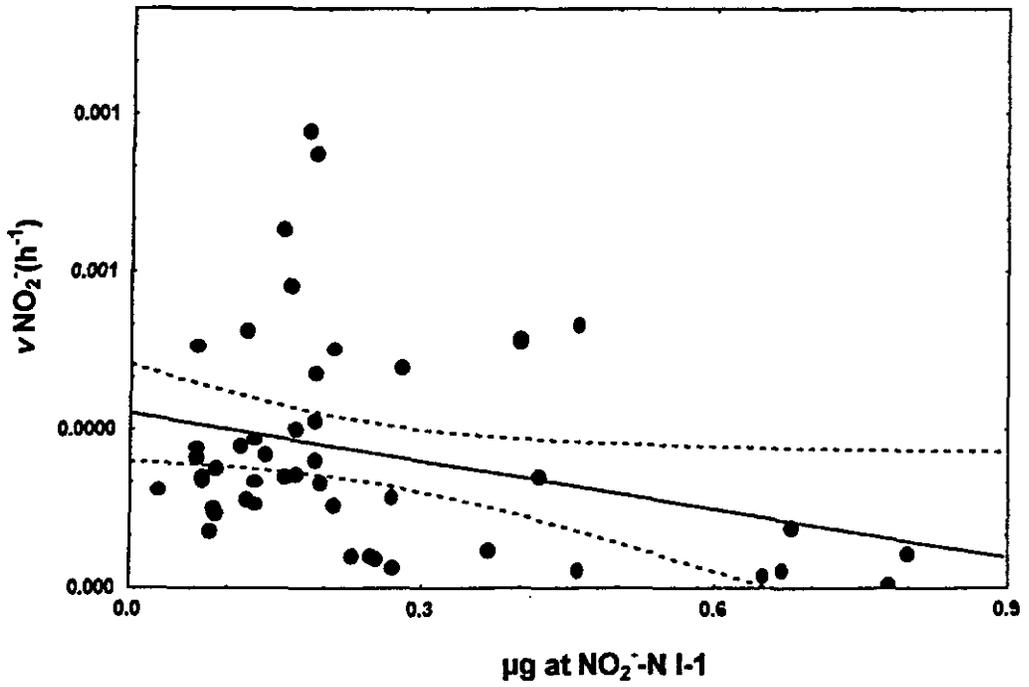


Fig. 4.28 Relation of  $v\text{NO}_2^-$  with nitrite concentrations

$$v\text{NO}_2^- = 0.00033 - (0.0003 * \text{nitrite concentrations}) \quad (r = -0.29, n = 48)$$

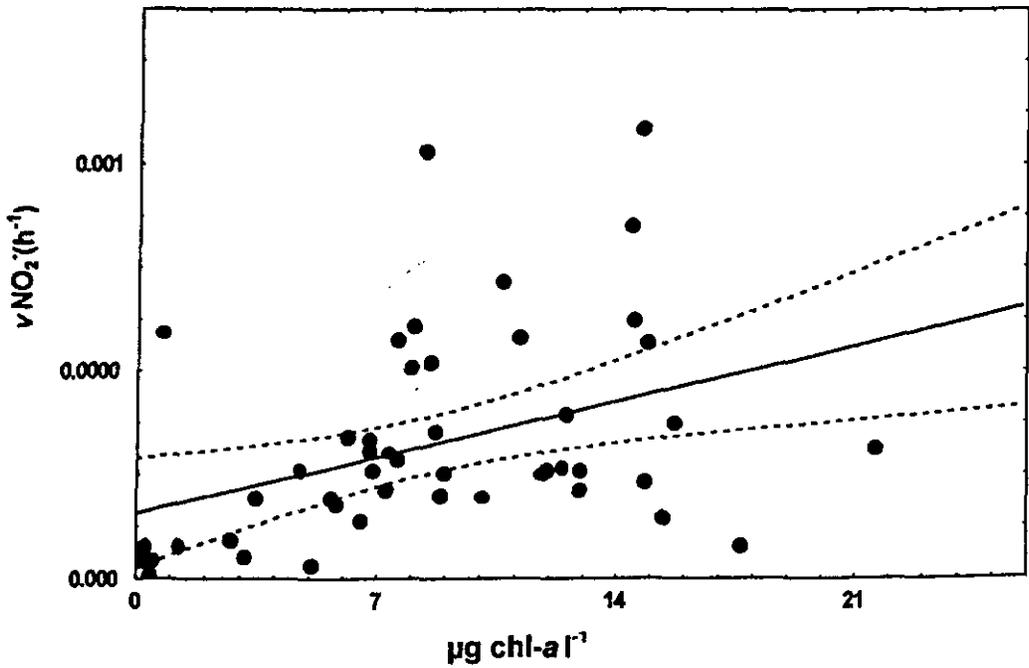


Fig. 4.29 Relation of  $v\text{NO}_2^-$  with chlorophyll-a concentrations

$$v\text{NO}_2^- = 0.00013 + (0.0002 * \text{chl-a}) \quad (r = 0.40, p = 48)$$

ambient nitrite concentrations, nitrite uptake rates were accelerated. This could be because of low concentrations of nitrogen nutrients and high demand of N by phytoplankton and will be discussed in detail (see below).

Interestingly, as that of nitrate uptake rates, the correlation between nitrite uptake rates and chlorophyll-*a* concentrations also shows a significant positive trend ( $r=0.40$ ,  $P=0.005$ ,  $n=48$ ) suggesting that nitrite uptake rates are dependent on the phytoplankton biomass (Fig. 4.29). In the post-monsoon months, the maximum  $\nu\text{NO}_2^-$  was related to the high phytoplankton biomass ( $10.2 \mu\text{g chl-}a \text{ l}^{-1}$ ) and the nitrite uptake pattern coincided with chlorophyll-*a* concentrations in this period. As the total N ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and urea) estimated in the dissolved pool during this period was relatively low ( $\Sigma\text{N}=2.5 \mu\text{g at N l}^{-1}$ ) compared to the pre-monsoon and monsoon seasons, (pre-monsoon:  $\Sigma\text{N}= 3.2 \mu\text{g at N l}^{-1}$  and monsoon:  $\Sigma\text{N}=10.1 \mu\text{g at N l}^{-1}$ ) seasons, nitrite was efficiently taken up in the post-monsoon months. This could have been due to the high demand of nitrogen by the rapidly growing phytoplankton community and the low availability of nutrients during this period. Therefore, to fulfil the N demand, the phytoplankton simultaneously utilized nitrite along with the other forms of nitrogen. Collos (1998) studied nitrite uptake during nitrate uptake in cultures as well as in natural populations, and found that nitrite uptake can equal or exceed nitrate uptake in several phytoplankton species. He attributed the high nitrite uptake rates to exhaustion of nitrate. Some studies have shown that at high N demand, when the ambient nitrogen concentrations are low, nitrate and nitrite

are significantly utilized by phytoplankton (Eppley and Coatsworth, 1968; Olson *et al.*, 1980; Bilbao *et al.*, 1981).

Comparing the post- and pre-monsoon seasons, in the pre-monsoon months, on an average, the chlorophyll-*a* concentrations did not change remarkably from the post-monsoon values (post-monsoon: 10.2  $\mu\text{g chl-a l}^{-1}$ ; pre-monsoon: 9.9  $\mu\text{g chl-a l}^{-1}$ ). Also, during these months, a significant decrease in the nitrite uptake rates were observed. This was due to the change in the phytoplankton taxonomic composition and cell size structure. Very few studies have been carried out on nitrite uptake in relation with species composition - Olson *et al.* (1980) on *Thalassiosira pseudonana*, Cresswell and Syrett (1982) on *Phaeodactylum tricornutum* and Eppley and Coatsworth (1968) on *Ditylum brightwelli*. These studies suggest that nitrite uptake by phytoplankton depended on the species composition. Fig. (3.9a-c) and Fig. (3.8) demonstrate succession of species with change in the cell size structure. The microphytoplankton (200-20  $\mu\text{m}$ ) dominated the population in the post-monsoon months (contributed 81% to the total chlorophyll-*a*) and accounted for 86% of the total nitrite uptake during this period (average  $v\text{NO}_2^- = 0.0007 \text{ h}^{-1}$ ; average  $\rho\text{NO}_2^- = 2.2 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). In the pre-monsoon months, the nanophytoplankton fraction was the major autotrophic form (66%), but accounted for only 26% of the nitrite uptake in this season (average  $v\text{NO}_2^- = 2 \times 10^{-5} \text{ h}^{-1}$ ; average  $\rho\text{NO}_2^- = 0.05 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). This decrease in the nitrite uptake rates from the post-monsoon to the pre-monsoon was probably due to the low preference of nitrite by the nanophytoplankton.

Therefore, it appears that the seasonal changes in the phytoplankton population and taxonomic composition (microphytoplankton → nanophytoplankton) significantly influenced the nitrite uptake pattern.

Interestingly, nitrite uptake rates showed negative correlations with nitrate concentrations ( $r=-0.38$ ,  $P<0.07$ ,  $n=48$ ) and ammonium concentrations ( $r=-0.06$ ,  $P<0.7$ ,  $n=48$ ). These relationships suggest that the increase in concentrations of nitrate and ammonium significantly inhibited the nitrite uptake rates. Studies of Cresswell and Syrett (1982) showed that nitrite uptake by the diatom *Phaeodactylum tricornutum* was inhibited by ammonium and nitrate. In the pre-monsoon months, on an average, high ammonium ( $0.7 \mu\text{g at N l}^{-1}$ ) and nitrate ( $2.2 \mu\text{g at N l}^{-1}$ ) concentrations were recorded compared with the post-monsoon months and this could have resulted in the lower nitrite uptake rates in the pre-monsoon months.

In the monsoon months, the  $\nu\text{NO}_2^-$  was very low. Although, nitrate and ammonium concentrations were high in these months, these did not appear to inhibit nitrite uptake as the total N uptake during this period was very low (monsoon: average  $\Sigma\nu\text{N}=0.002 \text{ h}^{-1}$ , pre-monsoon: average  $\Sigma\nu\text{N}= 0.008 \text{ h}^{-1}$ ; post-monsoon: average  $\Sigma\nu\text{N}= 0.007 \text{ h}^{-1}$ ). Hence, it appears that as with the case of nitrate uptake, nitrite uptake was also significantly influenced by the change in the climatic and hydrological conditions and low phytoplankton biomass in the monsoon months.

In the present study, spatial variations in the  $\nu\text{NO}_2^-$  were not significant. This was due to the lack of variations in the nitrite concentrations and the species composition at the three stations. The significant spatial variations in the  $\rho\text{NO}_2^-$  can be attributed to the significant spatial variations in the PON concentrations.

From the above discussion, the following conclusions can be drawn:

1. Nitrite uptake is not substrate limited.
2. Uptake of nitrite is dependent on the phytoplankton biomass, the taxonomic composition and cell size structure. The change in the species composition was responsible for the seasonal variations in the nitrite uptake rates.
3. The nitrate and ammonium concentrations significantly inhibit the nitrite uptake.
4. The changes in the climatic conditions in the monsoon months are responsible for the low nitrite uptake rates.

#### 4.4.4 AMMONIUM UPTAKE

The role of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the physiological ecology of phytoplankton has received considerable attention during the past decades. It has been well established that natural phytoplankton typically prefer the more reduced forms of nitrogenous nutrients (e.g. McCarthy *et al.*, 1977, 1982; Glibert *et al.*, 1982a,b; Glibert and McCarthy, 1984, Smith and Nelson, 1990; Owens *et al.*, 1991; Rees

*et al.*, 1995) and in fact,  $\text{NH}_4^+$  is the form of nitrogen most commonly used by phytoplankton in marine waters (Dugdale and Goering, 1967; Eppley and Peterson, 1979). These generalizations are valid even when reduced nitrogen constitutes only a small fraction of the total nitrogenous pool (Glibert *et al.*, 1982a, b). The importance of  $\text{NH}_4^+$  as a nutrient is especially great in the euphotic zone of oligotrophic waters where most of the primary production is based on the regeneration of nutrients. Although  $\text{NH}_4^+$  may represent < 1% of the total dissolved nitrogenous nutrient pool, it can account for 44-89% of the total N uptake and can suppress the assimilation of nitrate (Wheeler *et al.*, 1989). The simultaneous utilization of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  occurs, however, when insufficient  $\text{NH}_4^+$  is available to meet the entire nitrogen requirement of the phytoplankton (Price *et al.*, 1985; Collos *et al.*, 1989).

In the present study, ammonium was the most preferred nutrient for phytoplankton growth and accounted for 60% of the total annual uptake. This is consistent with the findings of the last two decades that phytoplankton, both in culture and as natural communities, generally prefer ammonium than other N nutrients (Dortch, 1990). Three other features in the seasonal changes of ammonium uptake are unique to this ecosystem: (i) phytoplankton growth is dependent on the availability of ammonium, (ii) uptake rates vary with the change in the species composition and (iii) the high proportion of ammonium uptake is related to an intense regeneration in the mangrove zone.

The observed specific uptake rates ( $3.6 \times 10^{-5}$  to  $0.014 \text{ h}^{-1}$ ) agree with other studies in the water column (Furnas, 1983; MacIsaac *et al.*, 1985; Wilkerson *et al.*, 1987) (Table 4.4 a). The very high  $\rho\text{NH}_4^+$  (3.8 to  $1188.4 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ) recorded here are comparable to those of coastal waters (Kaufman *et al.*, 1983) (Table 4.4 b).

The seasonal and spatial variations in the  $v\text{NH}_4^+$  and  $\rho\text{NH}_4^+$  were distinct (Fig. 3.17; Fig. 3.18). Maximum  $v\text{NH}_4^+$  was recorded in the pre-monsoon months (average  $v\text{NH}_4^+=0.006 \text{ h}^{-1}$ ), followed by the post-monsoon (average  $v\text{NH}_4^+=0.0014 \text{ h}^{-1}$ ) and monsoon (average  $v\text{NH}_4^+=0.0011 \text{ h}^{-1}$ ) seasons. However,  $\rho\text{NH}_4^+$  showed a slightly different pattern *i.e.* maximum uptake rates were recorded in the pre-monsoon season (average  $\rho\text{NH}_4^+ = 354.4 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ) and moderately high rates in the monsoon season (average  $\rho\text{NH}_4^+ = 111.6 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ). The lowest uptake was in the post- monsoon months (average  $\rho\text{NH}_4^+ = 77.7 \text{ ng at N } \Gamma^1 \text{ h}^{-1}$ ).

The strong positive correlations of  $v\text{NH}_4^+$  and  $\rho\text{NH}_4^+$  with ambient ammonium concentrations ( $v\text{NH}_4^+$  Vs. ambient  $\text{NH}_4^+$ : $r=0.81$ ,  $P<0.001$ ,  $n=48$  and  $\rho\text{NH}_4^+$  Vs. ambient  $\text{NH}_4^+$ : $r=0.67$ ,  $P<0.001$ ,  $n=48$ ) suggest that ammonium uptake is significantly dependent on substrate availability (Figs. 4.30a, b). Taking these correlations into consideration, the seasonal variations in the uptake rates can be explained. In the non-monsoon months (post-  $\blacktriangleright$  pre-monsoon months), the ammonium uptake rates increased gradually ( $v\text{NH}_4^+$ :  $0.0014 \blacktriangleright 0.006 \text{ h}^{-1}$ ). This

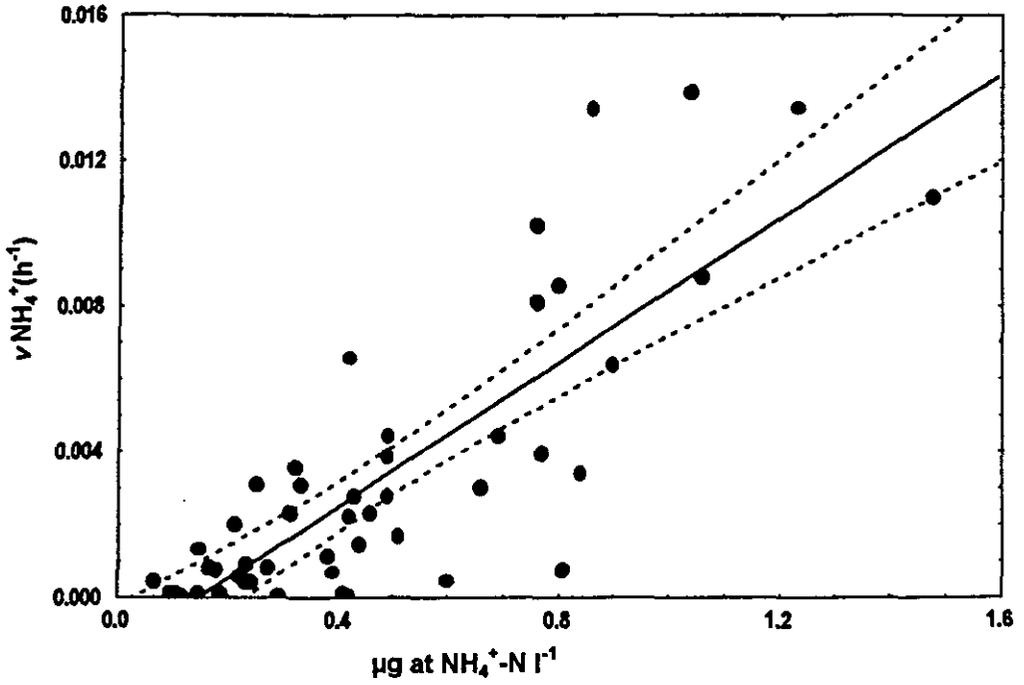


Fig. 4.30a Relation of  $v\text{NH}_4^+$  with ammonium concentrations  
 $v\text{NH}_4^+ = -0.0014 + (0.0098 * \text{ammonium concentrations})$  ( $r=0.82$ ,  $n=48$ )

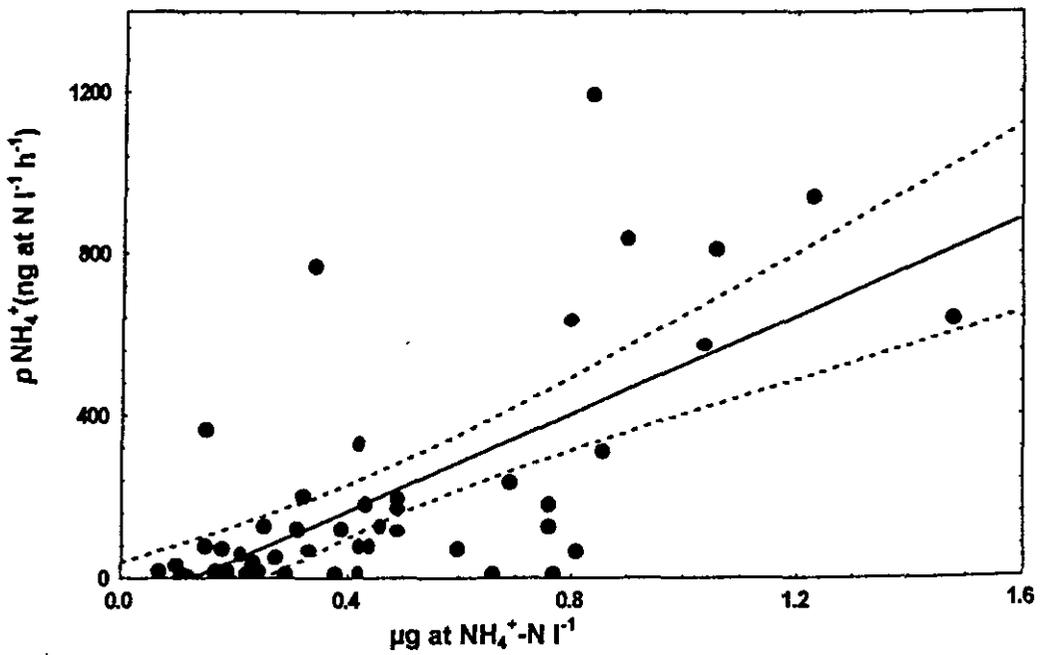


Fig. 4.30b Relation of  $p\text{NH}_4^+$  with ammonium concentrations  
 $p\text{NH}_4^+ = -75.29 + (600.8 * \text{ammonium concentrations})$  ( $r=0.67$ ,  $n=48$ )

ammonium concentrations (Fig. 4.30a,b). Therefore, it appears that in the non-monsoon months, the ammonium uptake rates are significantly dependent on the availability of ammonium. However, in the monsoon months, though the ammonium concentrations are sufficiently high ( $0.4 \mu\text{g at N l}^{-1}$ ) and therefore not a limiting factor, the  $v\text{NH}_4^+$  still shows a sharp fall (pre-monsoon:  $0.006 \text{ h}^{-1}$   $\rightarrow$  monsoon  $0.0011 \text{ h}^{-1}$ ). The sudden change in the climatic and hydrological conditions could have reduced the uptake rates significantly. Due to the southwest monsoon, freshwater advection into the estuary resulted in low salinity, low phytoplankton biomass, and low light intensity which adversely affected the ammonium uptake rates.

The significant positive correlation of  $v\text{NH}_4^+$  and chlorophyll-a ( $r=0.32$ ,  $P<0.03$ ,  $n=48$ ) (Fig. 4.31) suggests that chlorophyll-a also significantly influences the uptake rates. The shift from nitrate uptake in the post-monsoon months to ammonium uptake in the pre-monsoon months can be explained more clearly by the size-fractionated uptake study. It was observed that the nanophytoplankton efficiently utilized ammonium irrespective of the season, whereas, the microphytoplankton utilized ammonium only in the pre-monsoon season. In the present study, significant seasonal changes in the taxonomic composition and cell size structure were recorded (Fig. 3.8; Fig. 3.9a-c). In the post-monsoon months, the microphytoplankton dominated the phytoplankton biomass (81%), but their rate of ammonium utilization (average  $v\text{NH}_4^+=0.0001 \text{ h}^{-1}$ ; average  $\rho\text{NH}_4^+=0.61 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) was lower than the nanophytoplankton (average

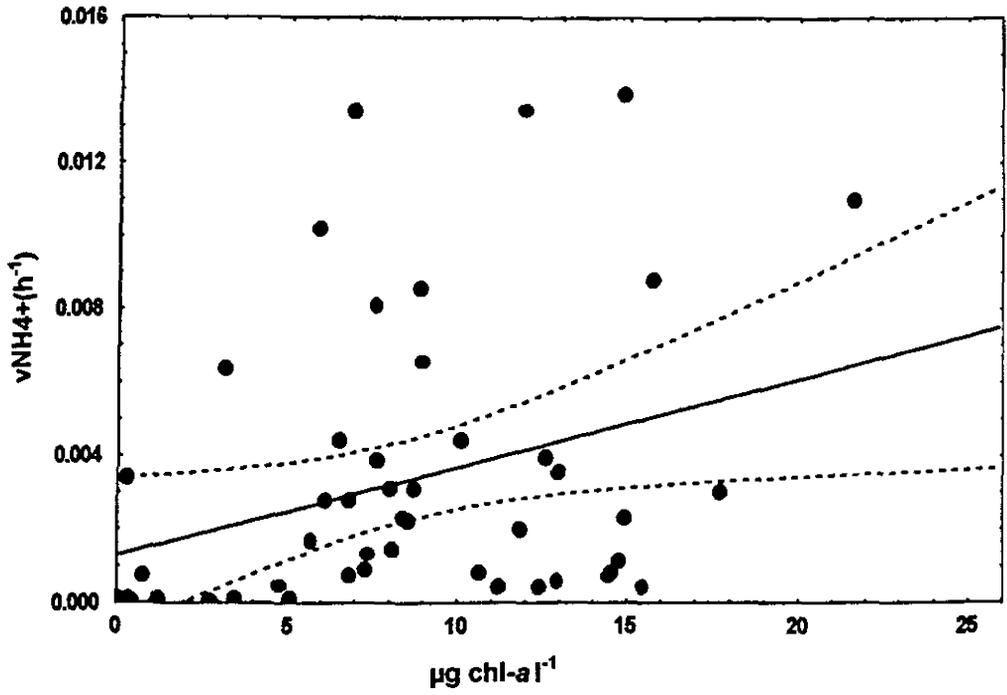


Fig. 4.31 Relation of  $vNH_4^+$  with chlorophyll-a concentrations  
 $vNH_4^+ = 0.00128 + (0.00024 * chl-a)$  ( $r = 0.32$ ,  $n = 48$ )

$v\text{NH}_4^+ = 0.003 \text{ h}^{-1}$ ; average  $p\text{NH}_4^+ = 14.1 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). In the pre-monsoon months, the community changed to nanophytoplankton which contributed 63% to the total chlorophyll-*a* and accounted for ~75% of the total ammonium uptake (average  $v\text{NH}_4^+ = 0.011 \text{ h}^{-1}$ ; average  $p\text{NH}_4^+ = 45 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). Therefore, it was recognized that the change in the species composition from microphytoplankton to nanophytoplankton remarkably increased the ammonium uptake rates: the nanophytoplankton population relied heavily on ammonium and hence, increase in the nanophytoplankton biomass could have been responsible for the high ammonium uptake rates in the pre-monsoon months. Numerous studies have shown that this group preferentially utilize ammonium (Malone, 1980; Glibert *et al.*, 1982a; Furnas, 1983; Probyn, 1985; Probyn *et al.*, 1990). For example, in seasonal studies, the specific ammonium uptake rates by phytoplankton in coastal waters of Vineyard Sound were largely by the nanophytoplankton population (Glibert *et al.*, 1982a). Similarly, in the Narragansett Bay, Furnas (1983) reported highest uptake by the nanophytoplankton fraction.

It is also interesting to note the relationship of ammonium uptake with ammonification. The very strong correlation of ammonium uptake rates with ammonification rates in the water column ( $r=0.82$ ,  $P<0.001$ ,  $n=48$ ) suggests that a high proportion of ammonium uptake is related to an intense regeneration in this ecosystem (Fig. 4.32 a,b). However, their rates are not always in balance, leaving a surplus of ammonium. Further, sediment ammonium regeneration rates (Dham, 2000) showed a strong relation with ammonium uptake in the

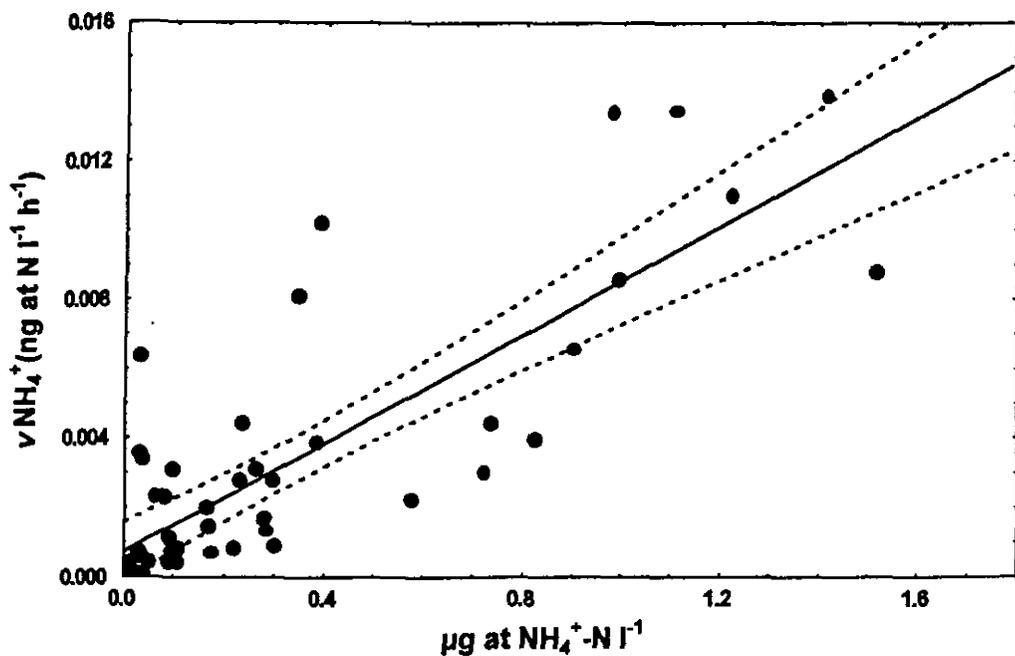


Fig. 4.32a Relation of  $v\text{NH}_4^+$  with ammonification rates

$$v\text{NH}_4^+ = 0.00071 + (0.00078 * \text{ammonification rates}) \quad (r=0.82, n=48)$$

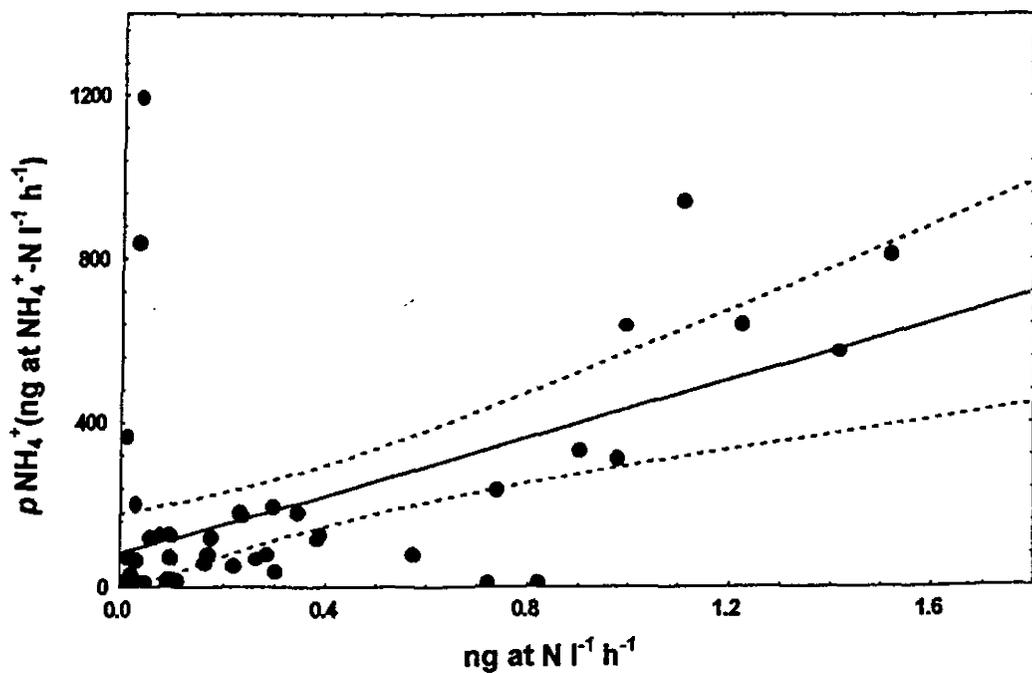


Fig. 4.32b Relation of  $p\text{NH}_4^+$  with ammonification rates

$$p\text{NH}_4^+ = 81.317 + (352.55 * \text{ammonification rates}) \quad (r=0.51, n=48)$$

water column ( $r=0.90$ ;  $P<0.001$ ,  $n=45$ ). It has been shown that benthic nutrient regeneration in shallow coastal estuarine systems is an important source of nitrogen made available to primary producers in the overlying waters (Blackburn and Henriksen, 1983; Billen and Lancelot, 1988).

The distinct spatial variations in the uptake rates could be explained by the variations in the ammonium concentrations, regeneration rates and the efflux of ammonium from the sediments at the three different stations.

In conclusion,

1. Ammonium uptake is substrate dependent and the change in the ammonium concentrations leads to variations in ammonium uptake rates.
2. The ammonium uptake rates are closely linked with phytoplankton biomass. The change in the phytoplankton community structure and cell size composition significantly influences the ammonium uptake rates.
3. The maximum uptake in the pre-monsoon months is due to the combined effect of high ammonium concentrations and high abundance of nanophytoplankton.
4. The change in the climatic and hydrological conditions and the low phytoplankton biomass in the monsoon months reduced the ammonium uptake rates.

5. The variations in the uptake rates are closely coupled with ammonium production rates (ammonification).
6. Spatial variations in the uptake rates are mainly due to the variations in the ammonium concentrations at the three different stations.

#### 4.4.5 UREA UPTAKE

Since the pioneering demonstration by McCarthy (1972) using  $^{15}\text{N}$  isotopes, urea has been recognized as an important nitrogen source for phytoplankton in coastal and oceanic waters (McCarthy *et al.*, 1977; Furnas, 1983; Kaufman *et al.*, 1983; Price and Harrison, 1988; Tamminen and Irmisch, 1996). McCarthy *et al.* (1977) estimated that urea contributed to about 20% of the total nitrogen uptake by phytoplankton in the Chesapeake Bay. Although not many studies are available on uptake of urea by phytoplankton, some information on the factors influencing urea mobilization are available. Several factors like light, substrate availability, substrate preference, competitive inhibition of urea uptake by other nutrients *etc.* have been considered important in the regulation of urea uptake.

Although urea has been known as a major form of regenerated nitrogen, its uptake by natural phytoplankton populations has not been studied as extensively as that of ammonium. Eppley and Peterson (1979) suggested that urea uptake could rise up to 50% of ammonium uptake. Kokkinakis and Wheeler (1988) showed that the uptake of urea depended on substrate availability. Conversely, Rees and Syrett (1979) reported an increased initial rate of urea

uptake at low nutrient concentrations. Kaufman *et al.* (1983) measured the uptake of nitrogenous nutrients by phytoplankton in the Barrier Island estuary, and found that urea was the nutrient most used by phytoplankton and concluded that the relatively high urea concentration was the major factor in the apparent preference for urea. The phytoplankton of Sargasso Sea showed a greater preference for urea as the N source rather than ammonium (Price and Harrison, 1988).

In the present study, urea uptake rates (average:  $0.0004 \text{ h}^{-1}$ ) were about ~6 times lower than those of ammonium (average:  $0.0024 \text{ h}^{-1}$ ) and nitrate (average:  $0.0028 \text{ h}^{-1}$ ) uptake and stood third in the order (7% of total annual uptake). The seasonal variations of specific urea uptake rates unlike those of absolute uptake were well marked. High uptake rate was recorded in the non-monsoon months (average  $v_{\text{urea}} = 0.0006 \text{ h}^{-1}$ ) and the lowest rates in the monsoon months (average  $v_{\text{urea}} = 0.0002 \text{ h}^{-1}$ ). Absolute urea uptake rates were maximum in the pre-monsoon months (average  $p_{\text{urea}} = 29.5 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ), moderate in the post-monsoon (average  $p_{\text{urea}} = 25.5 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and the lowest in the monsoon months (average  $p_{\text{urea}} = 13 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). The rates did not vary significantly between the stations.

The two unique features of the results are: (i) the significant correlation of specific uptake rates with chlorophyll-a (ii) the independence of uptake rates from the availability of substrate.

The significant correlation of chlorophyll-*a* and uptake of urea ( $r=0.68$ ,  $P<0.001$ ;  $n=48$ ) (Fig. 4.33 a, b) suggests that the uptake rates are closely related to primary production. In the present study, the high urea in the non-monsoon months corresponded to high chlorophyll-*a* concentrations (post-monsoon:  $10.2 \mu\text{g chl-}a \text{ l}^{-1}$  and pre-monsoon:  $9.9 \mu\text{g chl-}a \text{ l}^{-1}$ ). The rapidly growing phytoplankton population requires high N in order to sustain the high productivity. In the post-monsoon months, the total nutrient concentrations are low ( $\Sigma\text{N}=2.5 \mu\text{g at N l}^{-1}$ ), but the chlorophyll-*a* concentrations are high. Under such conditions, and in order to sustain the high phytoplankton biomass, urea is also utilized simultaneously along with the other nutrients, but at low rates as the ambient concentrations are low.

Unlike with ammonium uptake, change in species composition did not affect the urea uptake rates. The change in the phytoplankton community in the present study was well marked (Fig. 3.9a-c). In the post-monsoon months, the microphytoplankton formed a significant part of the population (81%) while, in the pre-monsoon months, the nanophytoplankton dominated and formed 66% of the total biomass (Fig. 3.8). Size-fractionated uptake showed that both these fractions utilized urea efficiently (Fig. 3.27; Fig. 3.28). In the post-monsoon season, the microphytoplankton contributed to 39.8% of the total urea uptake, while the nanophytoplankton accounted for 60.2%. In the pre-monsoon season, the phytoplankton population changed from microphytoplankton to

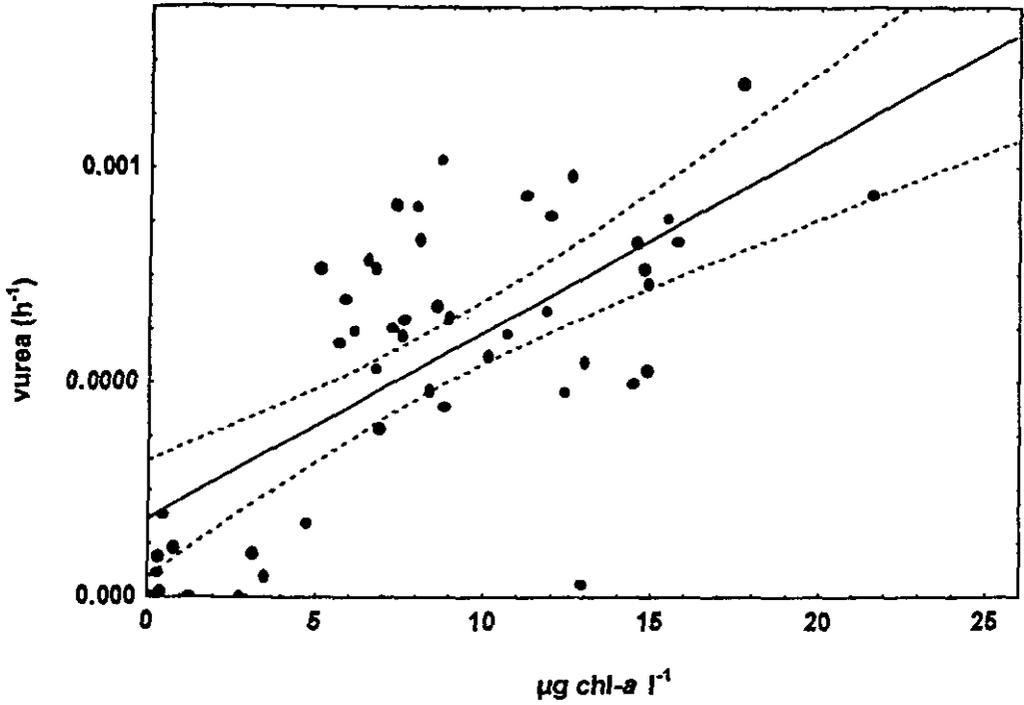


Fig. 4.33a Relation of  $v_{\text{urea}}$  with chlorophyll-a concentrations  
 $v_{\text{urea}} = 0.0015 + (0.00003 * \text{chl-a})$  ( $r=0.69$ ,  $n=48$ )

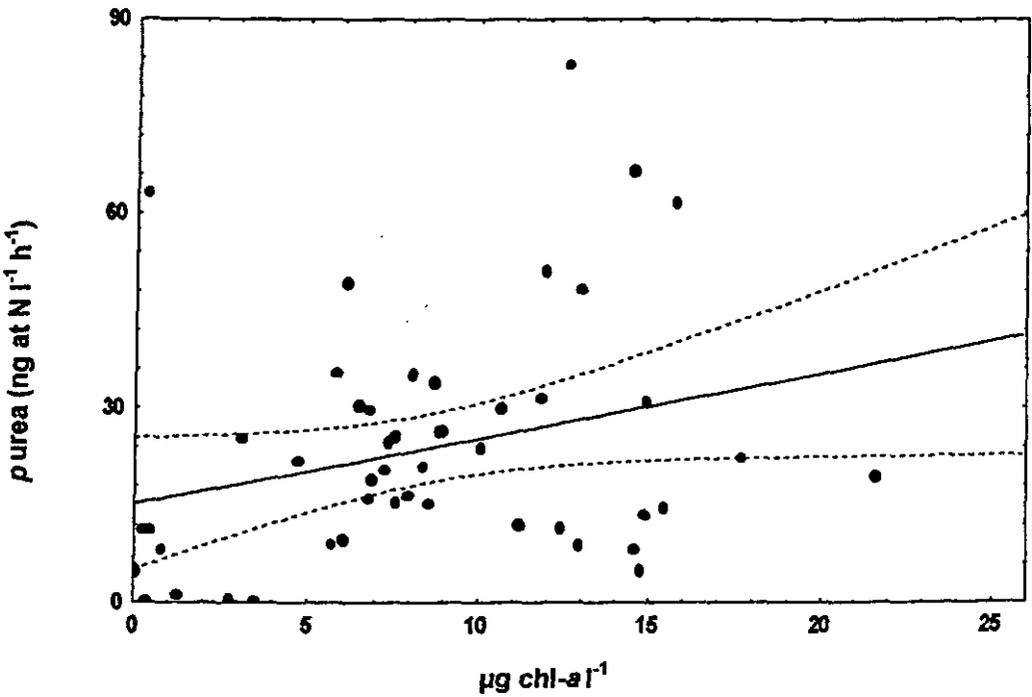


Fig. 4.33b Relation of  $p_{\text{urea}}$  with chlorophyll-a concentrations  
 $p_{\text{urea}} = 15.221 + (1.0011 * \text{chl-a})$  ( $r=0.28$ ,  $n=48$ )

nanophytoplankton which contributed to 37% and 63% of the total chlorophyll-a concentrations respectively. In this season, of the total urea uptake (average  $v_{\text{urea}}$ :  $0.0006 \text{ h}^{-1}$ ), the nanophytoplankton accounted for 70%, while the microphytoplankton, 30%. As urea concentrations were very low, the change in phytoplankton community structure did not alter the seasonal uptake rates significantly. However, in both the pre- and post-monsoon seasons, the nanophytoplankton showed a preference for urea, contributing to 70 and 60.2% of the total urea uptake. Earlier studies of Glibert (1982) and Furnas (1983) on size-fractionated uptake in coastal waters concluded that the nanoplankton formed the major group and utilized urea more efficiently than the microplankton. However, the study by Probyn (1985) in the field conditions reported high specific uptake by the microplankton fraction.

In the monsoon months,  $v_{\text{urea}}$  decreased sharply ( $0.0006 \text{ h}^{-1} \rightarrow 0.0002 \text{ h}^{-1}$ ). This sharp decrease in  $v_{\text{urea}}$  could be due to low phytoplankton biomass (average:  $4.2 \mu\text{g chl-a l}^{-1}$ ). The ambient urea concentrations did not appear to be limiting, as the urea concentrations were higher when compared with the non-monsoon months (average:  $0.3 \mu\text{g at N l}^{-1}$ ). This suggested that the uptake rates depended on the autotrophic biomass and its N demand. The change in the climatic and hydrological conditions (inadequate light supply, low salinity and high turbidity) could also have resulted in the reduced urea uptake rates, as was the case with nitrate, nitrite and ammonium uptake.

Another important observation was that urea uptake rates in the present study were independent of ambient urea concentrations. The urea concentrations in the non-monsoon months at all the stations were low and varied in a narrow range (0.15 to 0.2  $\mu\text{g at N}^{-1}$ ). Nevertheless, even at these low concentrations, the measured uptake rates are sufficient to satisfy about 15% of the N uptake by phytoplankton. Earlier studies of Kristiansen (1983) and L'Helguen *et al.* (1996) reported efficient uptake of urea at low ambient concentrations. Rees and Syrett (1979) also reported increased urea uptake rates at low concentrations. In contrast, Kokkinakis and Wheeler (1988) in their size-fractionated studies found a positive correlation between urea concentrations and its uptake.

Unlike other nutrients, vurea and purea did not show significant spatial variations. This could have been due to the insignificant variations of ambient urea concentrations between the stations.

In conclusion:

1. Urea uptake rates were not substrate dependent.
2. Urea uptake rates significantly depended on the phytoplankton biomass.  
Unlike the other nutrients, the change in cell size structure and taxonomic composition were not responsible for the variations in urea uptake rates.
3. In the monsoon months, climatic and hydrological changes significantly influenced urea uptake.

## CHAPTER V

### INTEGRATED DISCUSSION

Dynamics of nutrients and biological productivity in any ecosystem are controlled by more than one process at a given time. This is even more manifest in tropical coastal marine ecosystems which are located at the interface between sea and land and are influenced by the processes in both these biospheres. In earlier chapters, the dynamics of each nutrient and each process was discussed in detail. This section is an attempt to present a holistic account of the sources of nutrients, the interrelationships among them, and the influence of physical and biological processes on their abundance and distribution and their influence, in turn, on biological processes, both in space and time. The major pathways/processes involved in nitrogen cycling in mangrove and estuarine waters are depicted in Fig. 1.1.

Primary production and nutrient cycling in mangrove waters are different from those of other coastal waters that become seasonally stratified, in the sense that, both tend to reach maximum values in the pre-monsoon instead of the post-monsoon season (Devassy, 1983; Bhattathiri *et al.*, 1996; Gallegos *et al.*, 1997).

The salient findings of this study are:

- Litter is the major source of particulate organic nitrogen to the aquatic system of mangroves thus underlying the importance of terrestrial productivity on the planktonic nitrogen fluxes.
- Nitrate was the major nutrient in the dissolved assimilable nitrogen pool ( $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+ + \text{urea}$ ). The source for this new nitrogen (nitrate) during the monsoon months was freshwater advection.
- In the dry season, the dissolved nutrient pool was mainly maintained by microbial *insitu* regeneration processes, whereas in the wet season, freshwater advection was the major source of nutrients.
- The imbalance between the utilization (uptake) and production rates (*insitu* regeneration) resulted in the high nutrient concentrations in these waters.
- Porewater nutrients and remineralization processes in the sediments were also important in regulating the primary production of the water column in the non-monsoon months.
- Phytoplankton primary production was limited by a particular form of nitrogen:  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , rather than the total availability of nitrogen nutrients.
- The seasonal changes in phytoplankton species composition and cell size structure were due to the variations in nitrogen nutrient concentrations and salinity.

- The *insitu* processes (ammonification and nitrification) are substrate based.
- Nitrogenous nutrient uptake by phytoplankton depended on the availability of the substrate and phytoplankton biomass.
- High ammonium concentrations significantly inhibit the uptake rates of other nutrients.
- Seasonal shift in the nutrient uptake *i.e.* nitrate in the post-monsoon months to ammonium in the pre-monsoon months is due to change in the nutrient composition and phytoplankton community structure.
- The nanophytoplankton exclusively utilized the regenerated forms ( $\text{NH}_4^+$  and urea) whereas the microphytoplankton also utilized  $\text{NO}_3^-$ .

#### **Dissolved nutrient pool:**

Though mangroves are generally regarded as productive coastal marine ecosystems in the tropics (Qasim and Wafar, 1990), measurements of the concentrations of nutrients, especially of N, that sustain this production have been few and far between. Several among them are temporally discrete and only a few such as those in the mangroves of Australia (Boto and Wellington, 1988; Trott and Alongi, 1999); Pakistan (Harrison *et al.*, 1997), Mexico (Rivera-Monroy *et al.*, 1995a) and India (Krishnamurthy *et al.*, 1975) present seasonal cycles. Even in them, all assimilable forms of N, as in the present study, are not covered. The results of the present study demonstrate a clear seasonality in the changes of concentrations of nutrients. This is different from the monotonously

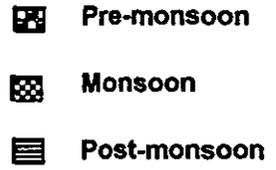
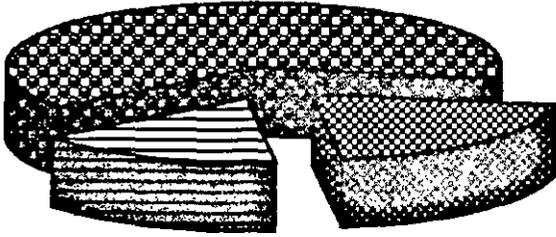
uniform concentrations observed during the dry season in the Australian mangroves (Trott and Alongi, 1999) or the lack of a pattern in Pakistan mangroves (Harrison *et al.*, 1997). An interesting feature in the seasonal changes of the N nutrients in this mangrove is the extent to which *in situ* processes control ambient concentrations.

In the monsoon months, allochthonous inputs actively added nutrients to the dissolved nutrient pool, whereas, in the non-monsoon months, autochthonous sources (microbial regeneration processes) were the main suppliers of nutrients.

Figs. 5.1 and 5.2 show the percent contribution and seasonal variations of the four nutrients estimated at the different stations. Nitrate was the major nutrient and contributed 72% to the total estimated dissolved nitrogen pool. This was followed by ammonium (16%) and nitrite and urea which contributed to 6% each. The seasonal variations in the nutrient pool were well marked with a pronounced peak of the reduced forms ( $\text{NH}_4^+$  and urea) in the pre-monsoon months, and that of the oxidized forms ( $\text{NO}_3^-$  and  $\text{NO}_2^-$ ), in the monsoon months. These variations in the nutrient levels were controlled by physical and biological means.

From Fig. 5.2, it is evident that the maximum nitrate concentrations in the monsoon months decreased considerably in non-monsoon months although its

a)



b)

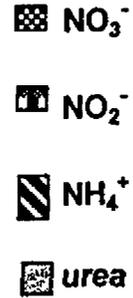
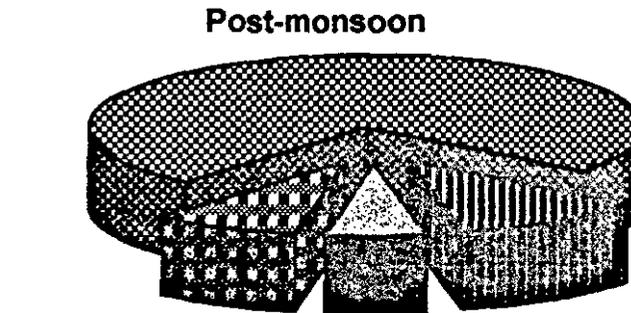
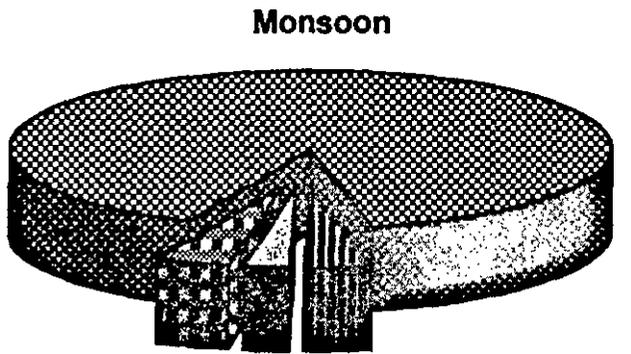
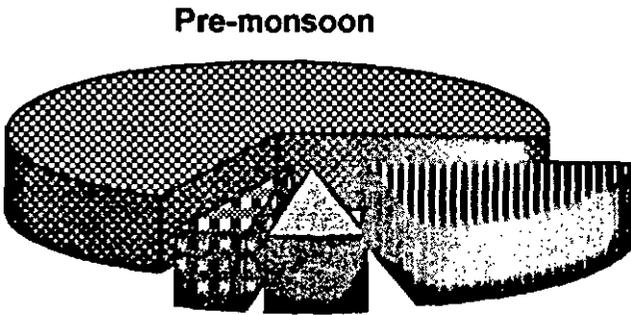


Fig. 5.1. Percent contribution of nitrogen nutrients in the estimated dissolved pool  
a) Total nutrient percent contribution over the seasonal cycle, b) Percent contribution of nitrate, nitrite, ammonium and urea over the seasonal cycle.

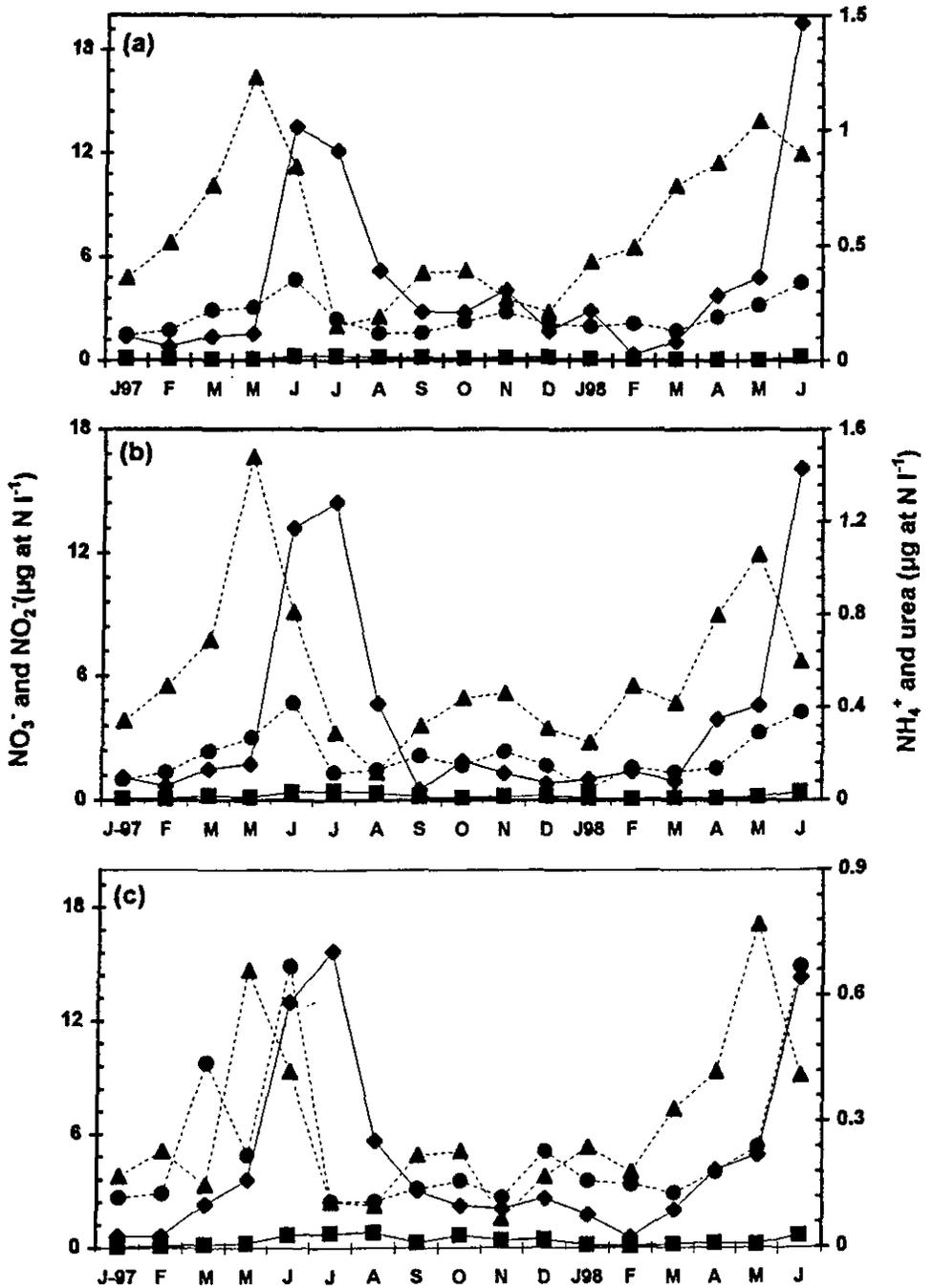


Fig. 5.2 Seasonal changes of the concentrations of nitrate (◆), nitrite (■), ammonium (▲) and urea (●) at the (a) reference, (b) middle and (c) mouth stations

percentage contribution remained high during this season (Fig. 5.1). The increase in ambient nitrate concentrations in the monsoon months was due to fresh water advection as confirmed by the plots of correlation with salinity (Fig. 4.1). In the non-monsoon months, when there was no freshwater inflow, the biological processes were responsible for the high percent of nitrate (Fig. 5.1) in the dissolved nutrient pool. The results of this study showed that the mangrove waters were active sites for nitrification (average:  $42.6 \text{ ng at N l}^{-1}\text{h}^{-1}$ ).

The positive relation of nitrate with nitrification ( $r=0.4$ ,  $P<0.05$ ;  $n=25$ ) and significant positive correlation with ammonium concentrations in the water column ( $r=0.34$ ,  $P<0.04$ ,  $n=37$ ) suggested that nitrate concentrations were closely inter-linked with those of ammonium. Ammonium, the substrate for nitrification, is supplied to the water column mainly by efflux from pore waters (Fig. 4.8) and *insitu* production in the water column (Fig. 4.7). The pore water, enriched with ammonium (Dham, 2000) enters the water column *via* diffusion. Earlier studies also showed that diffusion (Falcao and Vale, 1998), bioturbation (Kuwaer *et al.*, 1998) and water movement (Asmus *et al.*, 1998) regulated nutrient fluxes in shallow waters. The ammonium supplied is rapidly transformed to nitrate by the nitrifiers in the water column and this maintained the high nitrate concentrations even in the non-monsoon months. Hence, the sufficient supply of ammonium and its rapid oxidation (nitrification) were responsible for the high percent contribution of nitrate in the non-monsoon months.

In the post-monsoon months, nitrate concentrations on an average were lower (average:  $1.9 \mu\text{g at N l}^{-1}$ ) than in the other two seasons. However, its percent contribution to the total estimated dissolved pool remained high (Fig. 5.1b). One of the reasons for the high contribution of nitrate in the post-monsoon season was its accumulation as residual nitrate brought into the system by freshwater advection during the monsoon season. The rapidly growing phytoplankton utilized this nitrate as a major nutrient source and attained maximum uptake rates (average  $v\text{NO}_3^-$ :  $0.004 \text{ h}^{-1}$ ). Due to the high utilization of nitrate in the post-monsoon months, the ambient nitrate levels decreased rapidly, but their percent contribution to the total estimated dissolved pool was still high, as nitrification was also active. Therefore, the *insitu* production of nitrate and residual nitrate maintained the nitrate levels in the dissolved nutrient pool.

In the pre-monsoon months, the nitrification rates reached maximum values, and at the same time  $v\text{NO}_3^-$  decreased by ~68% (post-monsoon:  $0.004 \text{ h}^{-1}$  → pre-monsoon:  $0.001 \text{ h}^{-1}$ ). This led to an increase in nitrate levels in the dissolved pool. Hence, the imbalance between production (nitrification) and utilization resulted in the increase in the nitrate concentrations during this season (Figs. 5.3 and 5.4).

Ammonium concentrations in the water column were comparatively lower than those of nitrate. Although the ammonification rates were highest reported ever, the high uptake by phytoplankton and probably the mangroves could have resulted to the low ammonium concentrations in the dissolved pool. The

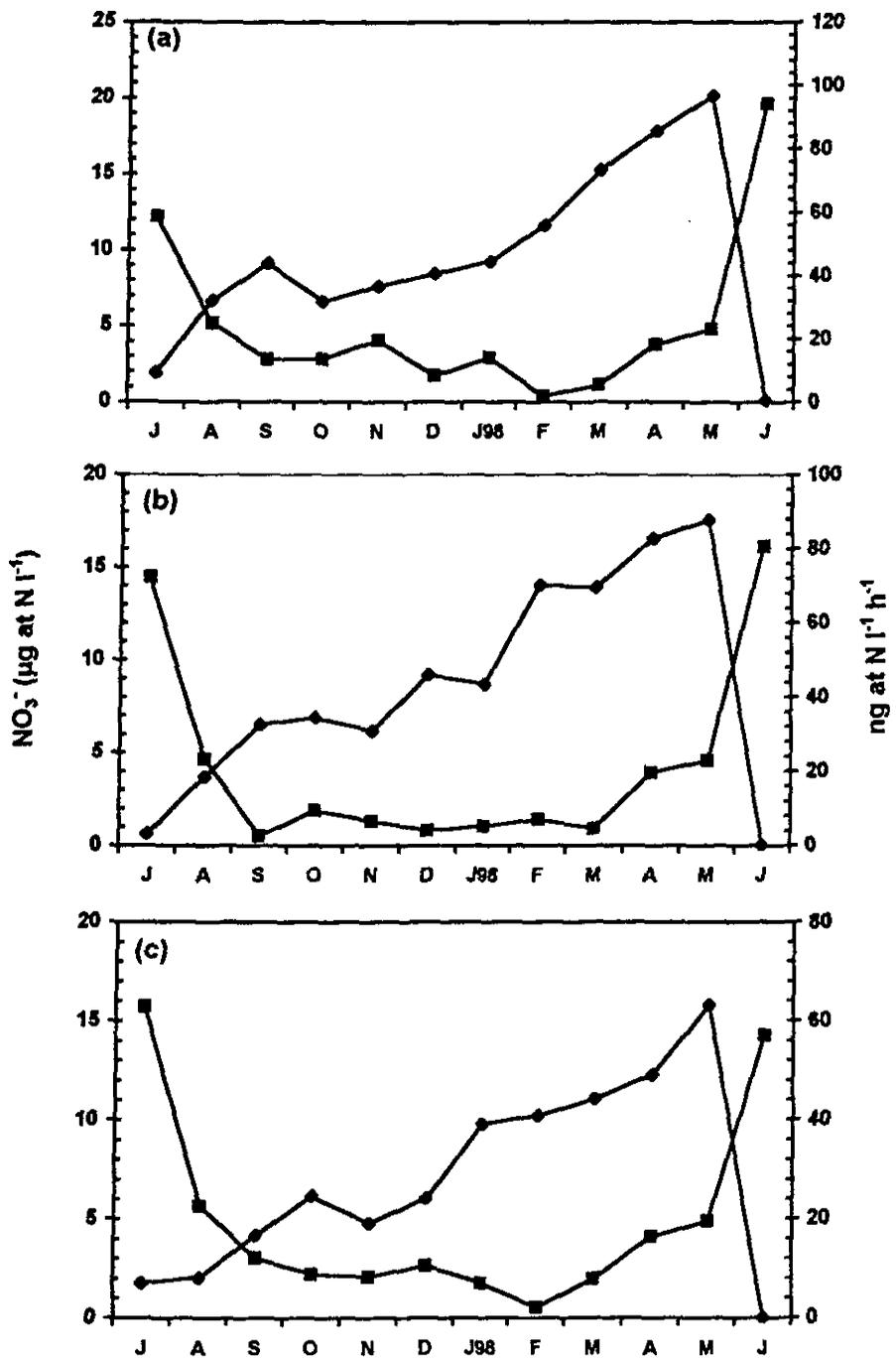


Fig. 5.3 Seasonal changes of nitrate concentrations (■) and nitrification rates (◆) at the (a) reference, (b) middle and (c) mouth stations

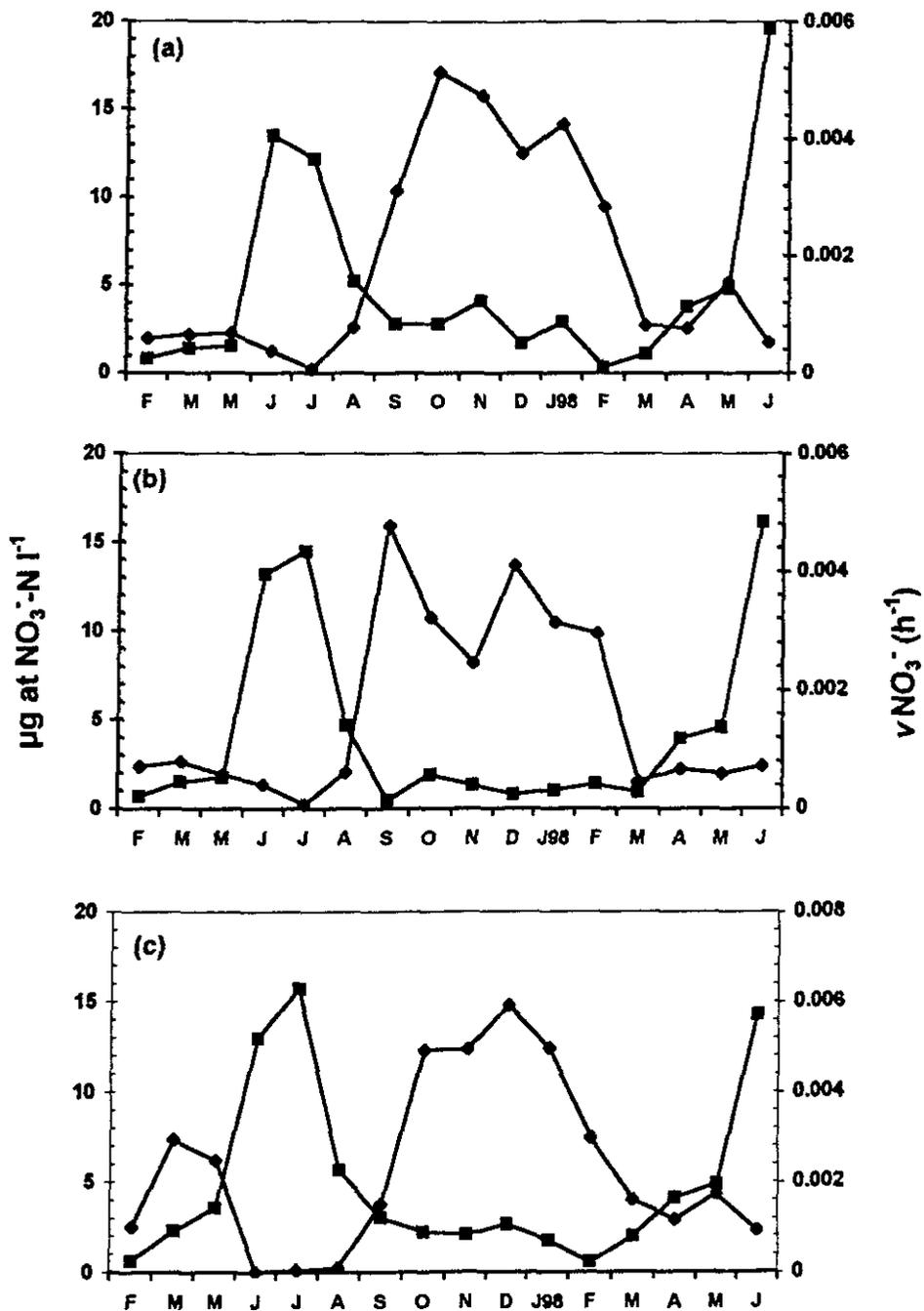


Fig. 5.4 Seasonal changes of nitrate concentrations (■) and specific nitrate uptake rates (◆) at the (a) reference, (b) middle and (c) mouth stations

significant relation between ammonium concentrations and ammonification rates (Fig. 4.7) clearly indicates that ammonium is mainly supplied by this process. Also, the significant positive correlation of ammonium concentrations in the pore water and the water column ( $r=0.55$ ,  $P<0.001$ ,  $n=48$ ) suggested that efflux of ammonium from the sediment to the water column significantly influenced the ammonium concentrations in the overlying waters. The increase in the porewater ammonium concentrations in the pre-monsoon months (Dham, 2000) may have increased efflux rates and thus could have been responsible for the high ammonium concentrations in the water column during this period ( $0.7 \mu\text{g at N l}^{-1}$ ). Other physical and biological factors such as water currents and bioturbation could have also enhanced the nutrient exchange rates (de Jonge and Colijn, 1994; Falcao and Vale, 1998).

Since ammonium was the most preferred nutrient ( $\text{RPI NH}_4^+ > 1$ ), the uptake of ammonium by phytoplankton was also an important factor influencing the ambient ammonium concentrations. In the post-monsoon months, the high demand for nitrogen by the rapidly growing phytoplankton community, the low supply of ammonium by *insitu* production (average ammonification rate= $130 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and the low ammonium concentrations in sediment ( $2.9 \mu\text{g at N (l pore water)}^{-1}$ ), resulted in minimum concentrations of ammonium during these months.

High ammonium uptake rates were recorded in the pre-monsoon months (average  $v\text{NH}_4^+$ :  $0.006 \text{ h}^{-1}$ ). Besides this, the ammonium concentrations were also high during these months because of increased *insitu* production in the water column. The high ammonification rates ( $663 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and efflux of porewater ammonium to the overlying waters also added to the dissolved nutrient pool (see section: 4.1.3).

As far as nitrite and urea are concerned, their contribution to the estimated dissolved nutrient pool was very low compared with  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (Fig. 5.1a). The low concentrations of these two nutrients can be explained by considering their supply and stability.

Nitrite is the intermediate compound derived from different biological processes (see section: 4.1.2). Nitrite can enter the system *via* freshwater influx, and is also produced by biological processes *i.e.* nitrification and denitrification. It has been reported that in mangrove waters, the process of denitrification is insignificant (Iizumi, 1986; Alongi, 1996; Kristensen *et al.*, 1998). Therefore, nitrification is the only likely process, which can supply nitrite to the water column in the non-monsoon months. In the present study, however, its correlation with nitrite was insignificant. In the pre-monsoon months, the decrease in the nitrite concentrations, in spite of the high nitrification rates suggested that nitrite produced *via* nitrification was probably lost to other pathways. The more or less inverse relation of nitrite concentrations and

nitrification rates during these months also suggests that the highly labile nitrite is rapidly oxidized to nitrate.

The decrease in the ambient nitrite levels through the post-monsoon months was due to enhanced uptake (Figs. 3.13 and 3.14). In the monsoon months, the high nitrite concentrations were due to freshwater input from the upstream region of the estuary. The significant negative correlation of nitrite concentrations with salinity supported this conclusion ( $r=-0.57$ ,  $P<0.001$ ,  $n=51$ ). Like nitrite, urea also accounted for 6% of the total estimated dissolved nutrient pool. The low *insitu* production of urea in the mangrove waters may be one of the reasons for the low concentrations. There are several sources through which urea is supplied (see section: 4.1.4). Like the other nutrients, high concentrations of urea were recorded in initial months of the monsoon season. This could mainly be attributed to freshwater advection which carries nutrients from land derived sources. The microbial degradation of particulate organic matter within the water column and sediment (Figs. 4.10 and 4.11) together with the increase in zooplankton biomass (Nair, 1979) appeared to have influenced the urea concentrations in the non-monsoon months.

It is important to mention that urea was continually taken up in the non-monsoon months and appeared to be an important nutrient source for phytoplankton growth. Hence, the high uptake rates would have also been responsible for the low urea concentrations in the dissolved pool.

### **Spatial and seasonal variations in the phytoplankton community and particulate organic load:**

The phytoplankton biomass and community structure showed large seasonal variations but no significant differences between the stations. Both the non-monsoon seasons showed high phytoplankton biomass (Fig. 3.7) and the community was characterized by large (200-20  $\mu\text{m}$ ) and small (20-0.8  $\mu\text{m}$ ) diatom species (Figs. 3.8). In the monsoon months, adverse climatic and hydrological conditions significantly affected the phytoplankton biomass and resulted in low chlorophyll-*a* concentrations.

Analysis of the environmental factors and phytoplankton community during the monsoon and non-monsoon seasons suggested that two different factors controlled the phytoplankton biomass in the two seasons. In the monsoon months, the physical factors such as light intensity, water stability, salinity and turbidity predominantly affected the phytoplankton biomass. Due to the southwest monsoon, fresh water advection reduced the salinity levels from 34  $\text{psu}$  to 0.2  $\text{psu}$  and this adversely affected the phytoplankton population (Qasim *et al.*, 1978; Fisher *et al.*, 1998). The freshwater that enters the system is highly turbid (Devassy, 1983; Mani, 1994), and together with high water movement (Asmus *et al.*, 1998), reduced the phytoplankton biomass. Various studies showed that light is an important factor that controls the phytoplankton biomass (Verlencar, 1982; Huszar and Caraco, 1998). In the present study, the changes in the climatic conditions *i.e.* low light intensity during this season (average:

443.3  $\mu\text{E s}^{-1} \text{M}^{-2}$ ) also resulted in decreased phytoplankton production. Hence, physical and climatic factors were responsible for the changes in the phytoplankton biomass in the monsoon months.

Nitrogen nutrient concentrations in the non-monsoon months (when the physical and climatic conditions are stable) showed large-scale variations that appeared to affect both the taxonomic composition and the size structure of the phytoplankton community. Ammonium and urea appeared to be the limiting nutrients for phytoplankton growth. These two nutrients showed a positive correlation with chlorophyll-*a* ( $\text{NH}_4^+$  Vs. Chl-*a*:  $r=0.37$ ,  $P<0.02$ ,  $n=35$ ; urea Vs. Chl-*a*:  $r=0.36$ ,  $P<0.02$ ,  $n=48$ ).

In the present study, total nitrogen nutrient availability did not appear to be a significant factor limiting biomass as the nutrient concentrations were high in all the seasons (pre-monsoon:  $\Sigma\text{N}=3.2 \mu\text{g at N l}^{-1}$ ; post-monsoon:  $\Sigma\text{N}=2.5 \mu\text{g at N l}^{-1}$  and monsoon:  $\Sigma\text{N}=10.1 \mu\text{g at N l}^{-1}$ ). The availability of a specific form of N however, did influence the chlorophyll-*a* concentrations. Among the estimated nutrients, nitrate was the most dominant form and contributed to 72% of the total estimated nutrient pool. In the post-monsoon months, nitrate appeared to be the major nutrient for phytoplankton growth. During this period, the rapidly growing phytoplankton community largely utilized nitrate (average  $\rho\text{NO}_3^- = 64.1 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ). Nitrate uptake related significantly with chlorophyll-*a* suggesting that autotrophic biomass influenced nitrate uptake (Fig. 5.5).

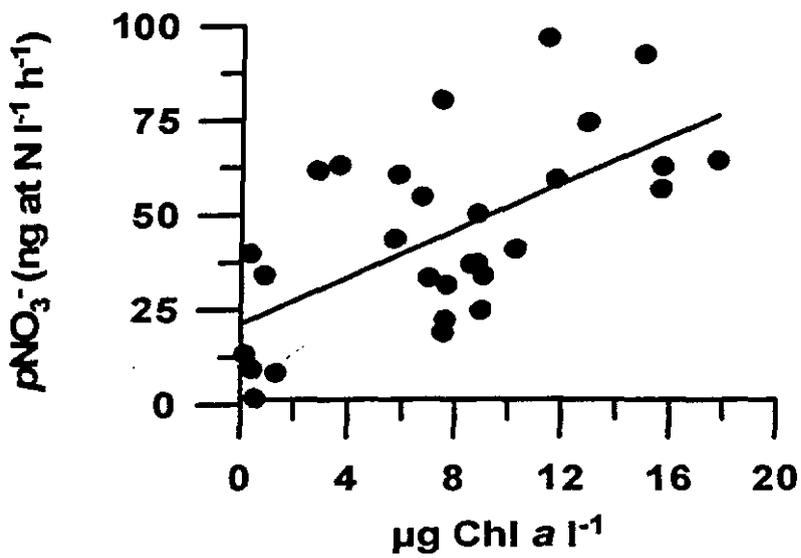
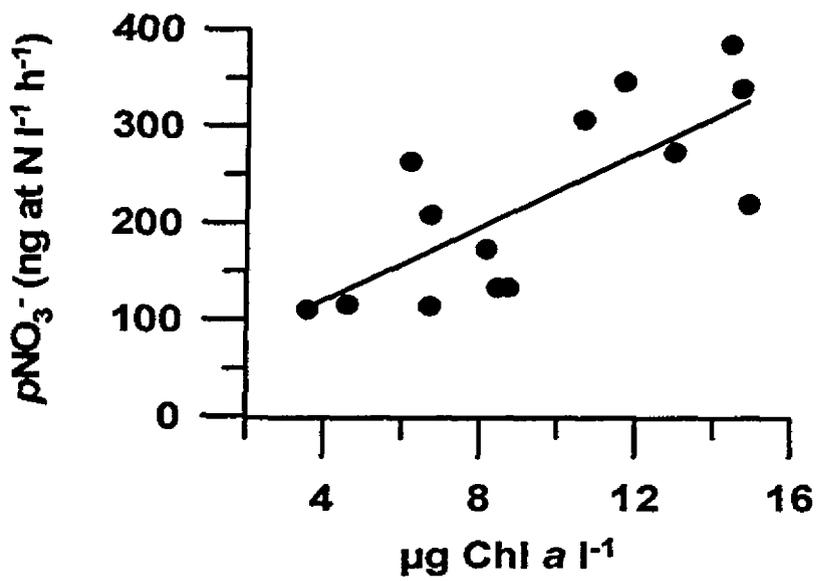


Fig. 5.5 Relation of nitrate uptake rates with chlorophyll *a*.

The size-fractionated study showed that the microphytoplankton dominated the phytoplankton population in the post-monsoon season contributing to 81% of the total chlorophyll-a, while the nanophytoplankton contributed a mere 19%. It has been well documented that larger cells preferentially utilize nitrate over the smaller cells (Glibert, 1982a; Probyn, 1985). Thus, it can be concluded that high nitrate availability during the post-monsoon season enhanced the rapid growth of the larger cells.

In the pre-monsoon season, the phytoplankton biomass was slightly lower than that of the post-monsoon season and the population was characterized by high nanophytoplankton (66%) and low microphytoplankton abundance (34%) (Fig. 3.8). This change in the species composition and cell size is the result of the ability of the phytoplankton species to grow in varying salinities (Andersson *et al.*, 1996) and their nutrient preferences (Gallegos *et al.*, 1997; Levine *et al.*, 1997). The availability of ammonium appeared to be the major nutrient influencing phytoplankton growth during these months (Fig. 4.20). Increased ammonium concentrations (post-monsoon:  $0.3 \mu\text{g at N l}^{-1}$  pre-monsoon:  $0.7 \mu\text{g at N l}^{-1}$ ) and salinity levels (post-monsoon: 30.2 pre-monsoon: 34.9 PSU) resulted in the change in the species composition and size structure (Figs. 3.8 and 3.9a-c). The nanophytoplankton community replaced the microphytolankton. The nanophytoplankton are sturdier, grow at high salinities (Andersson *et al.*, 1996) and efficiently utilize ammonium as the nutrient source (Glibert, 1982a; Probyn, 1985).

Another interesting feature is that chlorophyll-a concentrations showed a positive correlation with *insitu* regeneration (ammonification and nitrification), indicating that primary production was closely linked with *insitu* nutrient production (ammonification :  $r=0.50$ ,  $P<0.001$ ,  $n=48$ ; nitrification :  $r=0.45$ ,  $P<0.005$ ,  $n=36$ ).

Although phytoplankton biomass in the present study was high (average cell count:  $474 \times 10^3$  cells  $\Gamma^{-1}$ ), its contribution to the particulate organic nitrogen pool was insignificant as seen from the correlation of chlorophyll-a with PON concentrations (Fig. 4.12). In the present study, PON concentrations were very high (range: 12.1 to 207.9  $\mu\text{g at N } \Gamma^{-1}$ ), with maximum values in the monsoon season (116.5  $\mu\text{g at N } \Gamma^{-1}$ ). During these months, the high PON values in the water column were due to freshwater advection and sediment resuspension/erosion. Studies along the west coast of India (Shankar and Manjunatha, 1997) showed that detrital material was resuspended from marine sediments during the flood phase of the tidal cycle. Similarly, Alongi *et al.* (1999), in the Hinchinbrook mangroves concluded that heavy monsoonal rains and wind-induced wave action disturbed the sediments thus causing resuspension of organic matter. The strong negative correlation of PON with salinity ( $r= -0.81$ ,  $P<0.001$ ,  $n=51$ ) suggested that due to freshwater advection, the PON concentrations increased remarkably. However, the inverse trend of PON concentrations in water column with PON in the sediment (Fig. 4.16) clearly demonstrated that the increased PON in the monsoon months were

mainly due to erosion of sediments. In the non-monsoon months, the PON in the sediment also appeared to influence the PON concentrations in the overlying waters ( $r=0.52$ ,  $P<0.001$ ,  $n=34$ ). It is generally observed that the high PON concentrations in shallow waters are due to re-suspension of sediment particulate matter by water movement and tidal currents (de Jonge, 1995; Thomsen *et al.*, 1998; Clarks and Elliott, 1998).

### **Nitrogen recycling processes:**

#### Ammonification:

The two major microbial processes that are significantly active in the water column during the non-monsoon months are ammonification and nitrification. Ammonium regeneration rates are among the highest reported so far for nearshore waters (Glibert, 1982; Bode and Dortch, 1996) and are probably characteristic of high detritus ecosystems. Grazers (ciliates and flagellates) and DON mineralizers are the major producers of  $\text{NH}_4^+$  in the  $<200 \mu\text{m}$  fraction (Paasche and Christiansen, 1982; Probyn, 1987; Le Corre *et al.*, 1996). The relative importance of these groups is un-assessable with the present data, but the high PON content and the  $>50\%$  efflux of elements from the litter within 2 weeks of the onset of decomposition (Wafar *et al.*, 1997) suggest that mineralization of DON could have been an important source of  $\text{NH}_4^+$ . Among the environmental parameters, temperature, as expected from the pattern of seasonal changes (Fig. 3.1), had an influence ( $r= 0.65$ ,  $p<0.01$ ) on  $\text{NH}_4^+$  production.

Uptake and regeneration of  $\text{NH}_4^+$  in short term experiments are closely coupled but their rates are not always in balance. The U/R ratio may exceed one (ex. Harrison *et al.*, 1983), be less than that (e.g. Hanson and Robertson, 1988) or may vary as a function of season, depth and plankton size fraction (Le Corre *et al.*, 1996).

Ammonium production almost always exceeded its uptake and the daytime production was in excess of assimilatory N requirements (U/R ratio of 0.63). The good relationship of nitrification rates with  $\text{NH}_4^+$  concentrations (Fig. 4.22) (and  $\text{NH}_4^+$  production rates - see below) suggests that a part of  $\text{NH}_4^+$  production also fluxes to the nitrification pathway. When this was included along with uptake, the ratio increased through 0.07, leaving still excess  $\text{NH}_4^+$  production. Nevertheless, the ambient  $\text{NH}_4^+$  concentrations were low and the  $\text{NH}_4^+$  uptake by plankton was substrate-dependant. This suggests that other autotrophs (benthic algae and the mangrove vegetation) would constitute potential sinks for  $\text{NH}_4^+$  mineralized in the water column.

#### Nitrification:

Though subject to inhibition by light, nitrifying bacteria are known to be still active within the euphotic zone, especially near its base or at the primary  $\text{NO}_2^-$  maximum (Ward, 1986) and mineralize  $\text{NH}_4^+$  to an extent that even exceeds the rates of  $\text{NO}_3^-$  assimilation in the open ocean (Dore and Karl, 1996) and neritic (Gentilhomme and Raimbault, 1995) waters. Water column nitrification rates in

the marine environment are highly variable but most productive estuarine and coastal areas have higher rates than oceanic waters (Kaplan, 1983). Typical rates in the primary  $\text{NO}_2^-$  maximum in oceanic waters range between 20 (Olson, 1981; Ward *et al.*, 1984) and 40  $\text{nmol N l}^{-1} \text{d}^{-1}$  (Dore and Karl, 1996). In neritic waters, they are of an order of magnitude higher, up to 20  $\text{nmol N l}^{-1} \text{h}^{-1}$  (Gentilhomme and Raimbault, 1995). In unpolluted estuaries, they could be still one more order of magnitude higher, up to 1200  $\text{nmol N l}^{-1} \text{d}^{-1}$  (compilation in Berounsky and Nixon, 1993).

The average nitrification rate of 42.6  $\text{ng-at N l}^{-1} \text{h}^{-1}$  measured in this study, are among the few highest values reported (Berounsky and Nixon, 1993). What is remarkable is the fact that, unlike in other eutrophic environments, these high nitrification rates appear to be supported by low  $\text{NH}_4^+$  concentrations (Fig. 4.23). Though the latter did relate linearly with nitrification rates, the low ambient concentrations and the high planktonic uptake rates suggest that it would be rather the rate of  $\text{NH}_4^+$  production than its *in situ* concentrations that regulates nitrification. The relation between nitrification rates and  $\text{NH}_4^+$  regeneration rates did indeed obey the Michaelis-Menton kinetics (Fig. 5.6) and the V/S vs S plot was an extremely good fit ( $r= 0.95$ ,  $p<0.001$ ). The predicted maximum nitrification rate was 100  $\text{ng-at N l}^{-1} \text{h}^{-1}$ , which was attained in the month of May (96.7  $\text{ng-at N l}^{-1} \text{h}^{-1}$ ). The  $\text{NH}_4^+$  regeneration rate required to sustain half the maximum nitrification rate was about 225  $\text{ng-at N l}^{-1} \text{h}^{-1}$ .

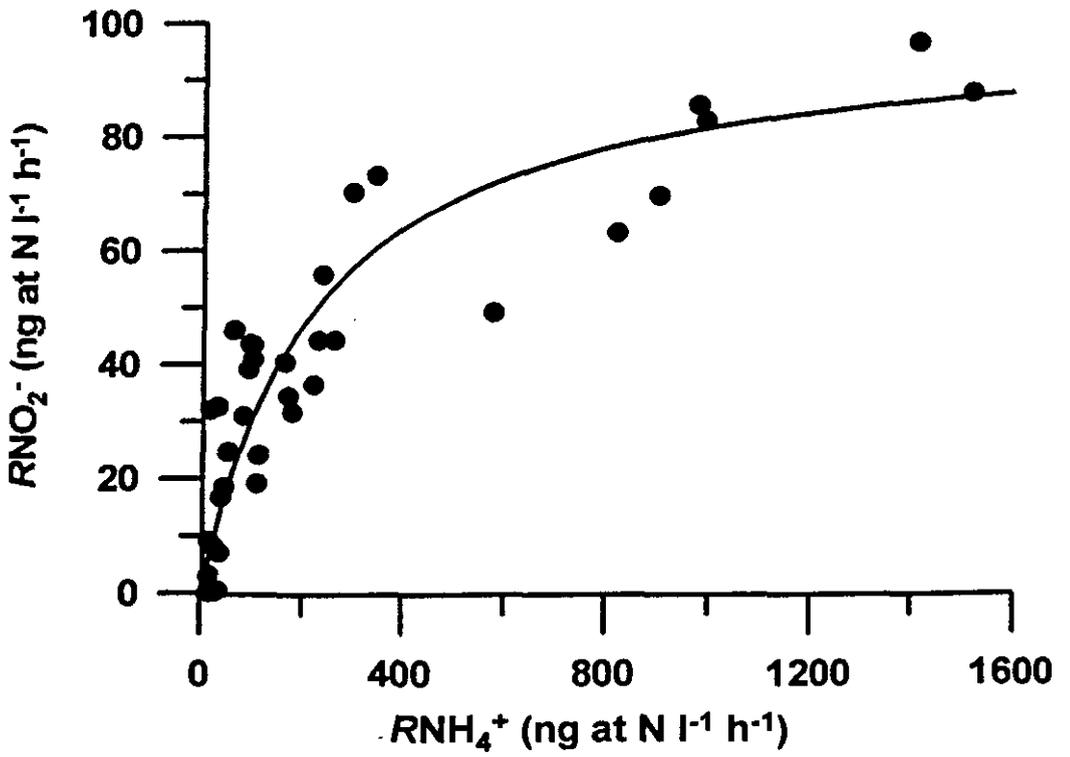


Fig. 5.6 Relation of ammonification and nitrification rates.

Other environmental variables that regulate nitrification are oxygen concentrations, temperature and suspended load. The percent saturation of dissolved oxygen at all stations was rarely below 100 and hence this could not have influenced nitrification rates. Besides, marine nitrifiers can grow and oxidize their substrate at very low oxygen tensions (Kaplan, 1983). Temperature, on the other hand, could have had an influence ( $r= 0.51$ ) but it could be fortuitous since the patterns of changes of temperature,  $\text{NH}_4^+$  concentrations and  $\text{NH}_4^+$  regeneration rates were more or less similar. Turbidity can also enhance nitrification rates (Helder and DeVries, 1983; Owens, 1986). This could have been an important factor regulating nitrification in the present study: though the analysis of variance did not show significant differences between nitrification rates among the 3 stations, there was still a gradient in the averages between the first two and the third (49.9, 47.3 and 30.6  $\text{ng-at N l}^{-1} \text{h}^{-1}$ ). This was proportional to the gradient in average PON concentrations (85.8, 72.5 and 48.6  $\text{ng-at N l}^{-1}$ ).

While the coupling between  $\text{NH}_4^+$  production and its utilization in the planktonic food chain was quite close, the same was not true with nitrification rates and uptake rates of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . The pattern of changes in them (Figs. 3.14, 3.16 and 3.12) showed that these rates were in balance only during February-May. As these were the months when the freshwater flow was virtually non-existent, uptake rates in excess of nitrification rates during the rest of the year could only have been supported by  $\text{NO}_3^-$  and  $\text{NO}_2^-$  input through freshwater advection.

Integration of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  uptake and  $\text{NO}_2^-$  production from July to January gave rates of 0.42 and 0.07 mg-at N  $\text{l}^{-1}$  respectively, indicating that about 80% of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  taken up during monsoon and post-monsoon was sustained by allochthonous inputs.

**Seasonal variations of nitrogen uptake regime: relation with autotrophic community structure and nutrient regime:**

Prodigious nitrate uptake (average  $v\text{NO}_3^- = 0.004 \text{ h}^{-1}$ , average  $\rho\text{NO}_3^- = 192.9 \text{ ng at N l}^{-1} \text{ h}^{-1}$ ) and predominance of new production ( $f\text{-ratio}=0.63$ ) characterized the post-monsoon uptake regime. The differences in the taxonomic composition and cell size structure and their preference for a particular nitrogen form are responsible for the variations in the uptake rates. The results indicated that the microphytoplankton (200-20  $\mu\text{m}$ ) relied largely on the oxidized forms of nitrogen, namely, nitrate and nitrite, while, the nanophytoplankton (20-0.8  $\mu\text{m}$ ), efficiently utilized the regenerated forms, ammonium and urea (Figs. 3.21 to 3.28).

In the post-monsoon months, the availability and relative contribution of ammonium and urea were small compared to that of nitrate (Fig. 5.1). Nitrate specific uptake rates were ~3 and ~8 times higher than ammonium and urea uptake rates respectively. Interestingly, the RPI for nitrate showed that it was the least preferred nutrient (Table. 4.3), whereas, the RPI for ammonium and urea ( $\text{RPI NH}_4^+$  and  $\text{RPI urea} \gg 1$  Fig. 5.7) indicated that these were more

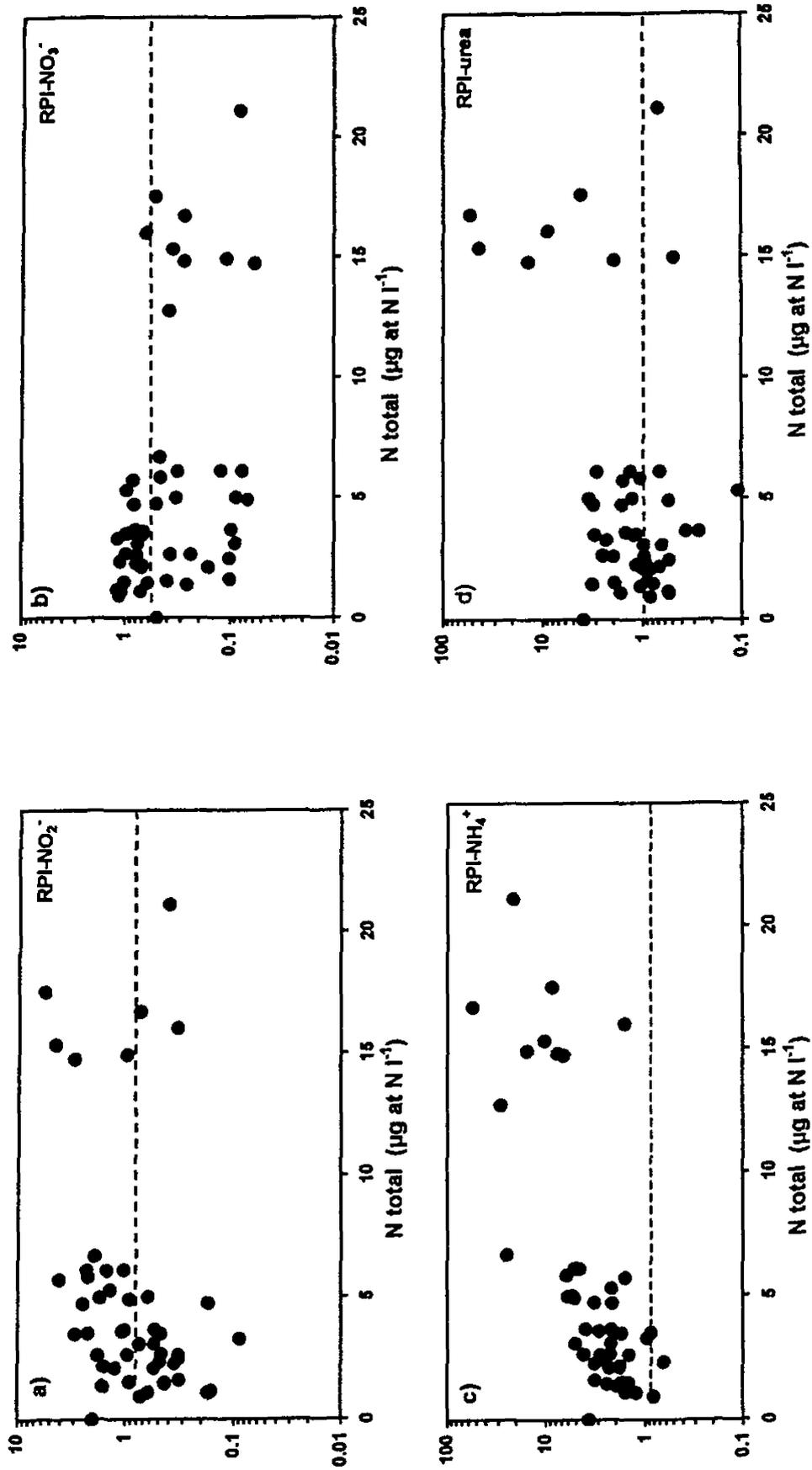


Fig. 5.7 Relative preference indices (RPI) for (a) nitrite, (b) nitrate, (c) ammonium and (d) urea

preferred nitrogen sources. The interpretation of the RPI however may not be true with the present study as Stolte and Riegman (1996) identified significant bias of RPI values at high nutrient concentrations and stressed that high values do not always reflect physiological preferences. Moreover, since the ambient nutrient concentrations are a balance between uptake and supply (Mengesha *et al.*, 1998), the low ammonium and urea uptake rates and their low ambient concentrations suggest a poor supply during this season (post-monsoon). Obviously, during the post-monsoon season, the nitrogen demand of the rapidly growing community was largely satisfied by nitrate. This is consistent with studies carried out in open ocean and well mixed waters (Olson, 1980; Smith and Nelson, 1990; Tupas and Koike, 1990; Goeyens *et al.*, 1991; Kristiansen *et al.*, 1992; Mengesha *et al.*, 1998).

In contrast, in the pre-monsoon season, ammonium uptake rates exceeded those of nitrate and the *f*-ratio values were on average  $<0.2$ , indicating predominance of regenerated production. This season was also characterized by increase in the ammonium availability (post-monsoon:  $0.3 \mu\text{g at N l}^{-1}$  pre-monsoon:  $0.7 \mu\text{g at N l}^{-1}$ ) (Fig. 5.1). The high uptake values (average  $v\text{NH}_4^+ = 0.006 \text{ h}^{-1}$ ) and the variations in the nutrient regime were paralleled by a change in the phytoplankton community structure from microphytoplankton to nanophytoplankton (Fig. 3.8).

In the pre-monsoon season, the nitrogen uptake at all the stations showed significant decrease in nitrate uptake rate, remarkable increase in ammonium uptake and a corresponding shift from predominantly new to regenerated production (Fig. 5.8). Interestingly, this seasonal shift occurred only when the phytoplankton community changed from microphytoplankton to nanophytoplankton. The change in the community structure and increased ammonium availability proved to be major factors driving the seasonal shift in nitrogen uptake (Fig. 5.9).

The change in the nutrient preference (nitrate to ammonium) was probably due to the increased ammonium levels in this season. The ambient concentrations could have reached the half saturation levels ( $K_s$ ) for the standing crop (phytoplankton). It is also important to note that the  $K_s$  values change in different environments, as well as with species (Table 4.2a, b). In the pre-monsoon months, the average ammonium concentrations were  $>0.3 \mu\text{g at N l}^{-1}$  and ammonium uptake reached its maximum. In the other two seasons (post- and monsoon seasons), ammonium concentrations averaged below  $0.3 \mu\text{g at N l}^{-1}$  and were apparently not enough to support maximum uptake. In the initial monsoon months, however, though the ammonium concentrations were  $>0.3 \mu\text{g at N l}^{-1}$ , maximum uptake was not attainable as the unfavorable physical and climatic conditions inhibited nitrogen uptake.

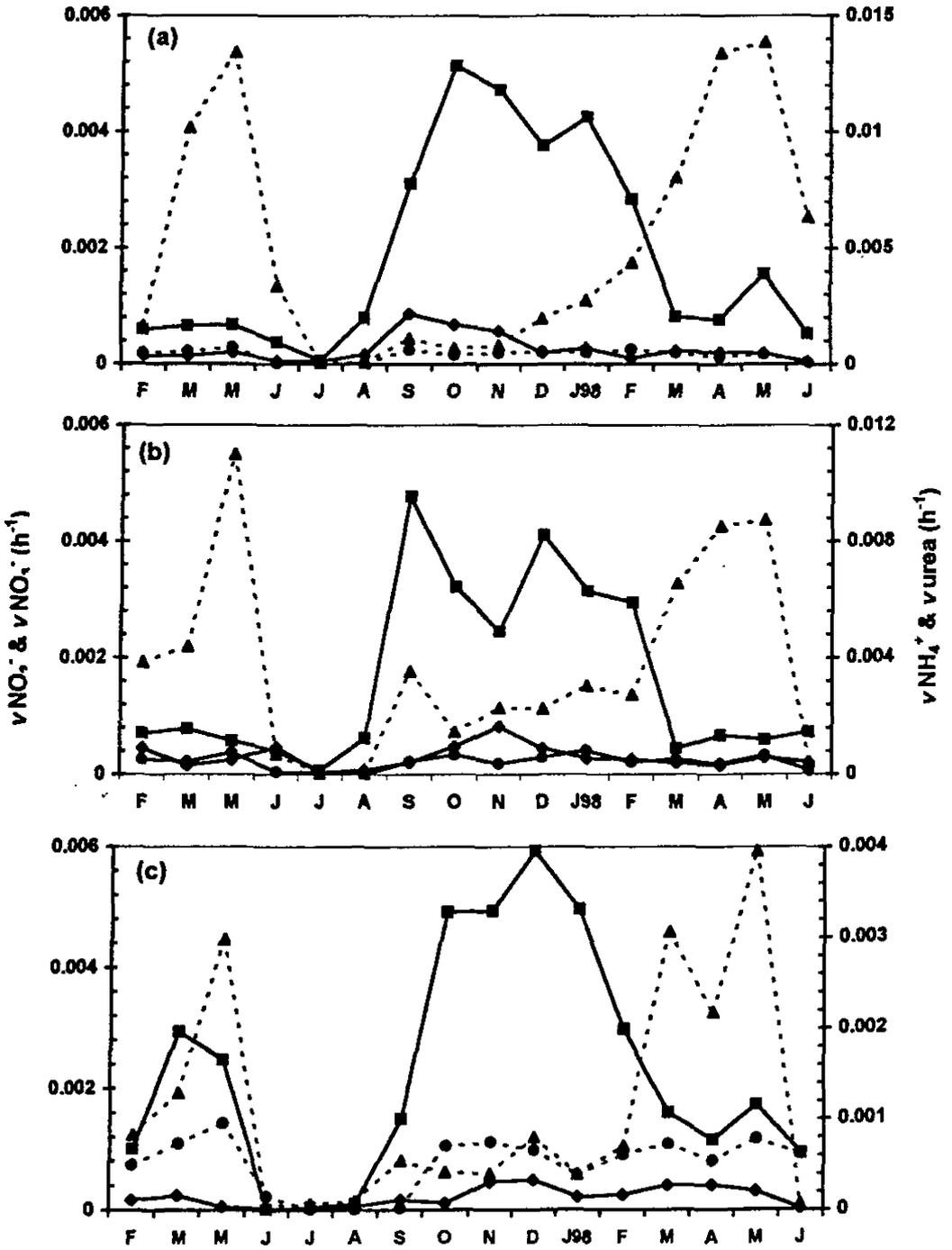


Fig. 5.8 Seasonal changes of uptake rates of nitrate (■), nitrite (◆), ammonium (▲) and urea (●) at the (a) reference, (b) middle and (c) mouth station

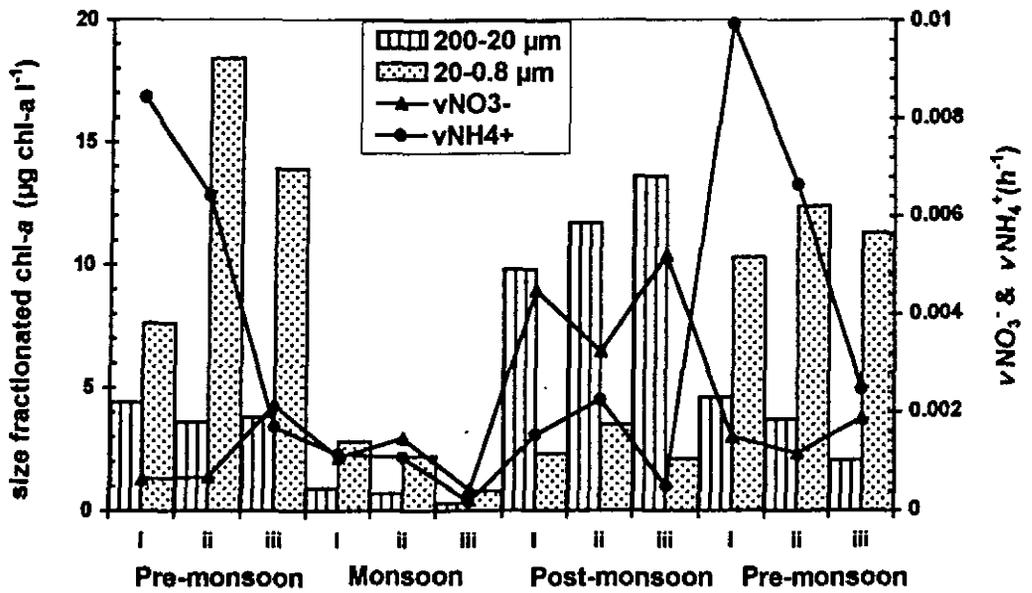


Fig. 5.9 Seasonal changes of chlorophyll-a concentrations of microphytoplankton (▨) and nanophytoplankton (▤) and specific uptake rates of nitrate (▲) and ammonium (●)

Amongst the three seasons, the average sum of nutrient concentrations were highest in the monsoon season ( $\Sigma N = 10.1 \mu\text{g at N l}^{-1}$ ) and almost all the nutrients showed extreme values in the initial period. Consequently, the remarkably low uptake rates during the monsoon season suggested that nutrients do not limit the uptake in these months. During this period, low light intensity and high turbidity reduced the uptake rates significantly. In the Amazon shelf waters, Demaster and Pope (1996), found that nearshore high turbidity inhibits autotrophic production because of light limitation. Therefore, the changes in the physical conditions would have led to a reduction in the uptake rates in the monsoon months.

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