

BIOLOGICAL NITROGEN FIXATION (ACETYLENE REDUCTION)  
ASSOCIATED WITH BLUE-GREEN ALGAL COMMUNITIES IN THE  
MGENI ESTUARY MANGROVE SWAMP

by

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ABSTRACT

Nitrogen fixation of blue-green algae associated with *Avicennia marina* (Forssk.) Vierh. pneumatophores and wet and dry surface sediments were investigated in the Mgeni Estuary mangrove swamp by means of the acetylene reduction technique.

Optimum partial pressures of acetylene ranged from 0,15 to 0,2 atm. for the different habitats. A lag phase of 3 h was observed in all habitats, followed by a period of linear ethylene production of 42 h for the pneumatophores and 72 h for the wet and dry mat areas. An assay period of 24 h was employed in all habitats.

Laboratory studies revealed percentage moisture and temperature to be the prime factors influencing ARA (acetylene reduction activity) in all habitats and rates were highest under submerged conditions and at 22°C. Short-term variations in ARA and salinity in the wet and dry mat areas, measured at 6 day intervals, were also related to percentage moisture. High concentrations of inorganic nitrogen (between 1 and 5 mg l<sup>-1</sup>) significantly depressed ARA in all habitats. Increases in ARA occurred with increase in light intensity up to 40  $\mu\text{E m}^{-2} \text{s}^{-1}$ , with negligible dark rates being recorded in the wet and dry mat areas. Significant dark rates of ARA and stimulation of ARA by sucrose in association with the pneumatophores indicated that bacteria may also be contributing to ARA in this habitat. No organic carbon stimulation was noted in the other sites. Salinity had little effect on ARA over the range generally experienced in each habitat.

Field studies revealed a marked seasonal variation in ARA, with summer maxima of 78, 678 and 341  $\text{nmol C}_2\text{H}_4 \text{ cm}^{-2} 24 \text{ h}^{-1}$  associated with the pneumatophore, wet and dry mat areas respectively. This coincided with maximum nitrogen-fixing blue-green algal numbers, temperature, light intensity and day length. No seasonal variations in organic carbon, inorganic nitrogen, salinity, percentage moisture or bacterial numbers were apparent.

Rates of bacterial ARA associated with decomposing litter of *A. marina* were highest under exposed conditions and reached a maximum of 25 935  $\text{nmol C}_2\text{H}_4 \text{ g dry wt}^{-1} 24 \text{ h}^{-1}$  after 3 weeks. Maximum rates of ARA under submerged conditions of 5394  $\text{nmol C}_2\text{H}_4 \text{ g dry wt}^{-1} 24 \text{ h}^{-1}$  were reached after 4 weeks of decomposition. An increase in percentage nitrogen occurred during decomposition and was greatest under submerged conditions. Rates of decomposition were highest under exposed conditions.

It was estimated that nitrogen fixation by blue-green algal communities supplies 23,8% of the annual nitrogen requirements of the mangrove swamp.

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## CHAPTER 1

### INTRODUCTION

The element nitrogen is an important constituent of proteins, amino-acids and nucleic acids of all living organisms. Unlike most other plant nutrients, nitrogen levels in the biosphere are not maintained simply by rock weathering and mineral recycling. Instead, losses through denitrification, leaching, sedimentation and volatilization require fresh inputs of nitrogen into the system.

Nitrogen gas makes up 78% of our atmosphere (Sprent, 1979), but is unavailable to plants in this form. Only a limited number of organisms are able to convert this nitrogen into an available form for other plants by the process of nitrogen fixation. This process involves the enzymatic conversion of nitrogen from the atmosphere to ammonium by the enzyme nitrogenase (Stewart, 1973), thereby supplying the biosphere with available nitrogen. The organisms responsible are known as diazotrophs which are comprised of bacteria and blue-green algae.

Biological nitrogen fixation is responsible for nearly  $\frac{2}{3}$  of the nitrogen input into the world's soils (Postgate, 1982). Diazotrophy is therefore fundamental to man's economy. It has been suggested that without the continuing input from biological nitrogen fixation, nitrogen levels would be depleted within a geologically short period of time (Brooks *et al.*, 1971). The process of nitrogen fixation has been shown to be important in a wide range of ecosystems including agricultural lands, tundra, grassland, deserts, forests, tropical lands and aquatic systems (Paul, 1978).

Nitrogen is considered to be one of the major nutrients limiting production in coastal and estuarine habitats (Ryther & Dunstan, 1971; Mauge *et al.*, 1974; Silver & Jump, 1975; Long & Mason, 1983). Biological nitrogen fixation is regarded as a major source of replacement for nitrogen which is lost from such systems. A number of studies have been made of the distribution and ecology of nitrogen fixation in several estuaries (Brooks *et al.*, 1971; Herbert, 1975) and salt marshes worldwide (Jones, 1974; Hanson, 1977a; Carpenter *et al.*, 1978; Dicker & Smith, 1980a&b). But these studies were mainly confined to the northern hemisphere. Only recently has a study been carried out on the nitrogen-fixing potential of the Swartkops Estuary, in South Africa (Talbot, 1982).

Nitrogen fixation has been shown to be important in certain high productivity environments such as coral reefs, salt marshes and seagrass beds (Potts, 1984). Mangrove forests are another high productivity environment of the marine shore, but little is known about the role of nitrogen fixation here (Kimball & Teas, 1975; Zuberer & Silver, 1978; Potts, 1979; Hicks & Silvester, 1985), and there is a need to establish the potential in such a system.

The mangrove swamps in the Mgeni Estuary were chosen for this study for the following reasons. Firstly, the convenient locality enabled frequent sampling and rapid transport of material to the laboratory for analysis. Secondly, the study complements research into other aspects of the mangrove ecosystem at the Mgeni Estuary, thus enabling a more complete understanding of the mangrove ecosystem. Litter decomposition studies have shown a nitrogen increase with time in litter bags (Steinke, *et al.*, 1983) and this could in part be attributed to nitrogen fixation by blue-green algae observed on the litter during this study. The zonation and

taxonomy of algae, including blue-green algae associated with pneumatophores of *Avicennia marina* (Forssk.) Vierh. are presently under study in this system. Due to the wide potential for nitrogen fixation in aquatic environments, only selected sites could be studied intensively. It was therefore decided to study the nitrogen-fixing potential of blue-green algal communities in the swamp. It has been suggested (Dugdale & Dugdale, 1962; Brooks *et al.*, 1971) that the contribution of blue-green algae to the nitrogen status of natural ecosystems and more specifically to salt marshes and estuaries (Stewart & Pugh, 1963; Jones, 1974; Bohlool & Wiebe, 1978) is probably much more important than the contribution by heterotrophic micro-organisms. In most of these studies, a variety of habitats were studied and significantly higher rates of nitrogen fixation were associated with large populations of blue-green algae.

The objectives were to conduct a study of nitrogen fixation in the Mgeni Estuary mangrove swamp and devise suitable methods in order to determine:

- a) the effect of variation of certain environmental parameters on nitrogen fixation i.e. moisture content, temperature, salinity, light intensity, organic carbon and inorganic nitrogen in relation to *in situ* recordings of these parameters;
- b) seasonal variation in nitrogen fixation over the period of a year and also to monitor physical and chemical parameters which may influence nitrogen fixation;
- c) the organisms responsible for nitrogen fixation in the selected sites and to monitor seasonal changes in diazotrophic populations

(blue-green algae and bacteria);

- d) levels of nitrogen-fixation and total nitrogen associated with decomposing mangrove litter;
- e) the annual contribution of blue-green algal communities to the nitrogen budget of the Mgeni Estuary mangrove swamp.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 THE NITROGEN STATUS OF ESTUARINE AND COASTAL ENVIRONMENTS

Nitrogen is an important nutrient in coastal environments (Ryther & Dunstan, 1971) and plays a critical role in determining the structure and function of ecosystems (Valiela & Teal, 1979). The rate of turnover in such systems at all trophic levels depends largely on the supply of nitrogen. The export of nitrogenous materials and organic matter to coastal waters from estuaries and salt marshes enhances productivity of these waters by providing nutrients for coastal organisms (Valiela & Teal, 1979). It should be noted that subsequent work has shown that net export or import of nitrogen varies widely from marsh to marsh (Nixon, 1980).

Nitrogen is generally considered to be a major nutrient limiting primary production in marine environments (Herbert, 1975; Bohlool & Wiebe, 1978; Lipschultz *et al.*, 1979). Ryther & Dunstan (1971) calculated that most temperate estuaries suffer nitrogen deficiencies. Various researchers have conducted fertilizer experiments in estuarine areas and the resultant increase in plant biomass was thought to provide proof of this. The plant biomass in salt marshes was shown to increase by 15% and 28% by Patrick & DeLaune (1976) and Buresh *et al.* (1980) respectively on application of nitrogenous fertilizer. Sewage sludge stimulated *Spartina alterniflora* Loisel production in a Georgia salt marsh (Hanson, 1977b). Bishop *et al.* (1984) showed levels of dissolved inorganic nitrogen to be limiting to phytoplankton in Georgia

nearshore waters. The literature indicates that nitrogen limitation to productivity is also apparent in mangrove swamps, which are generally accepted as highly productive areas. Significant growth responses have been noted in mangroves following enrichment of soils with bird guano (Onuf *et al.*, 1977) and ammonium (Boto & Wellington, 1983). Nitrogen limitation has also been studied and identified in communities of macroalgae (Mann, 1979) and seagrasses (Orth, 1977; Bulthuis & Woelkering, 1981).

Inorganic nitrogen levels in estuaries and coastal areas are generally low. Southern Californian coastal waters revealed low levels of ammonium ( $< 18 \mu\text{g l}^{-1}$ ) (Eppley *et al.*, 1979). Ammonium and combined nitrate and nitrite levels reported from the central Great Barrier Reef were 4,96 to 13,44  $\mu\text{g l}^{-1}$  and 9,6 to 20,16  $\mu\text{g l}^{-1}$  respectively (Wilkinson *et al.*, 1984). Low inorganic nitrogen levels have also been recorded in interstitial waters of the Swartkops Estuary (Talbot, 1982). Levels of inorganic nitrogen measured in the Mgeni Estuary were high, with ammonium and nitrate levels ranging from 764 to 802  $\mu\text{g l}^{-1}$  and 1039 to 1470  $\mu\text{g l}^{-1}$  respectively (Day, 1981). But this estuary is regarded as the most polluted estuary along the Natal coast and these values are ten times higher than values recorded for unpolluted Natal estuaries (Day, 1981). A number of researchers have reported low levels of nitrogen in the interstitial waters of salt marshes (Patriquin & Keddy, 1978; Lipschultz *et al.*, 1979; Casselman *et al.*, 1981) and waters of several mangrove swamps (Walsh, 1967; Nedwell, 1975; Boto & Wellington, 1984). Nixon (1980) estimated that most marsh sediments have a nitrogen content of between 5 and 20  $\text{g m}^{-2}$ .

However, more recently Boto *et al.* (1985) found that certain marsh

sediments contain high concentrations of nitrogen, mainly in the form of ammonium. This led them to conclude that the positive response of salt marsh plants and mangroves to fertilizer application was paradoxical. It was suggested that other factors may contribute to the lower standing crop biomass and these include low phosphorous availability, higher salinities and lower redox potentials (Boto & Wellington, 1984). The apparent nitrogen limitation may also be related to the anaerobic nature of most marsh soils (Boto *et al.*, 1985).

Combined inorganic nitrogen levels may appear high in certain instances, but nitrogen may not always be available in its optimal state. Most researchers believe that ammonium is taken up in preference to nitrate in the anaerobic muds of salt marshes and mangrove swamps (Boto & Wellington, 1983). More recently, Boto *et al.* (1985) showed that growth of *A. marina* was significantly enhanced by increased supply of nitrates. This is contrary to the generally accepted view. Measured nitrogen levels may also be high, but unavailable to plants as this element becomes adsorbed on to clay and silt particles (Beefink, 1977).

However, it is widely recognised that nitrogen is limiting in coastal and estuarine environments and it is in this light that the significance of the contribution by nitrogen fixation should be evaluated.

## 2.2 NITROGEN CYCLING IN ESTUARINE AREAS

Nitrogen cycling in estuarine and coastal environments, and the contribution of nitrogen fixation to nitrogen budgets in these areas, is poorly understood (Paerl *et al.*, 1981). Detailed studies have only

been conducted on the Great Sippewissett salt marsh (Valiela & Teal, 1979), Gulf Coast salt marshes (DeLaune & Patrick, 1980a; Smith *et al.*, 1982) and Colne Point salt marsh, U.K. (Abdul Aziz & Nedwell, 1986a, b). Estimates of nitrogen budgets were drawn up for these salt marshes. Comparative examples of nitrogen cycles for southern hemisphere estuaries are lacking. No known study has been conducted in mangrove swamps.

The above studies show significant exchange of nutrients between upland, marsh and coastal areas. Ground water is a major input of nitrogen in the Great Sippewissett salt marsh, while precipitation, nitrogen fixation, sedimentation and to a lesser extent defaecation provide other inputs. Nitrogen fixation provides 9% of the input of nitrogen to the Great Sippewissett marsh, while in the Gulf Coast salt marsh it is estimated to be the second largest input, sedimentation being the most important. Valiela & Teal (1979) also reported that tidal exchange resulted in a significant exchange of nutrients, although greater exports resulted in a net export of nutrients. In contrast, Abdul Aziz & Nedwell (1986a) and Smith *et al.* (1982) found tidal exchange to involve a minor portion of the total nitrogen cycled, as low levels of nitrogen were found to be exported from the salt marsh to the estuary by tides. Other outputs included denitrification, sedimentation and volatilization of ammonium. In the Great Sippewissett and Colne Point salt marshes, imports and exports of nitrogen were found to be balanced, whereas in the Gulf Coast salt marsh a net accumulation of  $21 \text{ g N m}^{-2} \text{ yr}^{-1}$  was apparent.

It is obvious that generalizations cannot be made when comparing one



system with another. Each system should be studied individually. The contribution of individual processes to the total nitrogen budget differs from one system to the next and is dependent upon local conditions.

Individual aspects of the nitrogen cycle of other systems have also been studied. Inputs by nitrogen fixation have been extensively studied and will be discussed in Section 2.5. Without studies of other inputs and exports of the nitrogen cycle in each system, the significance of nitrogen fixation to the total nitrogen budget cannot be assessed.

Denitrification has been studied in a number of salt marshes (Jones, 1974; Kaplan *et al.*, 1977) and marine and estuarine sediments (Sørensen, 1978; Kaspar, 1982; MacFarlane & Herbert, 1984).

Nitrification has been studied in estuarine sediments (MacFarlane & Herbert, 1984) and in an estuarine water column (Owens, 1986). DeLaune *et al.* (1983) studied losses from a salt marsh as a result of loss of plant material by export or mineralization of plant nitrogen and subsequent ammonium volatilization and/or nitrification - denitrification loss reactions. Sedimentation and its role in nutrient cycling has also been examined (DeLaune & Patrick, 1980b).

The influence of eutrophication on the nitrogen cycle has received attention. Many estuaries and other coastal marine ecosystems receive increased nitrogen from sewage, industrial wastes and agricultural runoff (Walsh *et al.*, 1981). The ecological consequences and fate of increased nitrogen inputs in coastal marine ecosystems is not well known. Research has shown increased rates of nitrification (Berounsky & Nixon, 1985) and denitrification (Seitzinger & Nixon, 1985) in eutrophicated systems. The effect of eutrophication on nitrogen

fixation is variable, as it is either stimulated or inhibited (Van Raalte *et al.*, 1974; Hanson, 1977b; Bohlool, 1978; Pearson & Taylor, 1978). Van Raalte *et al.* (1974) suggested that additions of combined nitrogen, such as may occur through sewage contamination, may change the structure of the nitrogen cycle in a salt marsh environment, replacing nitrogen fixation as a nitrogen input. Seitzinger & Nixon (1985) suggested that "the homeostatic capacity of coastal ecosystems can be exceeded by nitrogen inputs of a magnitude experienced by an increasing number of estuaries, bays and lagoons". The influence of pollution on the nitrogen cycle is not clearly understood and more studies are necessary in this regard.

### 2.3 THE RANGE OF NITROGEN-FIXING ORGANISMS

The organisms capable of biological nitrogen fixation are known as diazotrophs and belong to the kingdom Prokaryotae (Jensen, 1981). Nitrogen-fixing prokaryotes can be broadly divided into 3 groups: the heterotrophic bacteria, the photosynthetic bacteria and the blue-green algae. The last 2 groups are unique in being autotrophic and able to fix nitrogen. Reports that filamentous fungi and yeasts also fix nitrogen have been refuted after more thorough investigations (Millbank, 1969; Dixon & Wheeler, 1986).

Nitrogen fixation by bacteria was initially established by Hellriegel and Wilforth (1886 - 1888) in root nodules of legumes (Stewart, 1974). Non-symbiotic nitrogen fixation by bacteria was demonstrated by Winogradsky in 1895 (La Rue, 1977). Soils containing blue-green algae were first demonstrated to fix nitrogen by Frank in 1889 (Stewart, 1974).

The earliest pure nitrogen-fixing cultures of blue-green algae were obtained by 1928 by Drewes (Stewart, 1973).

Subsequently, many nitrogen-fixing organisms have been reported. Three main groups of blue-green algae exist, all having nitrogen-fixing representatives (Stewart, 1977). These are the unicellular, filamentous non-heterocystous and filamentous heterocystous forms. It was originally thought that only the heterocystous groups fixed nitrogen. The enzyme nitrogenase is oxygen-sensitive and breaks down in its presence and was therefore thought to be confined to the heterocysts (Wolk, 1982). Heterocysts lack the oxygen-evolving photosystem II of photosynthesis. This process is active in the vegetative cells only and in this way, oxygen-evolving photosynthesis is spatially separated from nitrogen-fixation.

More recently, non-heterocystous filamentous blue-green algae and unicellular blue-green algae have also been shown to fix nitrogen. Many reports need confirmation though, as axenic cultures of all species have not been obtained. Associated bacteria may be responsible for the observed nitrogen fixation (Taylor *et al.*, 1973).

Various mechanisms have been proposed as to how nitrogenase is protected from oxygen in bacteria and non-heterocystous blue-green algae. A discussion of these mechanisms is felt to be important as only non-heterocystous blue-green algae were identified in this study.

Obligately anaerobic bacteria such as *Desulfovibrio* spp. adopt a strategy of total avoidance of oxygen (Postgate, 1982). Facultatively

anaerobic bacteria such as *Klebsiella* spp. do not fix nitrogen in the presence of dissolved oxygen (Postgate, 1982). Dalton & Postgate (1969) proposed two oxygen-excluding processes for *Azotobacter* spp., an obligate aerobe. Firstly, "respiratory protection" which actively protects the functioning enzyme and secondly "conformational protection" which passively protects, though it reversibly inactivates the enzyme. Many aerobic diazotrophs fix nitrogen only under micro-aerophilic conditions. Such a "switch off/on" mechanism was proposed in *Oscillatoria* sp. by Stal & Krumbein (1985b). Other diazotrophic bacteria produce mucilage which may assist in impeding access of oxygen to the cell (Postgate, 1982). Clustering of bacteria may exclude oxygen more effectively than single cells and decrease areas of oxygen to central cells (Postgate, 1982). *Oscillatoria* (*Trichodesmium*) *thiebautii* forms bundles and it has been proposed that nitrogen fixation takes place in the centre and photosynthesis on the periphery (Carpenter & Price, 1976). Stal & Krumbein (1985a) proposed a temporal separation of oxygenic photosynthesis and nitrogen fixation in *Oscillatoria* sp. Stal & Krumbein (1985b) also gave evidence of high rates of synthesis of nitrogenase for *Oscillatoria* sp. under aerobic conditions in order to counteract losses of irreversibly inactivated enzyme.

The most commonly isolated nitrogen-fixing bacteria from estuarine and marine areas are species of *Azotobacter*, *Desulfovibrio*, *Klebsiella*, *Enterobacter* and *Clostridium* (Rodina, 1964; Werner *et al.*, 1974; Herbert, 1975; Zuberer & Silver, 1975; Dicker & Smith, 1980c; Uchino *et al.*, 1984). Both heterocystous (species of *Calothrix*, *Anabaena*, *Stigonema*, *Nostoc*, *Rivularia* and *Scytonema*) and non-heterocystous (species of *Lyngbya*, *Oscillatoria*, *Trichodesmium* and *Phormidium*) blue-green algae

are also thought to fix nitrogen in these areas (Jones, 1974; Dor, 1975; Carpenter & Price, 1976; Bohlool & Wiebe, 1978; Carpenter *et al.*, 1978; Potts, 1979). The importance of blue-green algal versus bacterial nitrogen fixation in marine and estuarine areas is discussed in Section 2.5.

#### 2.4 THE TAXONOMY OF THE BLUE-GREEN ALGAE

Blue-green algae are classified differently by bacteriologists and phycologists into the divisions Cyanobacteria and Cyanophyta respectively. This results from an unresolved argument between the two groups as to the position of the blue-green algae in the classification system. Phycologists regard any organism with chlorophyll a and a thallus not differentiated into roots, stems and leaves as an alga (Lee, 1980). This definition includes the blue-green algae. Bacteriologists regard the procaryotic cell structure and the structure of the cell wall, which is similar to gram -ve bacteria, as important, indicating a possible relationship between blue-green algae and bacteria (Lee, 1980). In this thesis, the phycologists viewpoint has been supported and subsequently reference will be made to blue-green algae.

The taxonomy of the blue-green algae is in a state of uncertainty. It was therefore felt that the following discussion would serve to highlight some of the difficulties encountered, and put into perspective the reasons for using the system followed in this thesis.

Difficulties in naming blue-green algae recognized by Desikachary (1959) arise as a result of lack of distinguishing characters between



taxa, the presence of intermediate characteristics and forms and the extreme variation which often exists within a species. The variability of these organisms has led to fundamentally different approaches to their taxonomy (Komárek, 1973).

Amongst the phycologists, two schools of thought are apparent: the "splitters" and the "lumpers". The "splitters" have created many genera and species based on morphological studies (Geitler, 1932; Papenfuss, 1955; Desikachary, 1959; Bourrelly, 1970; Bold & Wynne, 1978). The "lumpers" (Drouet, 1968, 1973, 1978; Humm & Wicks, 1980) have regrouped many taxa into a few species. They regarded many of the characters used by the former school as environmentally induced and not genetic and therefore taxonomically unsound. Drouet regarded many of the older names as being ecophenes of the same species that will revert to the same phenotype when grown under the same conditions. However, a number of culture studies do not support this concept (Stanier *et al.*, 1971). Humm & Wicks (1980), students of Drouet, followed his classification, and presented a very basic guide to the identification of marine blue-green algae. Criticisms of Drouet's work include the fact that in many cases, only herbarium specimens were investigated.

The bacteriologists have proposed that cyanobacterial identification should be based on the study of cultured material. Stanier *et al.* (1971) proposed an alternative system of classification based on physiological and biochemical characteristics of clonal axenic cultures. Such a classification has been attempted by Kenyon *et al.* (1972). One of the criteria they used was the ability to synthesise nitrogenase

aerobically or anaerobically. More work is necessary in this respect before the value of this type of classification can be assessed.

Both phycologists and bacteriologists recognise the importance of the development of a taxonomic system combining morphological and bacteriological characters based upon reference to cultures. A proposal was made to transfer the nomenclature of blue-green algae from the rules of the International Code of Botanical Nomenclature to that of the International Code of Nomenclature of Bacteria by Stanier *et al.* (1978). Until an intensive study of both aspects has been conducted, many bacteriologists and phycologists alike favour the classical taxonomic system of Geitler (1932) (Stanier *et al.*, 1971; Potts & Whitton, 1980; Dawes, 1981). In this study, Desikachary's system was used, which is regarded as a direct translation of Geitler (1932). Some reference was also made to Humm & Wicks (1980) when necessary. A more detailed discussion in this regard will be included in the text (Sections 5.2.1 and 5.3.1).

## 2.5 NITROGEN FIXATION IN ESTUARINE AND COASTAL ENVIRONMENTS

In reviewing nitrogen fixation in estuarine and coastal habitats, emphasis will be placed on the importance of nitrogen fixation in different environments and the relative contribution of blue-green algae and bacteria to nitrogen fixation.

Chapman, 1960 (in Dicker & Smith, 1980a) claimed that nitrogen fixation was insignificant in salt marshes because of the large amounts of organic matter (and presumably fixed nitrogen) present in such a

system - a maximum of 90% by dry weight.

In 1962, Turner and Grey isolated bacteria from a developing salt marsh, but no indication was given of the presence of diazotrophs. Blue-green algae were isolated from a developing salt marsh (Stewart & Pugh, 1963) and 17% were already known diazotrophs, implying salt marsh sediments to be a site of nitrogen fixation. The contribution of blue-green algae to the nitrogen budget of salt marshes has also been suggested by Fog (1973) and Chapman & Chapman (1973).

Nitrogen-fixing bacteria were first isolated from various types of mangrove sediments by Rodina (1964). *Azotobacter* spp., *Clostridium* sp., *Spirillum* sp. and desulfurizing bacteria were isolated and direct counts revealed the presence of *Azotobacter*-like cells in considerable numbers. Rodina concluded that these organisms were of great importance to the nitrogen balance of these soils and that the most important factor increasing their activity was a high supply of organic substances. Since this time, diazotrophic bacteria (Zuberer & Silver, 1975, 1978) and blue-green algae (Zuberer & Silver, 1975) have been isolated from mangrove sediments.

Brooks *et al.* (1971) provided the first real evidence of *in situ* bacterial nitrogen fixation in subtidal sediments of the Waccasassa Estuary in Florida and succeeded in isolating a diazotrophic *Clostridium* spp. from the sediments. However, rates of nitrogen fixation were low, and the phenomenon was not regarded as an important nitrogen source to overlying waters. Similar rates were found by Marsho *et al.* (1975) in intertidal and subtidal sediments of the



Rhode Estuary of Chesapeake Bay, where heterotrophic nitrogen fixation was estimated to account for less than 5% of the total influx of nitrogen into this system. Herbert (1975) and Nedwell & Abdul Aziz (1980) also showed low rates of heterotrophic nitrogen fixation associated with subtidal sediments of the Tay Estuary, Scotland and intertidal sediments of the Colne Point Estuary, Essex respectively. Herbert (1975) suggested that since the nitrogen content of the sediments was low, it was probable that heterotrophic nitrogen fixation does play a role in the nitrogen budget of the estuary. Herbert isolated species of *Azotobacter*, *Klebsiella*, *Enterobacter*, *Desulfovibrio* and *Clostridium* from estuarine sediments.

Nitrogen fixation by blue-green algae has been shown to be important and associated with several sites within salt marshes (Jones, 1974; Van Raalte *et al.*, 1974; Whitney *et al.*, 1975; Carpenter *et al.*, 1978). Gotto *et al.* (1981) reported that nitrogen fixation by blue-green algal mats in intertidal environments of the Texas Gulf Coast contributed significantly to the nitrogen economy of the shallow coastal environment. A study of intertidal mud flats of the Delaware Inlet, New Zealand, revealed that 50% of the total nitrogen fixed in this area could be accounted for by a mixed bloom of *Oscillatoria-Euglena* - photosynthetic bacteria occupying only 1% of the area (Bohlool & Wiebe, 1978).

Contrary to the above authors, Patriquin & McClung (1978) and Casselman *et al.* (1981) indicated limited importance of blue-green algal nitrogen fixation in a Nova Scotian and Gulf Coast salt marsh respectively.

Nitrogen fixation in mangrove swamps has been studied to a limited extent but little is known of the contribution of biologically fixed nitrogen to the mangrove ecosystem.

A correlation between rates of nitrogen fixation and the availability of organic material has been shown by Flordelis & Aspiras (1979) (in Potts, 1984) in a Philippine mangrove swamp and by Hicks & Silvester (1985) in a New Zealand mangrove swamp. Studies of Florida mangrove swamps (Kimball & Teas, 1975; Zuberer & Silver, 1975, 1978) showed that rates of nitrogen fixation were very low in plant- and litter-free sediments as opposed to plant-associated sediments.

Root-associated sediments have been demonstrated to have significantly higher rates of nitrogen fixation compared to root-free sediments (Zuberer & Silver, 1975). Root systems of three Florida mangroves, *Rhizophora mangle* L., *Avicennia germinans* (L.) Stern and *Laguncularia racemosa* Gaertn. were shown to have significant rates of nitrogen fixation (Zuberer & Silver, 1978). Associative symbiosis has been suggested as existing between these roots and bacteria, some of which are diazotrophs in the rhizosphere-rhizoplane (Zuberer & Silver, 1979). Van der Valk & Attiwill (1984a) also showed nitrogen fixation associated with live roots of *A. marina* from Australia.

Little attention has been paid to nitrogen fixation by organisms associated with pneumatophores. Hicks & Silvester (1985) concluded that the highest rates of nitrogenase activity in a New Zealand mangrove swamp were associated with excised pneumatophores. This activity was correlated largely with the presence of blue-green algae. Similarly,

in a study of Sinai mangroves, Potts (1979) showed high rates of nitrogen fixation associated with blue-green algal communities on pneumatophores of *A. marina*.

Significant rates of nitrogen fixation were found associated with decomposing mangrove leaf litter (Zuberer & Silver, 1978; Van der Valk & Attiwill, 1984a; Hicks & Silvester, 1985). The highest rates of nitrogen fixation reported in mangrove swamps are associated with decomposing leaves of *R. mangle* from Florida (Gotto & Taylor, 1976). Bacteria have mainly been claimed responsible for nitrogen fixation associated with decomposing leaves with the exception of *R. mangle* leaves from Florida where nitrogenase activity was shown to be light dependent.

The warty lenticellate bark of *Bruguiera gymnorhiza* (L.) Lam. has also been shown to contribute significantly to the input of nitrogen through nitrogen fixation (Uchino *et al.*, 1984). In this study, bacteria responsible for nitrogen fixation were isolated from the bark, and their capacity for nitrogen fixation confirmed.

Most researchers agree that nitrogen fixation, however limited, plays some role in the nitrogen budget of estuaries. In reviewing 20 years of research on the role of salt marshes in estuarine productivity, Nixon (1980) estimated that nitrogen fixation may add  $5 \text{ to } 28 \text{ g N m}^{-2} \text{ yr}^{-1}$ .

In the open sea, blue-green algae appear to be the primary agents of nitrogen fixation (Carpenter, 1973; Taylor *et al.*, 1973; Carpenter &

McCarthy, 1975). Although heterotrophic organisms are present in the open sea, their activity seems to be minimal (Fogg, 1978; Paerl *et al.*, 1981). The most important blue-green algal fixers appear to be *Trichodesmium (Oscillatoria)* spp. Heterocystous blue-green algae are sparse in the open sea (Fogg, 1978).

Results are conflicting with respect to nearly all aspects of the ecology of nitrogen fixation, including rates of nitrogen fixation in different vegetational areas, variation of fixation with depth, the contribution by blue-green algae and bacteria and the effect of oxygen and combined nitrogen on fixation.

Due to high temporal and spatial variations in nitrogen fixation (Marsho *et al.*, 1975, Dicker & Smith, 1980a), difficulties are encountered when extrapolating from localized data to the ecosystem as a whole. Caution has to be exercised in making generalizations of nitrogen fixation over a large area. This has been pointed out by a number of researchers (Bohloul & Wiebe, 1978; Carpenter *et al.*, 1978; Patriquin & McClung, 1978; Dicker & Smith, 1980a).

Bearing this in mind, tentative assessments of the significance of biological nitrogen fixation to the ecosystem as a whole have been made. Green & Edmisten (1974) related high primary productivity of several Gulf Coast salt marshes to high rates of nitrogen fixation. Similarly, Whitney *et al.* (1975) maintained that the high daily fixation of Flax Pond salt marsh was an indication that nitrogen fixation was an important contributor to the nitrogen budget.

## 2.6 THE EFFECT OF ENVIRONMENTAL FACTORS ON NITROGEN FIXATION

The effect of environmental conditions on nitrogen fixation has been reviewed in freshwater (Sprent, 1979; Fay, 1981; Paerl *et al.*, 1981), terrestrial (Stewart *et al.*, 1978; Sprent, 1979; Fay, 1981), marine (Sprent, 1979; Fay, 1981) and estuarine and coastal habitats (Paerl *et al.*, 1981). Environmental influences on blue-green algae (Stewart, 1974, 1977; Fay, 1981) and bacteria (Jensen, 1981) have also been reviewed.

In natural ecosystems, especially intertidal and estuarine environments, organisms are subjected to a variety of extreme and often rapidly fluctuating chemical, physical and biological conditions (Stewart *et al.*, 1978). In any ecological study, the question of how organisms adjust their growth and various processes such as photosynthesis, respiration and nitrogen fixation to a particular set of environmental conditions is raised. The effects of various conditions are commonly studied independently under laboratory conditions, but in a natural ecosystem, these conditions do not act independently. The difficulty of distinguishing the effects of different environmental conditions acting simultaneously has been pointed out by Jensen (1981). Conditions found in natural ecosystems often promote suboptimal rates of nitrogen fixation and the full potential is seldom realised (Jones, 1974).

Among the environmental parameters which may be important in determining nitrogen fixation in mangrove swamps are submergence, degree of exposure, temperature, salinity, light intensity, organic carbon and inorganic nitrogen. The importance of these factors is outlined below.

Moisture content is regarded as one of the most important factors influencing nitrogen fixation in a variety of habitats including



mangroves (Potts, 1979), tundra soils (Wojciechowski & Heimbrook, 1984), coastal dunes (Sprent, 1979), salt marshes (Carpenter *et al.*, 1978; Talbot, 1982) and desert algal crusts (MacGregor & Johnson, 1971). Stewart (1974, 1977) regarded moisture content as being the single most important factor influencing nitrogen fixation by blue-green algae.

Many nitrogen-fixing blue-green algae are well adapted to fluctuations in moisture content and have thick mucilagenous sheaths, enabling rapid absorption and slow loss of moisture (Stewart, 1977). In the Sinai mangrove swamps, Dor (1975) noted that the inner part of blue-green algal thalli were composed of thick, empty sheaths and suggested that this forms a capillary system absorbing water at high tide and retaining it during periods of exposure. Blue-green algal mats, dried for up to 2 years, have been shown to resume nitrogen fixation within 24 h of rehydration (MacGregor & Johnson, 1971; Whitton *et al.*, 1979; Scherer *et al.*, 1984). The inhibitory effect of desiccation on blue-green algal mats and surface sediments has been reported in salt marshes (Jones, 1974; Zedler, 1980; Talbot, 1982), mangroves (Potts, 1979) and terrestrial habitats (Ogan, 1983). In the absence of desiccation, temperature is regarded as a major factor controlling nitrogen fixation in blue-green algal mats (Jones, 1977a).

Nitrogen-fixing blue-green algae are abundant in a variety of habitats, ranging from hot springs to the cold arctic and antarctic regions (Stewart, 1974), where extremes in temperature range from 60°C to sub-zero. However, bacteria are mesophilic micro-organisms and exhibit a more limited range of distribution, being excluded from the hottest naturally occurring ecological niches such as hot springs (Jensen, 1981).

The distribution of bacteria is usually related to other factors such as pH, organic carbon and moisture (Jensen, 1981).

In reviewing the literature on soil blue-green algae, Stewart *et al.* (1978) concluded that a general correlation exists between the temperature responses of algae to nitrogen fixation and the temperatures of habitats where these organisms naturally occur. Nitrogen fixation can usually be detected at 54°C in hot springs (Sprent, 1979) and near 0°C in arctic and antarctic regions (Horne, 1972). However, optimum temperatures for blue-green algae generally occur between 32,5° and 35°C, which is higher than for most other algae (Stewart, 1977).

Marked variations in salinities in different marine and estuarine habitats render salinity a potential limiting factor to nitrogen fixation. Contradictory reports on the effects of salinity on nitrogen fixation in blue-green algae and bacteria appear in the literature (Jones, 1974; Werner *et al.*, 1974; Herbert, 1975; Potts & Whitton, 1977; Patriquin & Keddy, 1978; Ubben & Hanson, 1980; Dicker & Smith, 1981), although organisms appear to be adapted to ambient ranges in salinity.

In studying the distribution of blue-green algae in Sinai mangrove swamps, Dor (1975) reported these organisms to be well adapted to methohaline and hypersaline conditions. Marathe (1965) correlated high salt content in mangrove soils with the presence of lowest numbers of blue-green algal species, implying that salinity does affect the distribution of blue-green algae.

Light is recognised as an important factor influencing the distribution

and nitrogen fixation of photosynthetic micro-organisms as this process is principally dependent upon energy reserves derived during photosynthesis (Fay, 1981). The relationship between light intensity and nitrogen fixation is not very direct and is complicated by the influence of other factors such as availability of energy reserves (Head & Carpenter, 1975; Jones, 1977d; Finke & Seeley, 1978), *in situ* temperatures (Jones, 1977d) and nutrients (Carpenter *et al.*, 1978). The rate and duration of dark fixation by blue-green algae is dependent on conditions such as light intensity, CO<sub>2</sub> concentration and supply of energy reserves during the preceding light period (Fay, 1976; Jones, 1977d).

Generally, the relationship between light intensity and nitrogen fixation is asymptotic. Conflicting reports exist as to the effect of high light intensity on nitrogen fixation (Stewart, 1974; Jones, 1977b; Carpenter *et al.*, 1978; Stewart *et al.*, 1978; Fay, 1981; Talbot, 1982; Smith, 1984). Stewart (1974) and Stewart *et al.* (1978) suggested that blue-green algae adapt to high light intensities by increasing pigmentation or aggregating together to effect self-shading, resulting in an effective decrease in light intensity.

Organic carbon is one of the most important requirements for nitrogen fixation of diazotrophs. Autotrophic micro-organisms manufacture carbohydrates directly during photosynthesis. In this case, organic carbon availability is linked to light intensity. Heterotrophic nitrogen-fixing bacteria are capable of utilizing a wide range of low molecular weight carbon compounds, including organic acids (pyruvate, lactate, malate), simple alcohols, hydrocarbons (methane) and mono- and disaccharides (Jensen, 1981). These may be available as organic exudates



from roots (Jones, 1974; Hanson, 1977b; Zuberer & Silver, 1978), sugars in nodules (Parker, 1977) or dead organic matter (Zuberer & Silver, 1975, 1978).

Carbon compounds decompose easily and are utilized by numerous non-nitrogen fixing organisms. Consequently, these compounds are usually found in low concentrations in natural ecosystems and their availability will often be a limiting factor for nitrogen fixation. This has been demonstrated experimentally by the addition of decomposable carbon substances which have resulted in considerable increases in nitrogen fixation in salt marshes (Jones, 1974; Hanson, 1977a; Talbot, 1982), mangroves (Zuberer & Silver, 1975, 1978) and seagrasses (Patriquin & Knowles, 1972).

The inhibitory effects of combined nitrogen on nitrogen fixation are well documented in a number of habitats, including mangroves (Potts, 1979), salt marshes (Hanson, 1977a; Carpenter *et al.*, 1978; Patriquin & Keddy, 1978; Teal *et al.*, 1979; Dicker & Smith, 1980b; Casselman *et al.*, 1981), marine environments (Carpenter, 1973; Head & Carpenter, 1975) and lakes (Ogan, 1983). Ammonium and nitrate are the two most commonly cited suppressors of nitrogen fixation.

The relationship is not simple as the decline in nitrogen fixation is often related to factors other than ambient nitrogen concentrations, such as phosphorus availability (Flett, 1976 in Paerl *et al.*, 1981) and population density of organisms (Patriquin & Knowles, 1975). Responses have also been shown to vary seasonally (Dicker & Smith, 1980b).

The significance of inhibition of nitrogen fixation by high combined nitrogen levels in culture is often queried as addition of lower *in situ* levels are often not inhibitory (Hanson, 1977a, b; Teal *et al.*, 1979).

The addition of a variety of nitrogen compounds has been shown to decrease nitrogen fixation in salt marsh systems (Van Raalte *et al.*, 1974; Hanson, 1977a; Carpenter *et al.*, 1978). Other studies have noted an inverse correlation between ground water ammonium and nitrogen fixation (Head & Carpenter, 1975; Carpenter, 1978; Patriquin & Keddy, 1978; Teal *et al.*, 1979; Casselman *et al.*, 1981).

## 2.7 SEASONAL VARIATION IN NITROGEN FIXATION

Studies on seasonal variation in estuarine nitrogen fixation have mostly been conducted in cooler northern hemisphere climates where distinctive seasonal patterns of nitrogen fixation are evident (Green & Edmisten, 1974; Jones, 1974; Marsho *et al.*, 1975; Carpenter *et al.*, 1978; Dicker & Smith, 1980a; Casselman *et al.*, 1981). Maximum rates of nitrogen fixation generally occur from late spring to summer. Talbot (1982) reported high autumnal rates of nitrogen fixation in surface sediments of the Swartkops Estuary. Seasonal variation of nitrogen fixation in mangrove swamps has received little attention, the only known study being one conducted in New Zealand, where maximum rates were reported in summer (Hicks & Silvester, 1985).

Temperature has been claimed to be responsible for seasonal trends, especially in colder climates where winter temperatures are inhibitory to nitrogen fixation. Few other factors have been considered to play

a role (Dicker & Smith, 1980a; Talbot, 1982; Wojciechowski & Heimbrook, 1984). The possibility that the observed seasonal patterns result from changing diazotrophic populations has been considered by few researchers (Lean *et al.*, 1978; Dicker & Smith, 1980a; Talbot, 1982).

## 2.8 TECHNIQUES FOR THE MEASUREMENT OF NITROGEN FIXATION

A number of techniques may be used for the estimation of nitrogenase activity. Direct methods of measurement include the determination of increases in total nitrogen in a system using Kjeldahl or Dumas methods (Hardy *et al.*, 1973) and the use of radioactive isotope  $^{13}\text{N}$  and stable isotope  $^{15}\text{N}$  (Bergersen, 1980). The former method is not very sensitive to small nitrogen increases (Bergersen, 1980) and large samples and long time intervals are required (Gaskins & Carter, 1976). According to Hardy *et al.* (1973), the latter method is 1000 times more sensitive than the former, but is costly and time consuming. The  $^{13}\text{N}$  isotope is not widely used as it has a short half-life of 9.96 min (Bergersen, 1980).

Indirect methods of measurement are based on the ability of nitrogenase to reduce a number of substrates other than molecular nitrogen such as azide, cyanide, isocyanide and acetylene (Turner & Gibson, 1980). The acetylene reduction assay, involving the reduction of acetylene to ethylene, is widely used for measurement of nitrogenase activity. The application of this assay to samples from natural environments is described in detail by Stewart *et al.* (1967) and Hardy *et al.* (1968). This method is popular and advantages listed by Hardy *et al.* (1968)

include simplicity, rapidity, cost effectiveness and sensitivity (1000 times more sensitive than isotope methods). David *et al.* (1980) has proposed using ammonical silver nitrate to precipitate acetylene, thus permitting the gas chromatographic analysis of one sample in less than a minute. The acetylene reduction method has been used widely by researchers in estuarine areas and use of this method in the present study will facilitate comparison of results.

The technique basically involves the incubation of material in an air-tight assay chamber sealed with a rubber serum stopper in order to allow addition and removal of gas.. Material is exposed to an atmosphere containing acetylene for a set incubation time, which is dependent on the material being assayed and gas samples are then removed and analysed for ethylene using gas chromatography. The rates of ethylene production can then be converted to rates of nitrogen fixation using a conversion factor. A theoretical conversion factor of 3:1 (3 moles acetylene reduced to ethylene per mole of nitrogen reduced), which is based on the stoichiometric relationship between acetylene reduced and nitrogen fixed is often used (Gotto & Taylor, 1976; Casselman *et al.*, 1981).

However, the conversion factor has been measured in a number of systems using  $^{15}\text{N}$  uptake measurements and was found to range from 2 to 25:1 (Hardy *et al.*, 1973; Bergersen, 1970). The values used are generally in the range of 3 to 6 (Smith, 1980). Various researchers have proposed the use of 4 as a conversion factor rather than 3 (Peterson & Burris, 1976; Turner & Gibson, 1980; Jensen & Cox, 1983). Jensen & Cox (1983) explained that for every mole of nitrogen reduced, 1 mole of

hydrogen was produced. Acetylene inhibits hydrogen production and electrons involved are used to reduce acetylene to ethylene, raising the conversion factor to 4. Experimental evidence obtained from populations of blue-green algae support the use of 4 as a conversion factor (Peterson & Burris, 1976; Jensen & Cox, 1983). Peterson & Burris (1976) believe that the conversion factor depends on the organisms involved and the experimental conditions employed. Deviation from expected values can also arise when acetylene and/or nitrogen are supplied at less than saturating levels (Peterson & Burris, 1976; Jensen & Cox, 1983).

The acetylene reduction assay has been applied in a wide range of environments and under certain conditions, modifications of the basic techniques are required. Under waterlogged conditions, such as found in aquatic environments, estuaries and rice paddy soils, poor gas exchange results in a long lag phase prior to onset of ARA (acetylene reduction activity) (Rice & Paul, 1971; Matsuguchi *et al.*, 1978; Patriquin, 1978; Patriquin & Denike, 1978). This necessitates longer assay periods. Flett *et al.* (1976) suggested several modifications to the technique in aquatic environments, including agitation to ensure equilibration of gases and use of calculations to account for ethylene solubility in water.

The *in situ* technique, often involving the inversion of a bell jar-type container over the experimental material, appears advantageous, as the sample is undisturbed. Serious disadvantages of this technique have been outlined by Patriquin & Denike (1978) and include slow equilibration of gases and the inability to saturate the nitrogenase enzyme at non-

inhibitory partial pressures of acetylene, unless long assay periods in excess of 24 h were used. The technique also requires the use of an internal standard such as propane (Boddey *et al.*, 1978) or acetylene (McNabb & Geist, 1979) as gas losses occur from the lower region of the assay chamber. Modifications for use of the technique in remote areas are discussed by McNabb & Geist (1979).

It is apparent that the acetylene reduction technique, widely used because of its simplicity, must be used with caution and the assay procedure must be modified according to assay conditions.

## CHAPTER 3

### GENERAL MATERIALS AND METHODS

#### 3.1 THE MGENI ESTUARY MANGROVE SWAMP

This project was carried out prior to the devastating floods of 27-29/9/1987, which destroyed mangrove stands at the mouth of the Mgeni River. Conditions are therefore described as they existed prior to the flood.

##### 3.1.1 GENERAL FEATURES

The Mgeni River is about 235 km long (Day, 1981) and has its source in the Impendle area of the Drakensberg. The catchment area is 4871 km<sup>2</sup> and the average runoff  $707 \times 10^6 \text{ m}^3 \text{ yr}^{-1}$  (Midgley & Pitman, 1969). High annual rainfall and ground water sources at the source of the river maintain a constant flow of water all year round. The river can thus be regarded as perennial.

The Mgeni Estuary, situated at the mouth of the Mgeni River, is about 2,5 km long (Begg, 1978) and extends from 29°47'S to 29°49'S and from 31°00'E to 31°01'E.

The Beachwood Mangrove Nature Reserve (regarded as the Mgeni Estuary mangrove swamp in this study) is situated at the mouth of the Mgeni River and to the north thereof (Figure 3.1) and occupies an area of approximately 50 ha, of which 12,14 ha are under

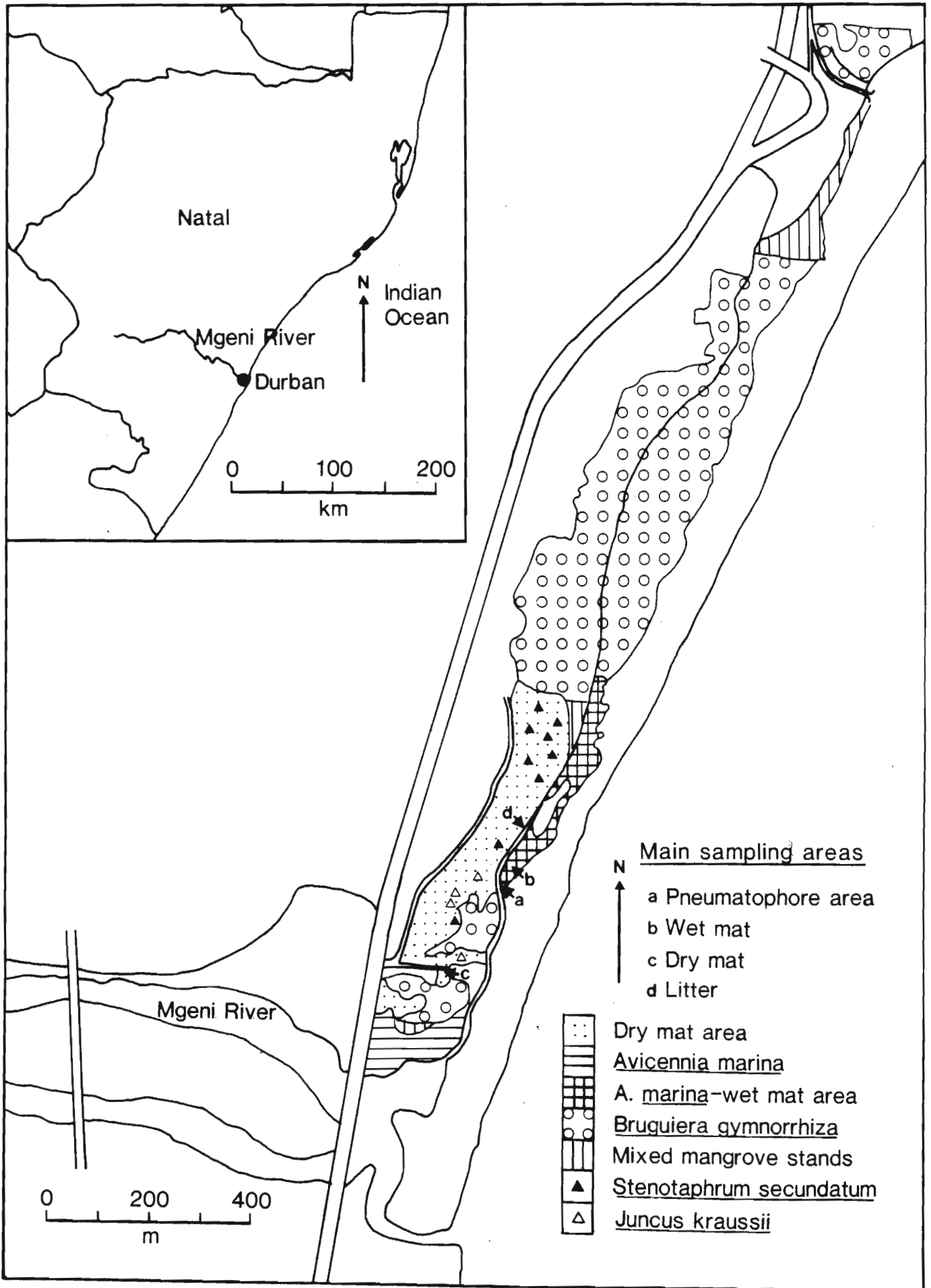


Figure 3.1 Map of the Mgeni Estuary mangrove swamp (Raiman, 1986).



mangroves (Beachwood Mangrove Nature Reserve Management Plan, 1986). The Beachwood Creek flows southwards through the Mgeni Estuary mangrove swamp for a distance of approximately 3 km (Raiman, 1986). The northern boundary is the Beachwood Golf Course. To the east, the area is bounded by a series of sand dunes, and stabilization by bamboo fences and the introduction of dune vegetation has been attempted. The Leo Boyd Highway forms the western boundary. The average altitude of the area is approximately 2 m (Raiman, 1986).

### 3.1.2 SUBSTRATE

Soil characteristics of mangrove stands in the Mgeni Estuary mangrove swamp were described by Naidoo (1980) as weakly acidic, high in clay, organic content and moisture at saturation.

*A. marina* soils had a slightly higher pH and organic content than *B. gymnorhiza* soils. The texture in the top 250 mm was regarded as sandy clay loam (Naidoo & Raiman, 1982). Surface soils contained a much higher clay fraction and organic content than sub-surface soils (Raiman, 1986).

### 3.1.3 SALINITY

Fresh water flows into the Beachwood Creek from the Beachwood Golf Course in the north and three stormwater drains from the Durban North residential area. Diurnal tidal variation results in the inflow of saline waters, and this influence has been measured 2840 m up the Beachwood Creek, where a salinity of

3<sup>0</sup>/oo was measured at high tide (Raiman, 1986). High salinities of above 30<sup>0</sup>/oo were recorded up to 1410 m from the mouth of the creek at high tide. Higher up the creek, salinities dropped to below 10<sup>0</sup>/oo.

Soil salinity is influenced by tidal inundation, distance from water courses and exposure (Raiman, 1986). Soils in the Mgeni Estuary mangrove swamp were saline, except for those above the range of tidal influence (Raiman, 1986).

#### 3.1.4 OXYGEN

Dissolved oxygen levels measured between 1979 and 1981 in the Mgeni Estuary were low and ranged from 3,3 mg l<sup>-1</sup> to 12,3 mg l<sup>-1</sup> (Begg, 1984). These levels are only slightly higher than the generally accepted minimum requirement of 4,0 mg l<sup>-1</sup> for aquatic life (Kemster *et al.*, 1980 in Begg, 1984). Higher levels of 10,2 mg l<sup>-1</sup> and 12,3 mg l<sup>-1</sup> were recorded from the mouth of the Beachwood Creek (Begg, 1984).

#### 3.1.5 CLIMATE

Temperature and rainfall data were obtained from unpublished records of the Botanic Gardens Meteorological Station, which is 6 km south-west of the Mgeni Estuary. Records of surf temperatures were obtained from Durban's North Beach.

TABLE 3.1 Air temperature ( $^{\circ}\text{C}$ ) at Durban Botanic Gardens for July 1974 to July 1987.

| Month       | Mean | Mean maximum | Absolute maximum | Mean minimum | Absolute minimum |
|-------------|------|--------------|------------------|--------------|------------------|
| January     | 24,9 | 28,8         | 37,8             | 21,1         | 12,0             |
| February    | 25,0 | 29,2         | 34,4             | 20,9         | 9,5              |
| March       | 24,1 | 28,2         | 35,6             | 20,0         | 9,5              |
| April       | 22,8 | 27,2         | 33,0             | 18,4         | 9,4              |
| May         | 20,3 | 25,5         | 34,4             | 15,2         | 9,4              |
| June        | 18,0 | 24,0         | 35,6             | 11,9         | 5,0              |
| July        | 18,0 | 23,5         | 32,0             | 11,8         | 5,6              |
| August      | 18,6 | 23,4         | 36,0             | 13,7         | 8,0              |
| September   | 20,0 | 24,0         | 33,3             | 15,9         | 6,7              |
| October     | 21,1 | 24,5         | 37,0             | 17,0         | 10,5             |
| November    | 22,2 | 26,1         | 36,0             | 18,3         | 13,0             |
| December    | 24,3 | 28,4         | 37,8             | 20,1         | 10,1             |
| YEARLY MEAN | 21,6 | 26,1         | 35,2             | 17,0         | 9,1              |

TABLE 3.2 Sea temperature ( $^{\circ}\text{C}$ ) at Durban's North Beach for January 1983 to November 1987.

| MONTH       | MEAN |
|-------------|------|
| January     | 24,3 |
| February    | 23,9 |
| March       | 23,9 |
| April       | 23,0 |
| May         | 22,0 |
| June        | 20,5 |
| July        | 19,6 |
| August      | 19,8 |
| September   | 20,7 |
| October     | 21,4 |
| November    | 22,4 |
| December    | 23,5 |
| YEARLY MEAN | 22,1 |

TABLE 3.3 Rainfall (mm) at Durban Botanic Gardens for 1934 to 1986.

| MONTH        | MEAN   |
|--------------|--------|
| January      | 138,3  |
| February     | 127,4  |
| March        | 114,3  |
| April        | 81,5   |
| May          | 65,7   |
| June         | 35,4   |
| July         | 31,2   |
| August       | 48,5   |
| September    | 62,2   |
| October      | 94,4   |
| November     | 118,8  |
| December     | 113,8  |
| YEAR (TOTAL) | 1031,5 |

The Mgeni Estuary experiences a warm temperate climate (Köppen-Geiger System of Climate Classification in Strahler, 1975), with a mean annual air temperature of 21,6°C (Table 3.1) and typically high humidity (Walter, 1977). Maximum air temperatures occur in February, whilst minimum temperatures occur in June and July. Surf temperatures are high and are influenced by the Agulhas current, which has a core temperature of 25°C in summer and 18°C in winter (Day, 1981). Mangroves are generally restricted to warmer tropical and subtropical regions, although their distribution can be extended by warm ocean currents.

*A. marina* is more tolerant of cold conditions than *B. gymnorhiza*, and, therefore has a wider distribution (Walter, 1977). Thus, sea temperature plays a major role in influencing the distribution of mangroves (Chapman, 1977b). Water temperatures are lower than air temperatures and the mean surf temperature for Durban's North Beach is 22,1°C (Table 3.2).

Rain occurs all year round, but 68,5% falls between October and March. The mean annual rainfall is 1031,5 mm (Table 3.3).

Frost is very rare along the Natal coast. The few reports are reviewed by Raiman (1986). Dew is common on calm winter nights and may provide an additional source of water, although evaporation occurs rapidly once exposed to direct sunlight.

### 3.1.6 POLLUTION

The Mgeni Estuary is regarded as one of the most polluted estuaries in Natal (Day, 1981). Pollutants include outflow from

industrial areas and the Zeekoei Lake, which acts as a maturation pond for sewage effluents. Industrial effluent is regarded as the major source of pollution (Begg, 1984).

### 3.1.7 VEGETATION

A detailed account of the vegetation of the Mgeni Estuary is provided by Raiman (1986). The vegetation is dominated by mangroves on either side of the Beachwood Creek, the dominant mangrove trees being *A. marina* and *B. gymnorhiza*. On the western fringe of the swamp, a drier, bare area interspersed with typical salt marsh communities of *Juncus kraussii* Hochst and *Stenotaphrum secundatum* (Walt.) Kuntze occurs. It is in this area, and areas dominated by *A. marina*, that extensive blue-green algal mats colonise the surface sediments at certain times of the year.

### 3.2 THE NITROGEN-FIXING SYSTEMS INVESTIGATED

The occurrence of nitrogen fixation has been demonstrated in a number of different habitats in mangrove swamps (Section 2.5). Only selected habitats could be studied extensively. Blue-green algae occur commonly in the Mgeni Estuary mangrove swamp (Lambert *et al.*, in prep.) and have been shown to be important nitrogen fixers in certain mangrove swamps (Gotto & Taylor, 1976; Potts, 1979; Hicks & Silvester, 1985), salt marshes (Jones, 1974) and estuaries (Bohlool & Wiebe, 1978). A study of the nitrogen-fixing potential of certain blue-green algal communities was therefore initiated. The study was confined to the lower reaches

of the Mgeni Estuary mangrove swamp, where 3 sites conspicuously inhabited by blue-green algae were identified. These are indicated in Figure 3.1.

- (a) Pneumatophores of *A. marina* in the intertidal region of the creek support a luxuriant growth of epiphytic blue-green algae (Figure 3.2a) and other algae such as reds, greens and browns (Lambert *et al.*, 1987; Lambert *et al.*, in prep). These will subsequently be referred to as the pneumatophore organisms.
- (b) Blue-green algal mats are conspicuous on the wet mud surface below *A. marina* stands, especially during the summer months (Figure 3.2b). Most of this area is submerged only at spring high tides, with the exception of the area along the creek which is subjected to daily tidal influences. Nevertheless, this area remains moist all year round. The mud and associated blue-green algae in this area often extend up the pneumatophores forming a collar around their bases. Decomposing litter on the surface of the mud also provides a substrate for attachment of blue-green algae. This area will subsequently be referred to as the wet mat.
- (c) Blue-green algal mats are also associated with mud surfaces in exposed areas on the periphery of the mangrove swamp (Figure 3.3a&b). They are also more conspicuous during the summer months. These areas are submerged to a lesser or greater extent at spring high tides. The mud dries out slowly, eventually forming deep cracks (Figure 3.3c), until the next spring high tide. Summer rains keep this area fairly moist in the summer months. This area will subsequently be referred to as the dry mat.



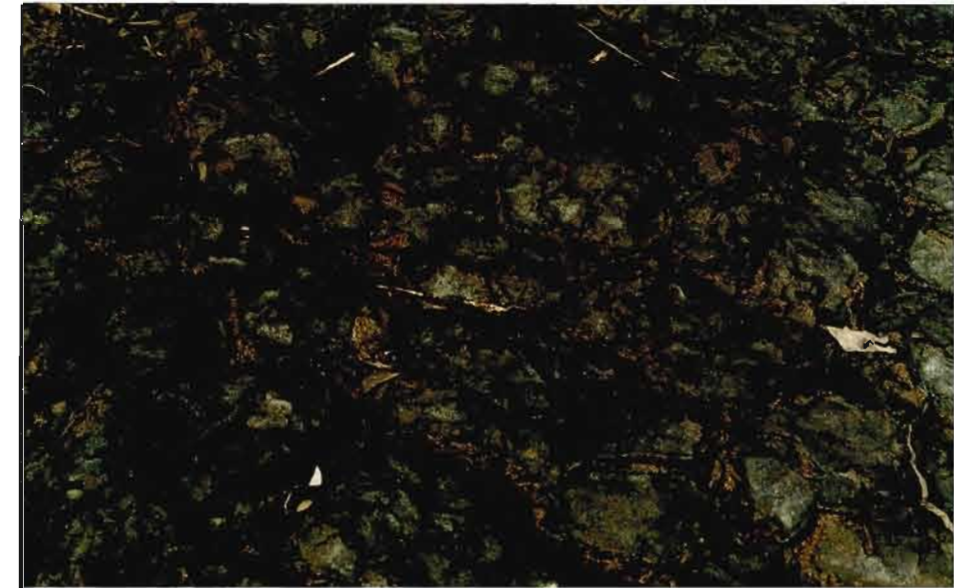
Figure 3.2 (A) Blue-green algae epiphytic on intertidal *A. marina* pneumatophores.

(B) Blue-green algal mats associated with the wet mud surface under *A. marina* trees.





- Figure 3.3 (A) A general view of exposed mud surfaces on the periphery of the mangrove swamp.
- (B) A close-up, showing blue-green algal mats associated with the exposed mud surfaces.
- (C) Deep cracks formed in the dry mud surface.



### 3.3 TECHNIQUES

#### 3.3.1 THE ACETYLENE REDUCTION ASSAY

##### 3.3.1.1 SAMPLE COLLECTION AND PREPARATION

Within each of the 3 sites chosen for study, a main sampling area was selected, having an area of 4 m x 8 m, 5 m x 8 m and 10 m x 10 m for the pneumatophore, wet and dry mat areas respectively (Figure 3.1). These areas were selected for easy accessibility and the presence of a representative cross-section of blue-green algal species possessing nitrogen-fixing ability.

Pneumatophores with epiphytic blue-green algae were randomly selected from the sampling area and cut off at the base (the soil-air interface). Segments of 10-15 mm were cut from the lower half of the pneumatophore for uniformity. Preliminary investigations in the Mgeni Estuary mangrove swamp have suggested a random distribution of blue-green algae on the pneumatophores (Strong, pers. comm.), but statistical confirmation is necessary. In the wet and dry mat areas, cores 2 mm deep were randomly removed with the aid of a plastic soil corer for the dry mat area and a metal corer with a sharp cutting edge in the wet mat area having areas of 1,188 and 1,208 cm<sup>2</sup> respectively. Two pneumatophore segments or cores were immediately placed into the assay chamber. At this stage water was added to the

chambers in certain experiments. This will be discussed in the relevant sections.

A wide variety of vessels have been used as assay chambers, ranging from 2 to 3 ml glass vials to 4 to 5 l jars (Turner & Gibson, 1980). In this study, 15 ml serum bottles were used in order to facilitate easy handling of numerous samples at any one time and to increase the sensitivity of the assay by having a reduced volume of air in the assay chamber (Flett *et al.*, 1976). Diffusion of gases into and out of samples is greater in small samples (Smith, 1980) resulting in higher rates of ARA (acetylene reduction activity).

The bottles were sealed with 12 mm rubber serum stoppers in order to facilitate easy addition and removal of gases. The bottles were vented back to atmospheric pressure at this point by inserting a 20-gauge hypodermic needle into the stopper for 10 sec. This was necessary as the large stoppers increased the pressure in the serum bottles when inserted.

The use of new caps was not always possible due to high cost. Used rubber stoppers have been shown to absorb ethylene and acetylene and later release these gases, causing unreliable results, especially when dealing with systems low in nitrogenase activity (Kavanagh & Postgate, 1970). Autoclaving of new rubber caps

(Jacobsen & McGlasson, 1970) and the use of terminating agents (Thake & Rawle, 1972) can also cause substantial release of ethylene into assay chambers. The use of terminating agents and autoclaving was avoided in this study. Decontamination of used caps was therefore necessary and was achieved by washing the caps thoroughly in distilled water, followed by subjection to a vacuum of 600 mm Hg for 24 h. Talbot (1982) found no release of ethylene from caps treated in such a manner. Another problem associated with the use of used caps was loss of gas through puncture holes. To prevent this occurring, the top of the stoppers was sealed with a silicone glue (Bostik marine).

Samples from all sites were incubated under aerobic conditions as this best simulated natural conditions. Acetylene was injected through the serum cap using a 5 ml gas-tight syringe preceded by removal of an equal volume of air to avoid increased pressure in the chamber (Gaskins & Carter, 1976; Bohlool & Wiebe, 1978). Commercial acetylene dissolved in acetone was used which had been passed through water and  $H_2SO_4$  scrubbers to remove contaminants (Turner & Gibson, 1980). Water removed acetone and  $SO_2$  and  $H_2SO_4$  removed  $PH_3$  and  $H_2S$  (Hardy *et al.*, 1973).

The optimum partial pressure of acetylene ( $pC_2H_2$ ) was determined as 0,15 atm. for the wet and dry mat areas and



0,2 atm. for the pneumatophores as outlined below. A control containing no acetylene was also set up for all the experiments. This was necessary in order to detect endogenous production of ethylene. In waterlogged soils, ethylene can attain high concentrations (Smith & Scott Russell, 1969) and this would lead to over-estimation of ARA. Blanks containing only seawater were also incubated with acetylene, but ethylene production was never apparent in these samples.

After addition of acetylene, samples were gently shaken by hand for 15 sec to aid solution of acetylene in samples containing water (Flett *et al.*, 1976).

#### PARTIAL PRESSURE OF ACETYLENE

Saturation of the enzyme nitrogenase by acetylene is important. Undersaturation results in an underestimate of ARA, whereas oversaturation could result in inhibition of ARA (Patriquin & Denike, 1978). The optimum  $p_{C_2H_2}$  required varies, depending upon the nature of the experimental material and has to be empirically determined in each system. In order to determine the optimum partial pressure required, material from each site was collected as previously described. Two ml 25<sup>0</sup>/oo seawater (pH 7,8) was added to each bottle in order to standardise moisture content of the samples, so as to eliminate one possible source of variation. Bottles

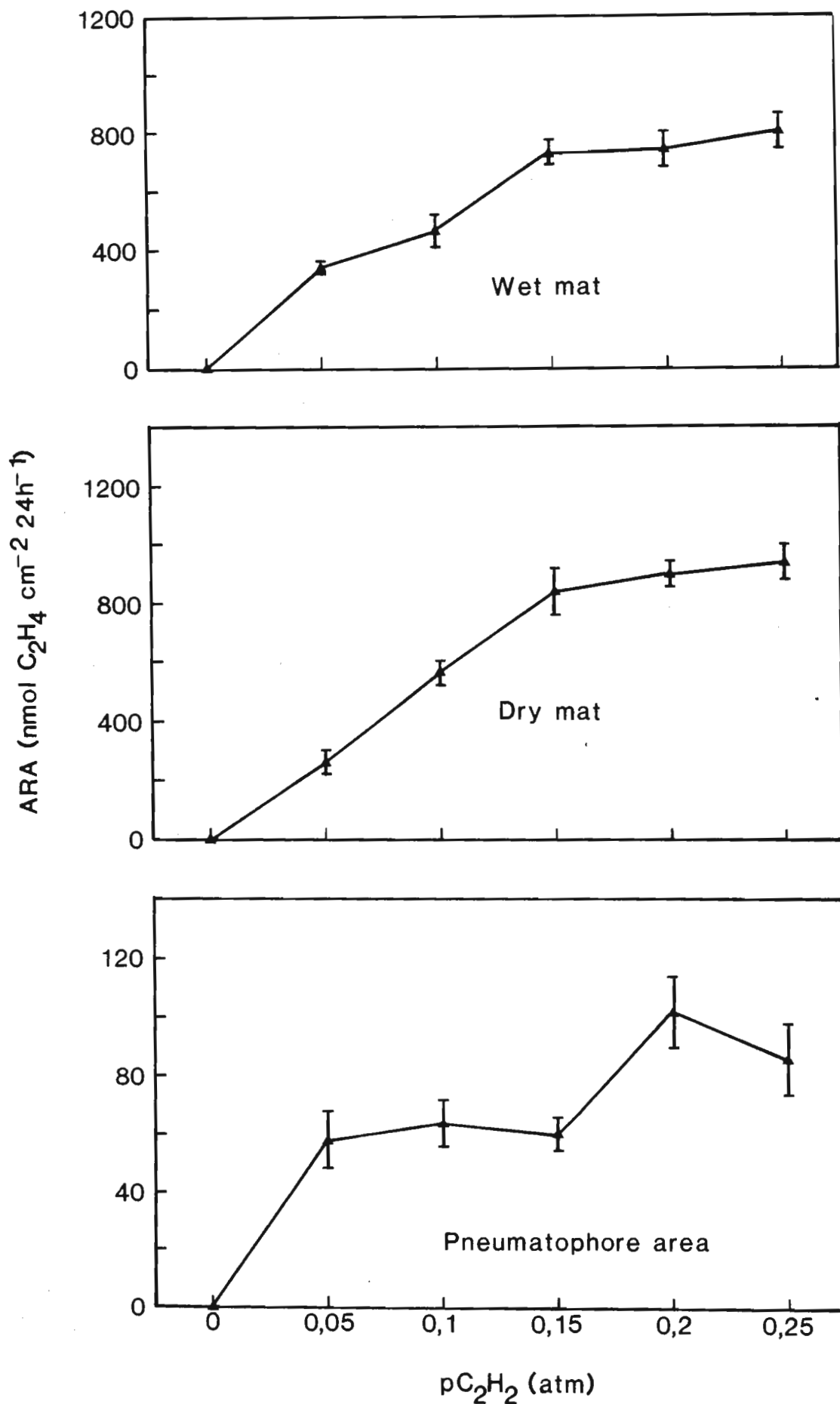


Figure 3.4 The effect of partial pressure of acetylene on acetylene reduction activity. The vertical bars represent one standard error.

were incubated at  $pC_2H_2$  of 0,00; 0,05; 0,10; 0,15; 0,20; 0,25 atm. Ten replications were used for each treatment. The samples were subsequently incubated at  $22^{\circ}C$  and  $200 \mu E m^{-2} s^{-1}$  for 24 h and assayed for ARA as described in Section 3.3.1.4.

Results (Figure 3.4) indicate a saturation of the nitrogenase enzyme between  $pC_2H_2$  0,2 and 0,25 atm. for the pneumatophores and between  $pC_2H_2$  0,15 and 0,25 atm. for the wet and dry mat areas. Analysis of variance showed no significant differences between these values. In each case, the lowest saturating  $pC_2H_2$  was selected for future experiments (i.e.  $pC_2H_2$  of 0,15 atm. for the wet and dry mat areas and 0,2 atm. for pneumatophores) to avoid inhibition at high partial pressures (Hardy *et al.*, 1968; Brouzes & Knowles, 1971; Patriquin, 1978; Patriquin & Denike, 1978). Patriquin (1978) suggested the use of the lowest  $pC_2H_2$  compatible with obtaining near maximal ARA. These values are within the range of partial pressures of acetylene reported in the literature (Hardy *et al.*, 1973). Partial pressures ranging from 0,02 atm. (Kimball & Teas, 1975) to 2,2 atm. (Potts, 1979) have been used in mangrove swamps.

### 3.3.1.2 INCUBATION OF SAMPLES

Samples were incubated either in the field under prevailing conditions of light and temperature, or in a



Fisons 600G3/THTL growth cabinet under controlled conditions. In the latter case, light provided by fluorescent and tungsten lamps and temperature was varied according to conditions required for each experiment. Details of experimental conditions are discussed in the relevant sections.

Pre-incubation and incubation periods were determined as outlined below. Samples were pre-incubated under assay conditions for 3 h after preparation. Acetylene was then injected, the samples shaken to ensure rapid diffusion of acetylene into the sample and ARA was measured over the next 24 h (i.e. between 4 and 28 h after sample preparation). This coincided with the period of linear ethylene production.

#### PRE-INCUBATION AND INCUBATION TIME

Length of incubation is an important factor influencing observed ARA in natural samples. Hardy *et al.* (1968) suggested a 1 h incubation period for soil cores. The choice of incubation period in estuarine ARA studies is highly variable, ranging from short incubation periods of 3 h or less (Goering & Parker, 1972; Van Raalte *et al.*, 1974; Carpenter *et al.*, 1978; Potts, 1979; Holter, 1984) to long incubation periods of 24 to 48 h (Jones, 1974; Patriquin & McClung, 1978; Zuberer & Silver, 1978; Teal *et al.*, 1979). In order

to determine a suitable assay time, it was necessary to determine representative time courses for the sites under investigation.

Samples (70) were collected from each site as previously described in Section 3.3.1.1 and submerged in 2 ml 25<sup>0</sup>/oo seawater made up to pH 7,8. Acetylene was injected and samples were subsequently incubated at 22<sup>0</sup>C and 200  $\mu\text{E m}^{-2} \text{s}^{-1}$  in a growth cabinet. Gas samples were analysed for ARA after 0, 3, 6, 18, 24, 42 and 72 h. Ten serum bottles were analysed for ethylene at each time as the headspace was not large enough to allow removal of 7 air samples from one bottle.

Representative time courses for ethylene production are presented in Figure 3.5. Two distinct phases of ARA were apparent in samples from all 3 sites. A lag of 3 h was apparent before the onset of ARA. Following this, rates were linear for up to 72 h for the wet and dry mat samples and 42 h for the pneumatophore samples. A third phase of enhanced rates was apparent for the pneumatophore samples. Three such phases have been reported under waterlogged conditions by various researchers (Rice & Paul, 1971; Herbert, 1975; Patriquin & Denike, 1978; Patriquin & McClung, 1978; Dicker & Smith, 1980a; Talbot, 1982).

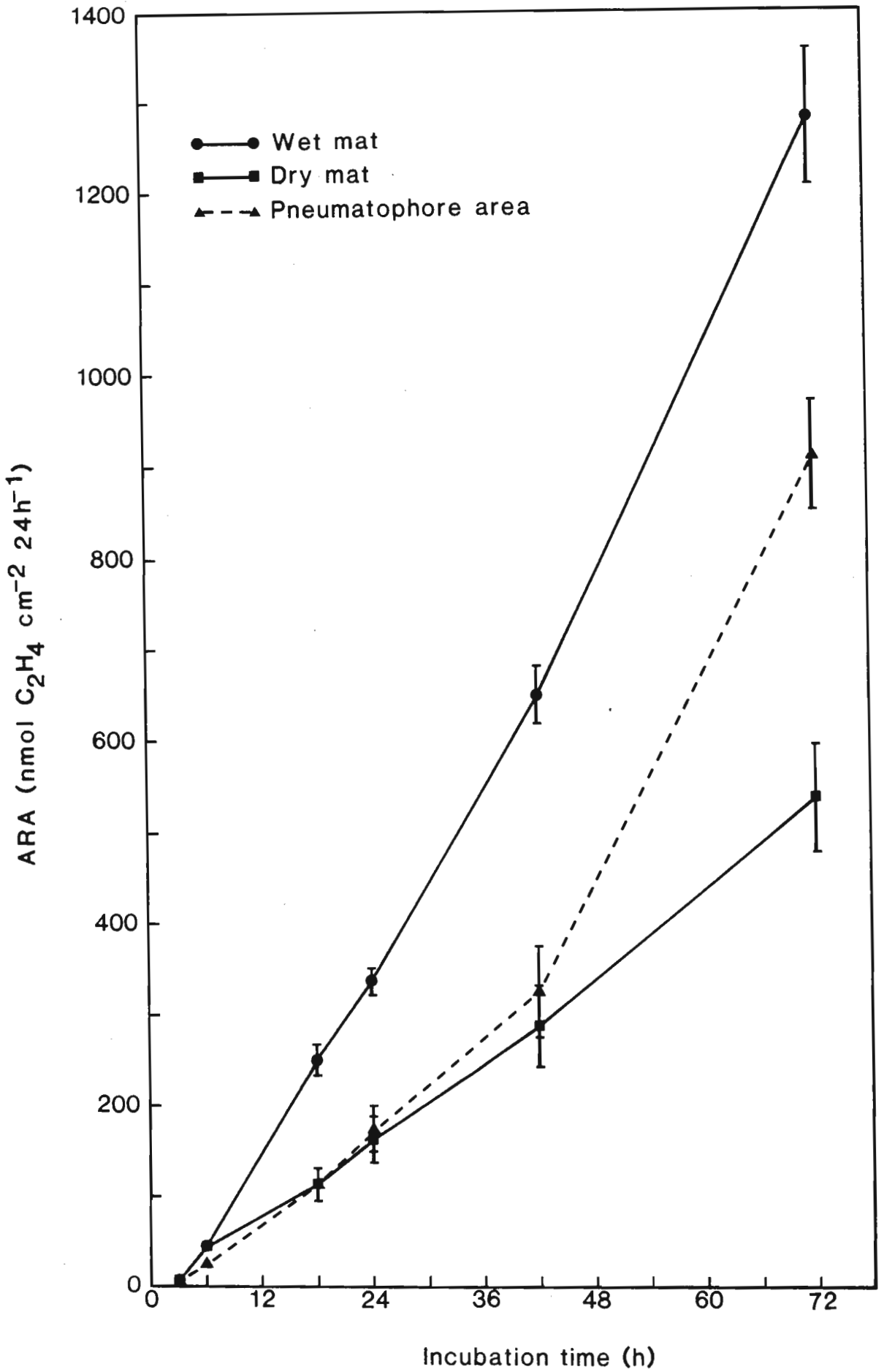


Figure 3.5 Time course for acetylene reduction activity.  
The vertical bars represent one standard error.

The lag phase has been interpreted differently by various researchers and may be a result of disturbance of nitrogen-fixers during sampling (Herbert, 1975; Patriquin, 1978; Teal *et al.*, 1979; Dicker & Smith, 1980a), physiological response to *in situ* ammonium and nitrate levels (Dicker & Smith, 1980b), oxygen inactivation of nitrogenase (Herbert, 1975; Patriquin, 1978; Casselman *et al.*, 1981; Capone, 1982) or slow diffusion of acetylene into the sample (Herbert, 1975; Casselman *et al.*, 1981; Capone, 1982). The importance of measuring ARA during the second phase, i.e. while rates are linear, is recognised by many researchers (Hanson, 1977a; Dicker & Smith, 1980a; Paerl *et al.*, 1981; Holter, 1984).

The third phase, observed only in the pneumatophore samples in this study may represent a so-called 'bottle effect' resulting in increased numbers of nitrogen-fixing organisms (Hardy *et al.*, 1973). This result may indicate that nitrogen fixation by bacteria, which divide rapidly, is important on the pneumatophores. In the wet and dry mat areas, blue-green algae, which divide slowly, are possibly the most important nitrogen-fixers, as enhancement of ARA, associated with increased numbers of nitrogen-fixing organisms, was not apparent.

The importance of a pre-incubation period before the addition of acetylene, has been recognised by various

workers in waterlogged systems in order to eliminate the lag phase (Brouzes & Knowles, 1971; Gaskins & Carter, 1976; Patriquin, 1978; Potts, 1979; Dicker & Smith, 1980a, b, c; Talbot, 1982; Wilkinson *et al.*, 1984). The introduction of acetylene immediately after preparation of samples has been shown to have an inhibitory effect on ARA in some experiments (Patriquin, 1978).

### 3.3.1.3 TERMINATION OF REACTION

Various methods have been employed in order to terminate ARA at the end of the assay, including the use of terminating agents such as NaOH (Taylor *et al.*, 1973), trichloroacetic acid (Stewart *et al.*, 1967; Carpenter, 1973; Carpenter & McCarthy, 1975),  $H_2SO_4$  (Burris, 1976), cupric sulphate (Burris, 1976) and acetone (Burris, 1976), refrigeration of the sample at  $4^{\circ}C$  (Jones, 1974) and removal and subsequent storage of a gas sample in a holding vessel (Schell & Alexander, 1970; Kimball & Teas, 1975; Potts, 1979; Grobbelaar & Rösch, 1981; Van der Valk & Attiwill, 1984a). The use of terminating agents is not recommended by various researchers for 2 reasons. Firstly, slow penetration into the sample results in failure of reagents to terminate the reaction immediately and this can cause errors (Hardy *et al.*, 1973). Secondly, ethylene is formed as a result of contact between terminating agents and rubber stoppers (Thake & Rawle, 1972). In this study, addition of gas

was staggered in laboratory studies and samples were analysed immediately, as recommended by Thake & Rawle (1972). For field studies, 2 ml gas was removed from each sample, injected into a 2 ml serum bottle by displacement of water (Grobbelaar & Rösch, 1981) and analysed in the laboratory within 1 h.

#### 3.3.1.4 ANALYSIS OF ETHYLENE

Before sampling, reaction vessels were shaken up for 10 sec in order to ensure equilibration of ethylene between liquid and vapour phases (Flett *et al.*, 1976). Gas samples of 1 cm<sup>3</sup> were removed from the reaction vessels using a gas-tight syringe at the beginning and end of the assay. This was necessary in order to determine the background levels of ethylene present at the start of the assay. Field gas samples were removed from the 2 ml serum bottles by displacement with water (Grobbelaar & Rösch, 1981). In order to determine amounts of ethylene produced during the assay, the background levels of ethylene were subtracted from the levels present at the end of the assay.

Analysis of ethylene was conducted on a Carlo Erba Fractovap Series 2150 gas chromatograph equipped with a flame ionization detector. The gas samples were carried by nitrogen (30 ml min<sup>-1</sup> flow rate) through a 2 mm stainless steel column, 2 m long, packed with 80 - 100

mesh Poropak R. The oven temperature was 70°C and the injector temperature 150°C. Retention times for ethylene and acetylene were 1,5 and 2 min respectively.

Ethylene peak heights were related to number of moles of ethylene from a standard curve (Hardy *et al.*, 1968; Zuberer & Silver, 1975; Talbot, 1982; Zeitsman, 1982) which was constructed as outlined below.

#### STANDARD CURVE FOR ETHYLENE

Standards were prepared to 4 different concentrations by injecting 1 cm<sup>3</sup> of pure ethylene into argon-filled flat-bottomed flasks of different volumes. Each flask was sealed with a rubber stopper into which a glass tube capped with a rubber serum cap was inserted. Flasks were left for 2 h at 25°C in order to allow thorough gas mixing. Gas samples of 1 cm<sup>3</sup> were removed from each flask and analysed as described above. The number of moles of ethylene was calculated for each peak height, using the ideal gas equation (Boikess & Edelson, 1978). The temperature and atmospheric pressure were accurately recorded at time of analysis. The flask volumes were determined as 141,026 cm<sup>3</sup>, 312,086 cm<sup>3</sup>, 575,22 cm<sup>3</sup> and 1027,104 cm<sup>3</sup> by displacing the air with water. Assuming that the density of water is 1 g m<sup>-2</sup>, the volume of air was calculated by subtracting the initial mass of the

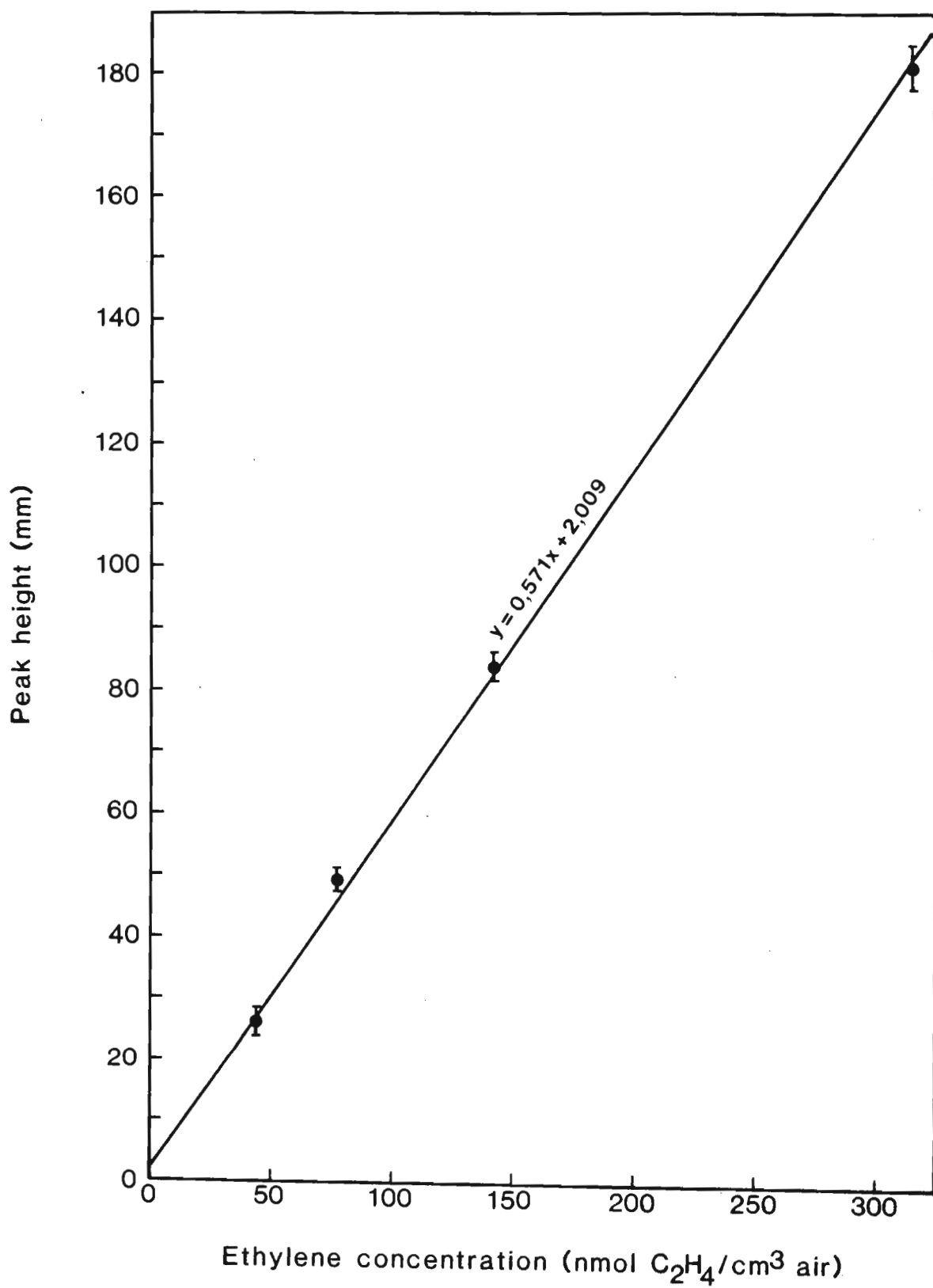


Figure 3.6 Standard curve for the determination of ethylene by gas chromatography. The vertical bars represent one standard error.



flask from the mass of the flask filled with water. A standard curve was constructed for regression analysis from the 4 data points, each being the mean of 10 replications (Figure 3.6).

Standards of ethylene in argon were maintained and used to detect any changes in sensitivity of the gas chromatograph every 2 months.

#### 3.3.1.5 EXPRESSION OF RESULTS

Results of ARA are expressed in terms of nitrogen fixed, acetylene reduced, or ethylene produced. The first method of expression requires empirical determination of a conversion factor using  $^{15}\text{N}$  measurements (Jones, 1974) or the use of the theoretical conversion factor of 3:1 (Gotto & Taylor, 1976; Casselman *et al.*, 1981). Variation in the conversion factor has been discussed in Section 2.8. Because of this variation, many researchers express results in terms of acetylene reduced or ethylene produced (Zuberer & Silver, 1978; Potts, 1979; Dicker & Smith, 1980a, b; Talbot, 1982; Hicks & Silvester, 1985). Facilities were not available for empirical determination of the conversion factor for this system and results were therefore expressed as nmoles ethylene produced per  $\text{cm}^{-2}$  per 24 h ( $\text{nMol C}_2\text{H}_4 \text{ cm}^{-2} \text{ 24 h}^{-1}$ ). Expression on a per unit surface area basis has been employed by many researchers (Van Raalte

*et al.*, 1974; Carpenter *et al.*, 1978; Teal *et al.*, 1979; Hicks & Silvester, 1985) and was decided upon as rates of ARA associated with nitrogen-fixing organisms on the mud and pneumatophore surfaces were being studied. Area of cores was constant and the surface area of each pneumatophore segment was calculated as  $2 \pi rh$  (Joseph & Keiffer, 1965). Other methods of expression include dry weight (Gotto & Taylor, 1976; Dicker & Smith, 1980a, b; Talbot, 1982), wet weight (Zuberer & Silver, 1978) and chlorophyll *a* (Potts, 1979).

#### 3.3.1.6 STATISTICAL ANALYSIS OF RESULTS

The standard error of each result was calculated. One or two-way analysis of variance and least significant differences were calculated for each experiment.

#### 3.3.2 DETERMINATION OF ENVIRONMENTAL PARAMETERS

Environmental parameters were recorded at a set time of day for comparative purposes. This will be discussed in the relevant sections.

##### 3.3.2.1 TEMPERATURE

The *in situ* temperature was recorded in  $^{\circ}\text{C}$  using a mercury thermometer. Air, mud and water temperatures were measured in the sun and shade. Care was exercised

in order to avoid direct sunlight falling on the thermometer. Mean monthly, maximum and minimum air temperatures were also obtained from the Botanic Gardens weather station. This gave a better indication of the range of temperatures involved.

#### 3.3.2.2 LIGHT INTENSITY

Photosynthetically active radiation (quantum flux density) was recorded using a Licor light meter graduated in  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Readings were taken in the sun and shade. Light intensity was recorded in the creek with the probe held about 2 cm below the surface of the water.

#### 3.3.2.3 PERCENTAGE MOISTURE

Moisture content of the samples was determined as a percentage of the dry mass. Samples were dried at  $80^{\circ}\text{C}$  until a constant mass was obtained and percentage moisture was calculated as:

$$\frac{\text{wet mass} - \text{dry mass}}{\text{dry mass}} \times \frac{100}{1} \quad (\text{Gardner, 1965}).$$

#### 3.3.2.4 SALINITY

Water from the creek and interstitial water from wet and dry mat areas was collected in McCartney bottles and the salinity determined directly as ‰ using a hand

held AO Model 10419 refractometer. Under dry conditions, the interstitial salinity of the wet and dry mat samples was determined as outlined below. Distilled water was added to mud (20 g wet mass) and stirred until a condition of saturation was reached (Bower & Wilcock, 1965). The sample was mixed by means of a mechanical shaker for 1 h and allowed to stand overnight. The salinity of free water droplets collecting on the surface of the mud was subsequently determined as described above. The increase in moisture resulting from addition of distilled water was determined as:

$$\frac{\% \text{ moisture of wet sample}}{\text{original } \% \text{ moisture of sample}}$$

The actual salinity of the sample was calculated as: salinity of wet sample X increase in moisture as determined above.

#### 3.3.2.5 ORGANIC CARBON

The organic carbon content of experimental sites was determined from samples used for ARA in the wet and dry mat areas. For the pneumatophore samples, the epiphytic organisms and adhering sediment were scraped off the pneumatophore segments and dried at 80°C until a constant mass was obtained. Cores from the wet and dry mat areas were treated similarly. Dried samples were

ground and thoroughly mixed using a pestle and mortar. Three replicate 0,5 g samples from each site were placed into crucibles and dried at 80°C to a constant mass which was accurately recorded. Dried samples were ashed at 450°C for 6 h and subsequently reweighed. The total amount of organic matter in the samples was determined in mg g dry mass<sup>-1</sup> as:

$$\frac{\text{dry mass} - \text{ashed mass}}{\text{ashed mass}} \times \frac{10^3}{1}$$

The organic carbon content was calculated as 45% of the total organic matter (Nixon, 1980).

### 3.3.2.6 INORGANIC NITROGEN DETERMINATIONS

Surface sediment samples from the wet and dry mat areas and a water sample from the creek were transported to the laboratory in sterile plastic bags on ice packs in a cooler box.

#### 3.3.2.6.1 PREPARATION OF SOIL EXTRACT

CaSO<sub>4</sub> (0,5 g) and water (250 ml) were added to 50 g sediment and placed on a mechanical shaker for 10 min (Bremner, 1965). The resulting solution was filtered through Whatman No. 42 filter paper several times until a clear filtrate was obtained. The

creek water was filtered in a similar way. This filtrate was analysed colorimetrically for nitrates, nitrites and ammonium as outlined below.

#### 3.3.2.6.2 NITRATE

Nitrate was determined by the phenoldisulphonic acid method (Bremner, 1965). A preliminary step to remove excess chlorides was necessary and was carried out as outlined below.

$\text{Ag}_2\text{SO}_4$  (0,1 g) was added to 100 ml extract and shaken for 15 min. Precipitation of  $\text{Ag}^+$  was then achieved by adding  $\text{Ca}(\text{OH})_2$  (0,2 g) and  $\text{MgCO}_3$  (0,5 g) and shaking for 5 min. The suspension was filtered through Whatman No. 4 filter paper and analysed as follows:

$\text{CaCO}_3$  (0,05 g) was added to 25 ml extract and evaporated in a boiling water bath to a point of dryness. Phenoldisulphonic acid (2ml), prepared by dissolving phenol (25 g) in concentrated  $\text{H}_2\text{SO}_4$  (225 ml), was added. Water (20ml) was added after 10 min and the solution stirred. A 50%  $\text{NH}_4\text{OH}$  solution was added until the extract was slightly alkaline (indicated by the development of a yellow colour). Thereafter, an additional 2 ml of the 50%  $\text{NH}_4\text{OH}$  was added to ensure an excess of this reagent.

The solution was then made up to 100 ml with water in a volumetric flask. Absorbance of the solution was measured using a Sequoia-Turner Model 340 spectrophotometer against a distilled water blank at 420 nm in 12 mm diameter cuvettes. The nitrate content was determined by reference to a calibration graph. The graph was drawn up by measuring the absorbance of a range of nitrate standard solutions ( $50 \mu\text{g l}^{-1}$  to  $1600 \mu\text{g l}^{-1}$ ).

#### 3.3.2.6.3 NITRITE

Nitrite was determined using a modified Griess-Ilosavy method (Bremner, 1965) as outlined below. Water (20 ml) and 1 ml diazotizing reagent (sulphanilamide (0,5 g) in 2,4 N HCl (100 ml) ) were added to 25 ml extract and stirred. After 5 min 1 ml coupling reagent (N - (1-naphthyl) - ethylene diamine hydrochloride (0,3 g) in 0,2 N HCl (100 ml) ) was added and stirred. After a further 20 min the solution was made up to 50 ml with water. Absorbance was measured against a distilled water blank at 520 nm and the nitrite content determined by reference to a calibration curve. The concentration of standard solutions ranged from  $40 \mu\text{g l}^{-1}$  to  $300 \mu\text{g l}^{-1}$ .

#### 3.3.2.6.4 AMMONIUM

Ammonium was determined by the phenolhypochlorite method (Solórzano, 1969) as follows: to 50 ml extract the following reagents were successively added: 2 ml phenol solution (phenol (10 g) in 95% v/v ethyl alcohol (100 ml) ), 0,5% solution sodium nitroprusside (2 ml) and 5 ml oxidising solution (100 ml sodium citrate solution (trisodium citrate (100 g) and sodium hydroxide (5 g) in water (500 ml) ) and hypochlorite solution (25 ml)). A blue colour was developed at room temperature for 1 h and absorbance subsequently measured against a distilled water blank at 640 nm. The ammonium content was determined by reference to a calibration curve. The concentration of standard solutions ranged from  $40 \mu\text{g l}^{-1}$  to  $600 \mu\text{g l}^{-1}$ .

#### 3.3.2.7 TOTAL NITROGEN DETERMINATIONS

Total nitrogen was determined by the micro-Kjeldahl method (Horwitz, 1965). Milled, dried leaves (0,05 g) were digested by heating in a 30 ml micro-Kjeldahl flask containing  $\text{K}_2\text{SO}_4$  (2 g),  $\text{HgO}$  (0,4 g),  $\text{H}_2\text{SO}_4$  (2,5 ml) for 1 to 1,5 h. A blank sample was incubated in each batch. A small quantity of water was added to the cooled digest in order to dissolve the solids



and subsequently transferred to the distillation apparatus. The flask was rinsed several times with water to remove traces of digest. A 125 ml erlenmeyer flask containing a saturated solution of  $H_3BO_3$  (5 ml) and 4 drops of indicator (2 parts 0,2% methyl red solution mixed with 1 part 0,2% methylene blue solution) was placed under the condenser with the tip submerged.

To the still, 8 - 10 ml NaOH -  $Na_2S_2O_3$  solution (NaOH (60 g) and  $Na_2S_2O_3 \cdot 5H_2O$  (5 g) diluted to 100 ml in water) was added, 15 ml distillate collected and diluted to 50 ml. This solution was titrated with HCl until a violet colour was achieved and the percentage nitrogen in each sample calculated as:

$$\frac{(\text{ml HCl} - \text{ml blank}) \times \text{Normality} \times 14,007 \times 100}{50 \text{ mg sample}}$$

### 3.3.3 IDENTIFICATION OF BLUE-GREEN ALGAE

Blue-green algae were identified with the aid of keys presented by Desikachary (1959) and Humm & Wicks (1980). The recognition of certain diagnostic features is necessary in order to identify species. These become more apparent with the use of the stains outlined below:

- a) Thickened cross-walls and protoplasmic granules are stained by grams iodine (saturated iodine solution to which 1% KI is added) (Humm & Wicks, 1980).

- b) End walls and cross walls are stained by briefly adding sulphuric acid-potassium dichromate ( $K_2 Cr_2 O_7$  (30 g) dissolved in water (100 ml) and made up to 1 l with concentrated  $H_2SO_4$ ) (Humm & Wicks, 1980).
- c) Sheaths are stained by methylene blue (Johansen, 1940).
- d) Certain sheaths are stained blue by chlor-zinc iodide (KI (16 g) and  $Zn Cl_2$  (50 g) added to water (17 ml) An excess of iodine is added to this solution) (Desikachary, 1959).

Cell dimensions are also important diagnostic features.

Measurements were made of 10 cells of each species present at each seasonal sampling date with the aid of an eyepiece graticule. Mean length and width of cells were calculated. Permanent slides were made of the different species by mounting the specimens in glycerine jelly.

These are stored in the Botany Department of the University of Durban-Westville. Photographs of each species were taken with the aid of a Reichert photo-microscope.

### 3.3.4 ENUMERATION OF NITROGEN-FIXING COMMUNITIES

#### 3.3.4.1 BLUE-GREEN ALGAE

Three techniques are commonly used to measure blue-green algal abundance within a sample. These are

dilution and plating techniques, pigment analysis and direct examination and counting by light microscopy (Stewart, 1980). The last method, although time-consuming, is regarded as the most satisfactory (Stewart, 1980) and was employed in this study.

In order to accurately count cell or trichome numbers, a random distribution of material is desirable (Guillard, 1973). Two common species present in the samples, *Microcoleus chthonoplastes* and *Lyngbya confervoides* (described in Section 5.2.1), possess thick mucilaginous sheaths, which bind the filaments together into interwoven mats. A number of methods were attempted in order to achieve a random distribution of material.

Chemical breakdown of the mucilaginous sheath in order to release the trichomes of *M. chthonoplastes* and *L. confervoides* into suspension was attempted. Frey-Wyssling & Stecher (1954) (in Drews, 1973) reports the sheaths to be composed partly of cellulose. In an attempt to break down these sheaths, cellulase was added to trial samples at concentrations of 1, 5 and 10%. This caused extensive cellular damage, as the cell walls are also composed of cellulose, and these were presumably broken down as well. Addition of a weak solution of  $H_2SO_4$  had a similar effect.

Thus a mechanical separation of the filaments was attempted. Three glass beads (mean diameter of 6 mm) were added to McCartney bottles containing algal material, which was collected as described below and the sample was homogenised for 1 min using

a vortex mixer. This time period enabled some degree of separation, without their total fragmentation. As no method could be devised to separate the filaments more satisfactorily, the latter method of mechanical separation was used and a random distribution of clumps of filaments had to suffice for counting.

The use of various counting devices is reviewed by Guillard (1973). The use of a 0,1 mm deep hemacytometer was attempted for counting in this study, but a random distribution of material could not be achieved, as the clumps of filaments settled in the entry slit. This method is suitable for cells of 2 - 30  $\mu\text{m}$  in length (Guillard, 1973), but filaments to be counted in this study were up to 3525  $\mu\text{m}$  long. Counting chambers such as the Sedgwick-Rafter or the Palmer-Maloney may have been suitable, but were not readily available for this study. As no other suitable methods were found in the literature, a method was devised relevant to the samples to be analysed (See below).

#### METHODS

Four 2 cm pneumatophore segments and 4 core samples from each of the wet and dry mat areas were collected as previously described (Section 3.3.1.1). Algae scraped off the surface of each pneumatophore segment and core from the wet and dry mat areas were teased apart with dissecting needles, placed into McCartney

bottles and made up to a volume of 5 ml using 0,4% formalin as a preservative. This provided an adequate dilution for enumeration of the algae. Material was mechanically separated as described above. For each of the 4 samples from each site, 3 slides were prepared as follows: the homogenate (50  $\mu$ l) was pipetted onto a slide and covered with a square coverslip (4,84  $\text{cm}^2$ ). The slide was pre-washed in alcohol to reduce surface tension.

Counts were carried out with the aim of determining number of cells  $\text{cm}^{-2}$ , i.e. number of cells per unit surface area of pneumatophore and wet and dry mats and involved different procedures for abundant and infrequent species. A 94  $\text{mm}^2$  eyepiece graticule, occupying nearly the entire field of view of a Cooke Troughton and Simms compound microscope was used for counting. The abundant species were counted along 2 belt transects of 22 mm x 0,94 mm each at x100 magnification across and down the middle of the coverslip. For infrequent species, the entire coverslip was counted.

The following criteria were used for counting purposes:

- a) If 50% or more of the trichome length occurred within the belt transect, the trichome was counted.

- b) If less than 50% of the trichome length occurred within the belt transect, the trichome was not counted.

As fragments of one trichome of a species may have been counted several times due to harsh sample preparation, and as trichome length varied greatly from one individual to another, individual presence of trichomes would have been misleading. The length of each trichome was therefore also recorded and from the above data, the number of cells in each trichome was calculated as:  $\text{trichome length} \div \text{mean cell length}$ .

In order to measure mean cell length, 10 cells of each species were measured in each sample. The total number of cells of each species were therefore calculated for each belt transect (for the abundant species) and for each coverslip (for the infrequent species).

The volume of liquid under one transect was calculated as 2,13  $\mu\text{l}$  and the volume of liquid under the entire coverslip as 50  $\mu\text{l}$ . In order to determine the total cell number of each species in each 5 ml sample, the following calculations were carried out:

For abundant species:

$$\frac{\text{cell number in 1 transect}}{1} \times \frac{\text{total volume of sample (5 ml)}}{\text{volume counted (2,13 } \mu\text{l)}}$$

For infrequent species:

$$\frac{\text{cell number under each coverslip}}{1} \times \frac{\text{total volume of sample (5 ml)}}{\text{volume counted (50 } \mu\text{l)}}$$

Mean cell number was then calculated from the 3 slide replications for each of the 4 samples collected from each site. In order to determine the cell number  $\text{cm}^{-2}$  of sample, the total cell number of each species in each 5 ml sample was divided by the surface area of pneumatophore and cores from the wet and dry mat areas (Section 3.3.1.5). Results were expressed as log cell number  $\text{cm}^{-2}$ .

The procedure of enumeration was very lengthy and did not permit studying more than 3 replications per sample.

#### 3.3.4.2 BACTERIA

Three sets of samples were collected from each experimental site, as described in Section 3.3.1.1 and 2 soil cores or pneumatophore segments were placed into each sterile McCartney bottle. The wet mass of the samples was recorded and the volume made up to 10 ml with 0,85%



saline. Three sterile glass beads with a mean diameter of 6 mm were added to each bottle. Samples were homogenised using a vortex mixer and serial 10-fold dilutions were prepared by transferring 1 ml successively to each of 5 McCartney bottles under sterile conditions. Samples were homogenised well after each dilution. A 0,1 ml sample was pipetted from each dilution onto agar plates and spread using a bent glass rod. Three replications were made from each dilution.

A number of different genera of nitrogen fixing bacteria have been identified, although only 5 occur commonly in estuarine regions (Discussed in Section 2.3). In this study, an estimation of total numbers of diazotrophs was considered sufficient in order to give an idea of relative numbers present at each sampling date. *Azotobacter* spp. are commonly reported nitrogen-fixers in estuarine environments (Herbert, 1975; Zuberer & Silver, 1975; Dicker & Smith, 1980c) and is one of the few aerobic nitrogen-fixing bacteria. Culturing specifically for this genus was also conducted as it is probable that it would predominate in the aerobic environments being studied. In order to estimate total bacterial numbers present, a general medium for heterotrophic bacteria was also employed.

The technique most widely used for investigating changes in diazotrophic populations is isolation of organisms on nitrogen-free media and subsequent colony



enumeration by the most probable number count, or direct colony count method (Herbert, 1975). The former method was employed by Talbot (1982) in the Swartkops Estuary, but yielded erratic results. The latter method was therefore adopted and has been employed by Zuberer & Silver (1975).

With the exception of the *Azotobacter* medium, the media were similar to those employed by Zuberer & Silver (1975) for culturing bacteria in a Florida mangrove swamp. An outline of the media employed for the various groups of bacteria is presented below:

a) Aerobic and anaerobic diazotrophs

Modified nitrogen-free medium of Centifanto & Silver (1964) (Table 3.4). Concentrations of  $\text{KH}_2\text{PO}_4$  and  $\text{K}_2\text{HPO}_4$  were increased 10x and 5x respectively. NaCl (12 g) was added to increase the salinity to 25‰. The medium was made up with Difco bacto-agar ( $15 \text{ g l}^{-1}$ ).

b) Aerobic and anaerobic heterotrophs

Tryptone soy agar ( $38 \text{ g l}^{-1}$ ) prepared with a modified synthetic seawater medium of Lyman & Flemming (1940) (in Kinne, 1976). NaBr was substituted for KBr as the latter was unavailable (Table 3.5).

c) Azotobacter

Modified nitrogen-free mannitol broth of Harrigan & McCance (1966) (Table 3.6). Salinity was adjusted to 25<sup>0</sup>/oo by increasing NaCl to 20 g l<sup>-1</sup>. The medium was made up with Difco bacto-agar.

The pH of all media was adjusted to 7,8 before autoclaving. Anaerobic environments were obtained for heterotrophs and diazotrophs by incubating cultures in anaerobic gas jars and using Anaerocult anaerobic gas kits. Samples were incubated at 25<sup>0</sup>C (Zuberer & Silver, 1975). *Azotobacter* spp. and diazotrophic bacteria were incubated for 48 h and heterotrophic bacteria for 24 h. The bacterial populations were enumerated by standard plate counts (Collins & Lyne, 1984) and results were expressed as log colony number cm<sup>-2</sup>.

TABLE 3.4 Modified nitrogen-free medium for diazotrophs  
(Centifanto & Silver, 1964).

| ADDITIVE   | CONCENTRATION (g l <sup>-1</sup> ) |
|--|------------------------------------|
| sucrose  | 30,00                              |
| K <sub>2</sub> HPO <sub>4</sub>                      | 4,00                               |
| KH <sub>2</sub> PO <sub>4</sub>                      | 2,00                               |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O                 | 0,20                               |
| Ca SO <sub>4</sub> ·2H <sub>2</sub> O                | 0,01                               |
| Fe SO <sub>4</sub> ·7H <sub>2</sub> O                | 3,25 × 10 <sup>-3</sup>            |
| Na <sub>2</sub> Mo O <sub>4</sub> ·2H <sub>2</sub> O | 0,21 × 10 <sup>-3</sup>            |
| NaCl   | 12,00                              |

TABLE 3.5 Modified synthetic seawater medium  
(Lyman & Flemming (1940) in Kinne, 1976).

| ADDITIVE                             | CONCENTRATION (g l <sup>-1</sup> ) |
|--------------------------------------|------------------------------------|
| NaCl                                 | 23,477                             |
| MgCl <sub>2</sub> ·6H <sub>2</sub> O | 10,626                             |
| Na <sub>2</sub> SO <sub>4</sub>      | 3,917                              |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 1,459                              |
| KCl                                  | 0,664                              |
| Na HCO <sub>3</sub>                  | 0,192                              |
| Na Br                                | 0,083                              |
| H <sub>3</sub> BO <sub>3</sub>       | 0,026                              |
| Sr Cl <sub>2</sub>                   | 0,024                              |
| NaF                                  | 0,003                              |

TABLE 3.6 Modified medium for *Azotobacter*  
(Harrigan & McCance, 1966).

| ADDITIVE                             | CONCENTRATION (g l <sup>-1</sup> ) |
|--------------------------------------|------------------------------------|
| Mannitol                             | 10,0                               |
| K <sub>2</sub> HPO <sub>4</sub>      | 0,5                                |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O | 0,2                                |
| NaCl                                 | 20,0                               |
| MnSO <sub>4</sub> ·4H <sub>2</sub> O | 5,0 x 10 <sup>-3</sup>             |
| FeSO <sub>4</sub> ·7H <sub>2</sub> O | 10,9 x 10 <sup>-3</sup>            |

## CHAPTER 4

### THE EFFECT OF ENVIRONMENTAL FACTORS ON ARA

#### 4.1 MATERIALS & METHODS

Material was collected from the main sampling areas at about 09h00, as described in Section 3.3.1.1, and the effect of selected environmental conditions on ARA was studied as outlined below. For all the experiments, except those investigating percentage moisture and salinity, 2 ml of sterile seawater (25‰, pH 7,8) was added to each serum bottle in an attempt to standardise conditions of salinity and moisture in all the experiments for comparative purposes. High rates of ARA were evident under submerged conditions (Section 4.1.1) and it was therefore felt that under such conditions, the effects of various environmental conditions would be more clearly visible. All experiments, with the exception of temperature and light intensity, were incubated in a growth cabinet at a temperature of 22°C (the mean annual air temperature is 21,6°C and the mean surf temperature is 22,1°C (Section 3.1.5)) and a continuous light intensity of  $200 \mu\text{E m}^{-2} \text{s}^{-1}$  (the maximum light intensity that could be obtained in the growth cabinet). Results were analysed as described in Sections 3.3.1.4 and 3.3.1.6.

##### 4.1.1 PERCENTAGE MOISTURE

Sets of samples were incubated under conditions of submergence, saturation (samples were submerged, followed by removal of all excess water) and varying degrees of dehydration (samples were exposed to a fan at 25°C for various time periods). One set

remained unchanged, representing field conditions at the time of sampling. Percentage moisture of each sample was determined as described in Section 3.3.2.3.

#### 4.1.2 TEMPERATURE

Samples were incubated at temperatures of 12, 17, 22, 27, 32 and 37°C.

#### 4.1.3 SALINITY

Core samples from the wet mat area and pneumatophore segments were incubated under submerged conditions at salinities ranging from 0 to 45‰. This covers the range of salinities that these sites are subjected to in the field. Core samples from the dry mat area are often subjected to higher salinities as a result of dehydration of the mud and were therefore incubated at salinities ranging from 0 to 80‰. Lower salinities were obtained by diluting seawater with distilled water and higher salinities by adding hypersaline water.

#### 4.1.4 LIGHT INTENSITY

Samples were subjected to light intensities of 40, 80, 120, 160 and 200  $\mu\text{E m}^{-2} \text{s}^{-1}$ . One set of samples was covered in aluminium foil in order to simulate dark conditions. Different light intensities were achieved by varying the distance of the samples from the light source and using shade cloth.

#### 4.1.5 ORGANIC CARBON

Seawater made up to a salinity of 25<sup>0</sup>/oo and a pH of 7,8 was amended with sucrose to give 0; 0,05; 0,5 and 5% solutions. Samples were subsequently submerged in the above solutions.

#### 4.1.6 INORGANIC NITROGEN

In order to investigate the effect of inorganic nitrogen on ARA, solutions of ammonium and nitrate were made up in a modified synthetic seawater medium of Lyman & Flemming (1940) (in Kinne, 1976) to give concentrations of 0; 0,1; 0,5; 1; and 5 mg l<sup>-1</sup> (Table 3.5). Samples were subsequently submerged in the above solutions.

### 4.2 RESULTS

#### 4.2.1 PERCENTAGE MOISTURE

The effect of moisture content on ARA is represented in Figure 4.1. The pneumatophore organisms and the wet mat area exhibited significantly higher rates of ARA under submerged conditions ( $P < 0,01$ ). Under saturated conditions, rates of ARA of the pneumatophore organisms and the wet mat area decreased to 21,1% and 47,6% of the rate recorded under submerged conditions respectively (regarded as being 100% ARA). Rates of ARA decreased significantly thereafter with increasing dehydration. In the wet mat area, rates of ARA decreased to 18,3% of the maximum under field conditions at time of collection



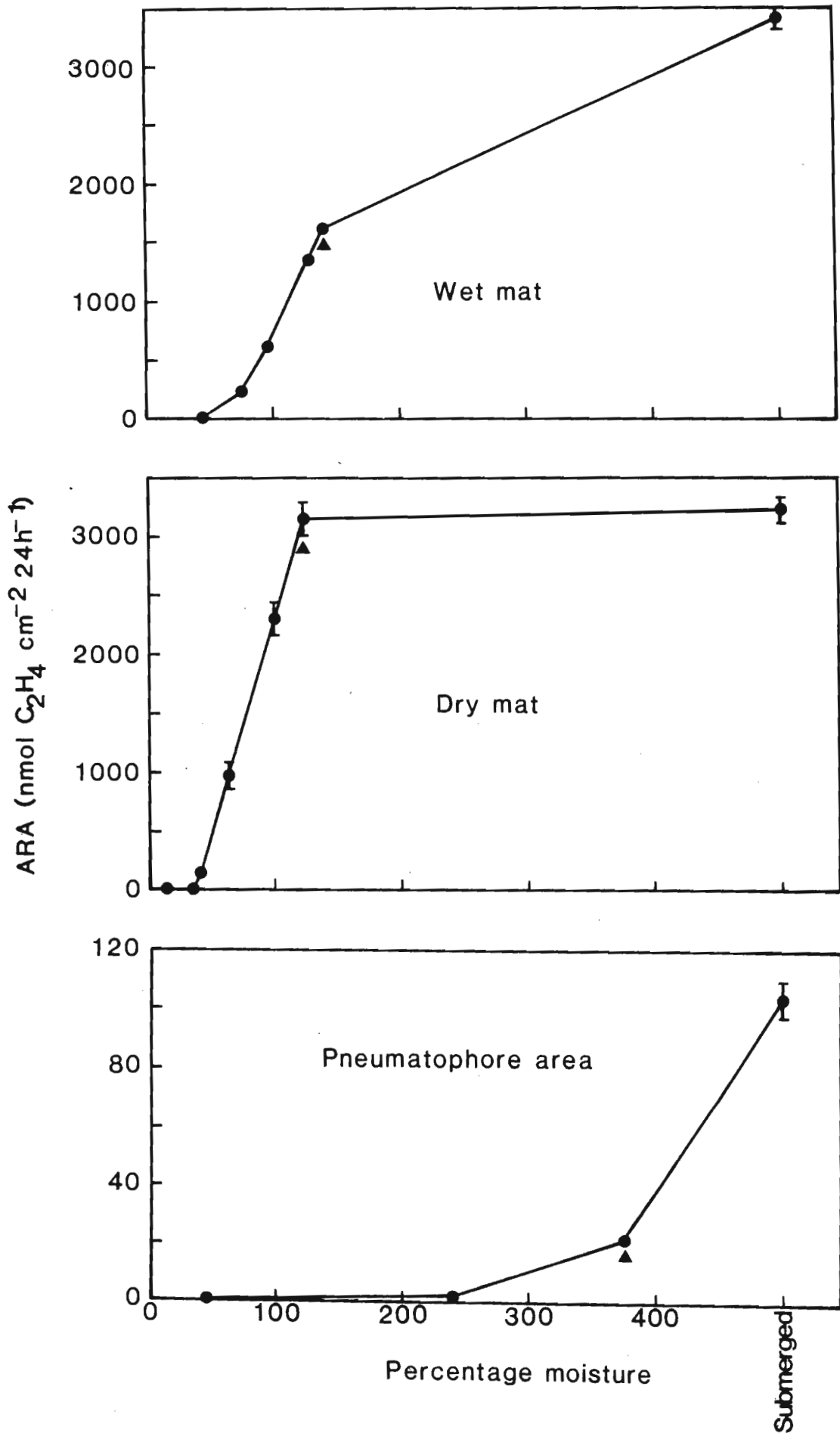


Figure 4.1 The effect of moisture content on acetylene reduction activity. The vertical bars represent one standard error. The arrow (▲) indicates the saturated condition.

(97% moisture). In the dry mat area, highest rates of ARA occurred under saturated and submerged conditions, with no significant differences between these values. Under saturated conditions, rates of ARA were 98,2% of submerged rates. This was followed by a sharp decrease in ARA with increasing dehydration. At 44 and 37% moisture, ARA ceased in the wet and dry mat areas respectively. Rates of ARA of the pneumatophore organisms decreased to 0,5% of the maximum rate at 44% moisture.

#### 4.2.2 TEMPERATURE

The influence of temperature on ARA is shown in Figure 4.2 and similar trends were evident in the three habitats under investigation. Micro-organisms were mesophilic, exhibiting maximum rates of ARA at 22°C. At 12 and 37°C, ARA could not be detected, with the exception of the dry mat area, where very low rates (0,6% of the maximum rate) were recorded at 37°C.

#### 4.2.3 SALINITY

The pneumatophore organisms exhibited no significant differences in rates of ARA over the experimental range of salinities (Figure 4.3). Maximum rates of ARA in the wet mat area occurred between 15 and 35‰, with no significant differences being recorded between these values. A slight, but significant decrease in rates of ARA ( $P < 0,01$ ) was evident at lower salinities of 0 and 5‰, where rates of between 79,5 and 81% of the maximum rates were recorded. In the dry mat area,

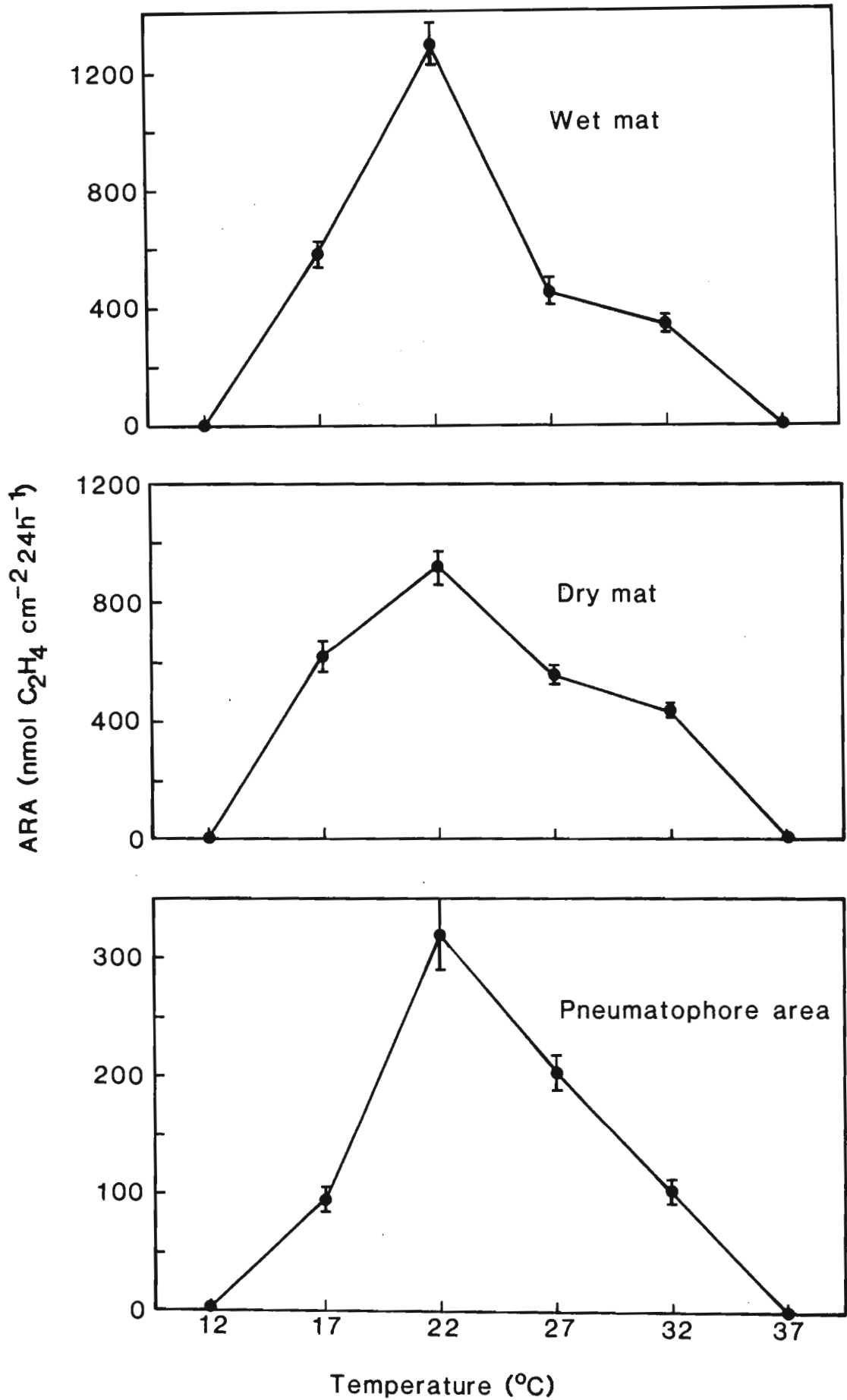


Figure 4.2 The effect of temperature on acetylene reduction activity. The vertical bars represent one standard error.

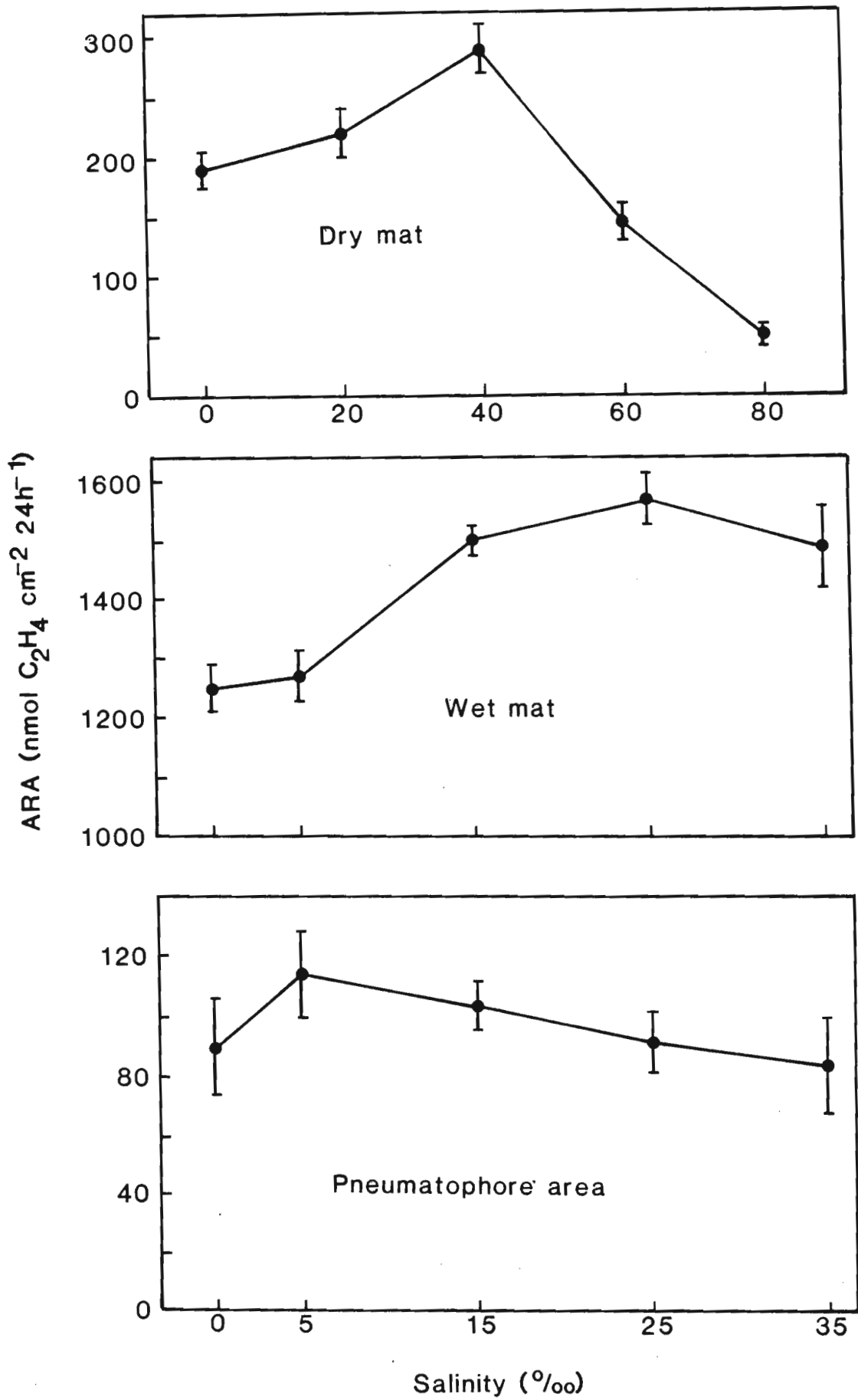


Figure 4.3 The effect of salinity on acetylene reduction activity. The vertical bars represent one standard error.

significantly higher ARA rates occurred at 40<sup>0</sup>/oo ( $P < 0,01$ ). At 0 and 20<sup>0</sup>/oo, rates of ARA decreased to 66 and 76% of the maximum rate respectively, with no significant differences between these values. Progressively marked decreases in ARA occurred at salinities higher than 40<sup>0</sup>/oo.

#### 4.2.4 LIGHT INTENSITY

In all sites, ARA increased with increases in light intensity (Figure 4.4). Saturation occurred at 40  $\mu\text{E m}^{-2} \text{s}^{-1}$  and no significant increase in ARA was evident thereafter at experimental light intensities. The highest light intensity (200  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) was not inhibitory to ARA. In the dark, rates of ARA decreased to 5,46 and 7,47% of the maximum in the wet and dry mat areas respectively. The pneumatophore organisms showed no significant difference between rates of ARA in the dark and at 26  $\mu\text{E m}^{-2} \text{s}^{-1}$  and dark rates were 48,83% of the maximum rate.

#### 4.2.5 ORGANIC CARBON

The wet and dry mat areas showed no response to sucrose amendment (Figure 4.5). The pneumatophore organisms showed a slight stimulation at low concentrations of sucrose (0,05 and 0,5%), where a significant increase in ARA occurred ( $P < 0,05$ ).

#### 4.2.6 INORGANIC NITROGEN

Amendment with high concentrations of nitrate and ammonium caused

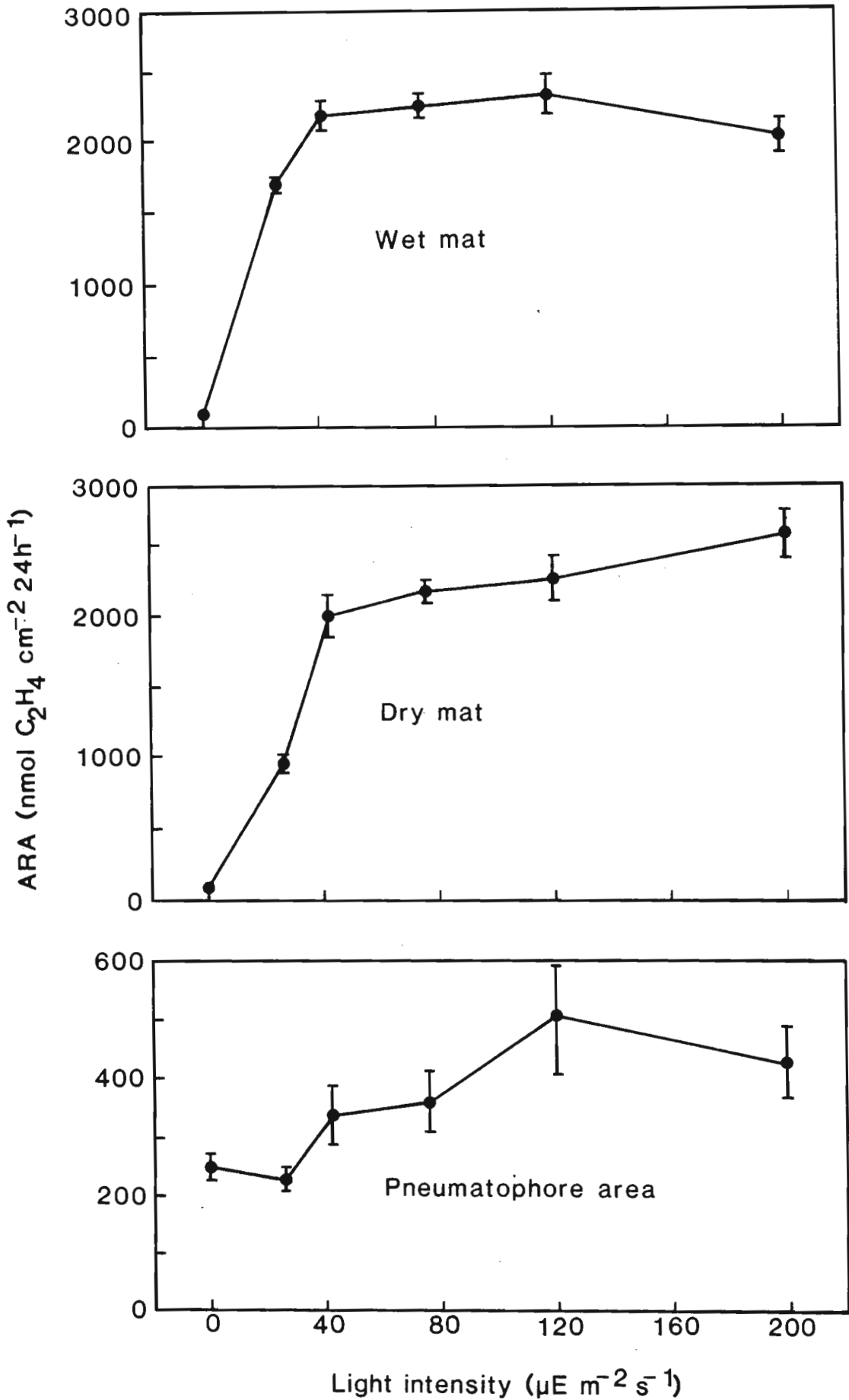


Figure 4.4 The effect of light intensity on acetylene reduction activity. The vertical bars represent one standard error.

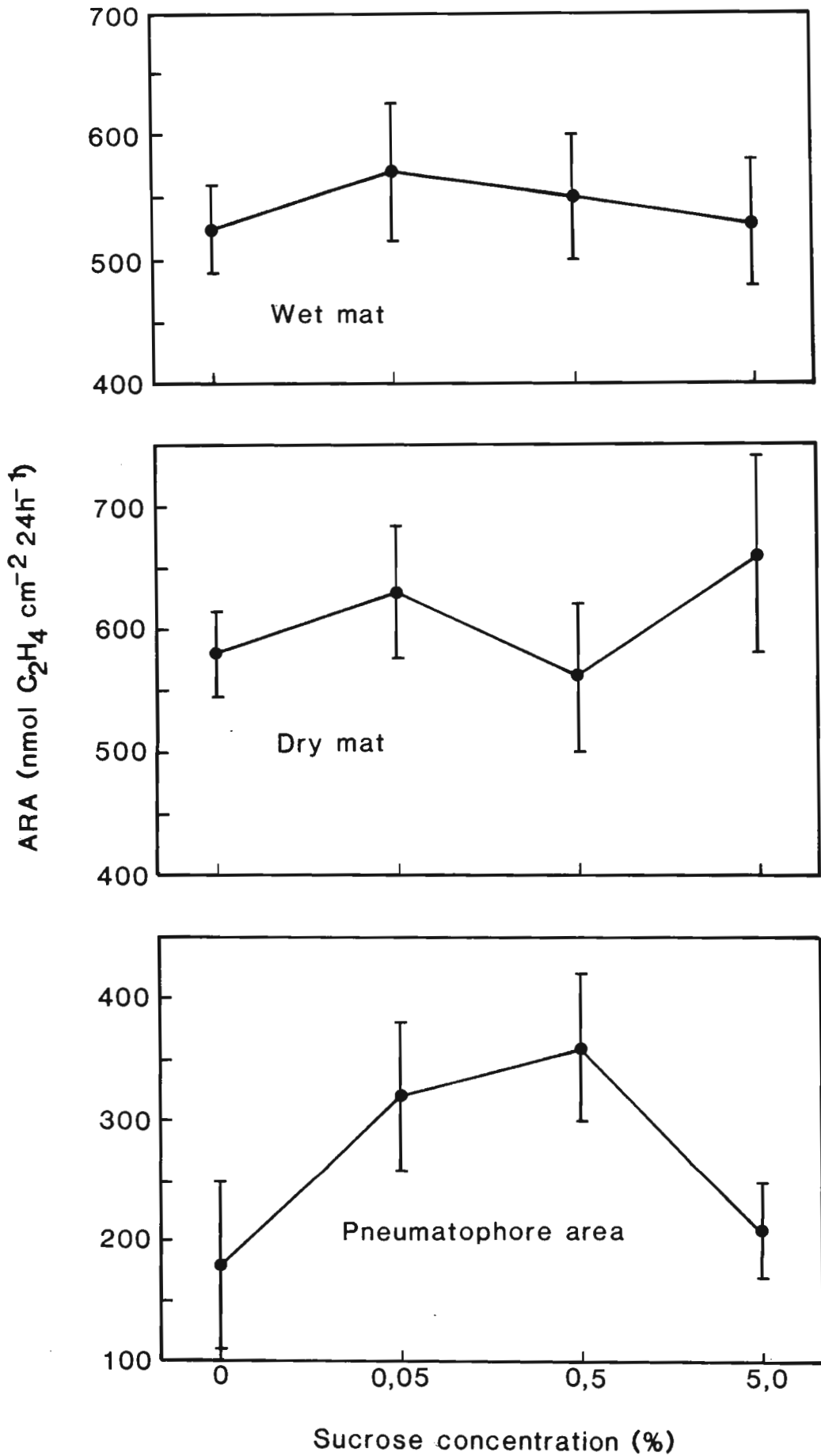


Figure 4.5 The effect of organic carbon amendment on acetylene reduction activity. The vertical bars represent one standard error.

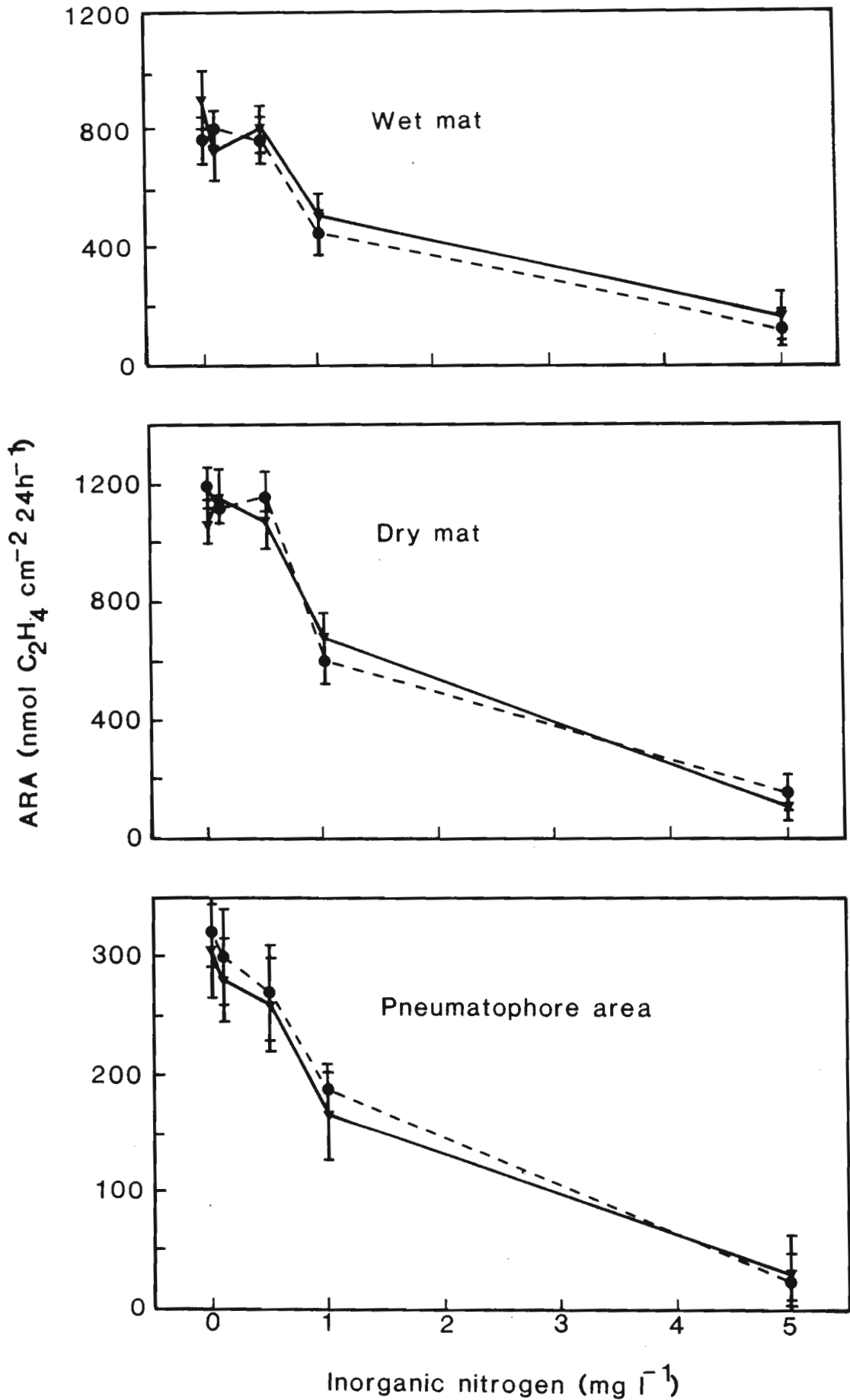


Figure 4.6 The effect of ammonium (  $\triangle$ --- $\triangle$  ) and nitrate (  $\bullet$ --- $\bullet$  ) on acetylene reduction activity. The vertical bars represent one standard error.



inhibition of ARA in all sites, which is evident in Figure 4.6. At  $1 \text{ mg l}^{-1}$ , partial inhibition of ARA occurred and rates decreased to between 49 and 59% of the maximum rate. At  $5 \text{ mg l}^{-1}$ , almost total inhibition of ARA occurred and rates decreased to between 9 and 18% of the maximum rate.

### 4.3 DISCUSSION

#### 4.3.1 PERCENTAGE MOISTURE

Moisture appears to be a major factor influencing ARA in the 3 habitats under study. High rates recorded under submerged conditions support research of Talbot (1982) in the Swartkops Estuary salt marsh, but are in contrast to findings of several other workers. The depressive effect of surface water on ARA has been reported in paddy soil (Matsuguchi *et al.*, 1978), salt marshes (Jones, 1974), decomposing mangrove litter (Van der Valk & Attiwill, 1984a) and estuarine areas (Paerl *et al.*, 1981). Dor (1975) suggested that the presence of numerous heterocystous blue-green algae on the exposed parts of Sinai pneumatophores may indicate that the exposed community performs nitrogen fixation, whilst the lower, submerged non-heterocystous algae do not fix nitrogen. Talbot (1982) regarded high rates of ARA under submerged conditions as indicative of the adaptation of salt marsh organisms to waterlogged conditions and that this stimulatory effect may be as a result of a decrease in oxygen supply.

The pneumatophore organisms are subjected to tidal submersion on a daily basis and during this time, rates of ARA are maximal. Moisture becomes severely limiting during exposure at low tide. The wet mat algae are submerged only at spring high tides, but often retain a surface film of water for a number of days thereafter, or may be covered by rainwater. During these periods, maximal ARA is evident. This site maintains a high moisture content of 70% to submerged, as is evident from results in Chapters 5 and 6 and the potential therefore exists for ARA, though somewhat reduced at times, most of the year. This area is continually shaded by *A. marina*, preventing rapid evaporation of moisture.

The dry mat algae are similarly only submerged at spring high tides, but dry out rapidly subsequently due to exposure to direct sunlight, which causes increased surface temperatures. The lowest moisture content recorded in July was 25% and this resulted in decreased rates of ARA (Section 6.3).

Summer rains also provide moisture and during these periods, high rates of ARA would be expected. During dry periods, when rainfall is limited, ARA is low, although the development of dew at night in the winter may exert an influence.

The most dominant blue-green algae in the dry mat area and on the pneumatophores possess thick mucilaginous sheaths (Section 5.2.1), which possibly aid in water retention during exposed periods (Stewart, 1977).

#### 4.3.2 TEMPERATURE

Temperature also has a major influence on ARA. In warm temperate climates, as exists along the Natal coast, maximum ARA usually occurs between 30 and 35°C (Jones, 1977c; 1981; Smith & Hayaska, 1982; Talbot, 1982; Hicks & Silvester, 1985). However, optimal temperatures recorded for all sites fall into the 20 to 25°C range of temperatures, which are the commonly recorded maxima in cool, temperate climates (Jones, 1974; Blasco & Jordan, 1976), although optimum temperatures in this range have been recorded in warm temperate climates of the south-eastern U.S.A. (Hanson, 1977a; Thomson & Webb, 1984).

The pneumatophore organisms fix nitrogen maximally under submerged conditions surrounding high tide (Section 4.2.1), and an adaptation to ambient creek water temperatures would thereafter be expected. Temperatures ranging from 15,5 to 27°C, with a mean of 21,25°C have been recorded during this study. This corresponds to the optimum temperature at which ARA occurs and the pneumatophore organisms appear to be well adapted to *in situ* temperatures.

The dry mat area is exposed to full sunlight and as a result, high temperatures are reached at midday during summer. Mud temperatures ranging from 12 to 36°C have been recorded in this area during the study, although lower air temperatures are experienced during winter nights (5°C has been recorded at the Botanic Gardens as the absolute minimum for June (Section 3.1.5). It was not possible to record temperatures in the

field over a 24 h period. The maximum and minimum mud temperatures are inhibitory to ARA. This could result in a diurnal variation in ARA. Jones (1977a, b) demonstrated that high midday temperatures of 40°C were inhibitory to ARA in blue-green algal mats, causing a decrease in rates at this time.

In the wet mat area, permanent shading by the *A. marina* canopy promotes lower temperatures. Mud temperatures ranging from 13 to 26,5°C have been recorded during the course of this study. These algae seem reasonably well adapted to *in situ* temperatures, although lower winter temperatures may be inhibitory.

The range of temperatures over which positive ARA was recorded is smaller than previously recorded in estuarine areas (Hanson, 1977a; Talbot, 1982). This may result from experiments being conducted under submerged conditions. It is possible that different trends would be evident under exposed conditions. This needs to be determined.

#### 4.3.3 SALINITY

Estuarine environments are subjected to marked fluctuations in salinity as a result of tidal inundation, evaporation and flooding. Micro-organisms in all three study areas appeared to be euryhaline, as ARA was not markedly affected by a range of salinities, supporting work on intertidal blue-green algae (Potts & Whitton, 1977) and salt marsh micro-organisms

(Patriquin & Keddy, 1980; Ubben & Hanson, 1980).

The pneumatophore organisms are subjected to a wide range of salinities between high and low tide (Section 6.4). They are well adapted to an intertidal habitat as they exhibited no significant differences in ARA with salinities ranging from 0 to 35<sup>0</sup>/oo. The effect of higher salinities was not recorded, although higher salinities may be achieved on pneumatophores under conditions of extreme evaporation during exposure at low tide. However, increases in salinity above 35<sup>0</sup>/oo would be accompanied by dehydration and under such conditions, ARA has been shown to be limited.

Maximum rates of ARA in the wet and dry mat areas occurred at *in situ* salinities to which these organisms are most frequently exposed, supporting work of Ubben & Hanson (1980) and Dicker & Smith (1981). The range of salinities recorded in the wet mat area was 4 to 48<sup>0</sup>/oo, although salinities generally varied from 10 to 35<sup>0</sup>/oo (Sections 5.2.2 and 6.3). Maximum rates of ARA occurred within this range of salinities. In the dry mat area, salinities ranged from 0 to 47<sup>0</sup>/oo (Sections 5.2.2 and 6.3). In this area, rain has an important influence on ARA by increasing the percentage moisture (Section 6.3 and 6.4). This also results in a decrease in salinity. It is significant that relatively high levels of ARA were still maintained at salinity levels of 0<sup>0</sup>/oo. A salinity of 85<sup>0</sup>/oo has been recorded in this area (29/5/85), although such a high salinity would usually be accompanied by prohibitively low moisture levels for ARA. It is significant that micro-organisms in this area are able to survive

such high salinities, although other factors are likely to exert a greater limiting effect on ARA at this stage.

#### 4.3.4 LIGHT INTENSITY

Saturation of ARA occurred at low light intensities in all sites. In the Swartkops Estuary, Talbot (1982) reported saturation at higher light intensities of between 300 and 390  $\mu\text{E m}^{-2} \text{s}^{-1}$  in surface sediments.

The wet mat and pneumatophore organisms would be expected to exhibit an adaptation to lower light intensities because of shading and submergence. The wet mat organisms occur naturally in shaded areas under the canopy of *A. marina* trees, where light intensities of between 16 and 150  $\mu\text{E m}^{-2} \text{s}^{-1}$  were commonly recorded (Sections 5.2.2 and 6.3). Higher light intensities (265  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) were sometimes recorded as a result of sun flecks. The pneumatophore organisms generally also occur in partly shaded areas, although they are subjected to full light intensities for limited periods each day. Light intensities during periods of submergence are important as it is under these conditions that maximum rates of ARA occur. Low submerged light intensities of between 22 and 45  $\mu\text{E m}^{-2} \text{s}^{-1}$  have been recorded under shaded conditions (Section 5.2.2).

The dry mat algae naturally occur in full sun for most of the day and would therefore be expected to show an adaptation to higher light intensities. Full sunlight intensities of between



250 and 860  $\mu\text{E m}^{-2} \text{s}^{-1}$  have been recorded at this site (Section 6.3). Saturation at lower light intensities is therefore surprising and cannot be fully explained. These micro-organisms possibly show a tolerance to a wide range of light intensities. Unfortunately because of limitations imposed by the growth cabinet, it was not possible to determine experimentally the effect of higher light intensities on ARA. Other possibilities include fixation only at lower light intensities experienced in the early morning and evening, under submerged conditions during tidal submergence or on overcast and rainy days. On hot, sunny days, high midday temperatures and low moisture content would be dominant factors inhibiting ARA in this area, adding weight to the above arguments. The inhibitory effect of high light intensities on ARA in blue-green algae has been suggested by Stewart *et al.* (1978) and Smith (1984). In other cases, high light intensities were shown to have no inhibitory effects (Jones, 1977b; Carpenter *et al.*, 1978). Jones (1977b) reported that high light intensities experienced in southern Africa at midday were not responsible for inhibition of ARA in mats of blue-green algae. Talbot (1982) reported no inhibitory effects of high light intensities in surface sediments of the Swartkops Estuary.

Contradictory reports appear on dark rates of ARA in blue-green algae communities and surface sediments. A marked decrease in ARA in the absence of light has been reported by workers on seagrasses (Capone & Taylor, 1977), macro-algae (Capone *et al.*, 1977), intertidal lagoons (Bohloul & Wiebe, 1978),

*Trichodesmium* sp. (Taylor *et al.*, 1973) and salt marshes (Jones, 1974; Hanson, 1977b). An 80% decrease in light rates was noted by Hicks & Silvester (1985) on mangrove pneumatophores in the dark. A 50% decrease in light rates was recorded by Potts & Whitton (1977) in an intertidal lagoon and by Kimball & Teas (1975) in mangrove surface sediments. Potts (1979) observed only a slight decrease in mangrove blue-green algal ARA in the dark. Talbot (1982) reported a lag of 24 h before light and dark rates differed significantly. It has been suggested that the occurrence of high rates of dark fixation is an indication of heterotrophic nitrogen fixation (Kimball & Teas, 1975; Thomson & Webb, 1984; Hicks & Silvester, 1985). If this is the case, it would appear that ARA is primarily due to blue-green algae in the wet and dry mat areas, whereas a significant portion of ARA associated with pneumatophores could be accounted for by heterotrophic bacteria. However, high night time rates of ARA have been recorded in blue-green algal communities (Carpenter *et al.*, 1978). Dark fixation is strongly linked to the availability of organic compounds (Taylor *et al.*, 1973; Fay, 1976). The influence of organic carbon will be discussed further in the Section below.

#### 4.3.5 ORGANIC CARBON

Organic carbon is most frequently implicated as an important factor limiting ARA (Talbot, 1982). In this study, the supply of organic carbon does not appear to be a major factor limiting rates of ARA, especially in the wet and dry mat areas, where



no response to sucrose supplementation was shown. In habitats rich in organic carbon, such as mangrove roots and litter, limited response to carbon amendment has been shown (Zuberer & Silver, 1978). Zuberer & Silver (1978) and Hicks & Silvester (1985) related ARA to *in situ* levels of organic carbon in mangrove soil. Higher rates of nitrogen fixation in plant-associated mangrove surface sediment were correlated with the presence of plant derived organic matter. Various mangrove sediments have been shown to possess high levels of organic carbon (Walsh, 1967; Hicks & Silvester, 1985).

In the wet mat area, organic carbon levels are high, ranging from 50,8 to 94,7 mg g dry mass<sup>-1</sup> (Sections 5.2.2 and 6.3) and these levels may not be limiting to ARA. However, in the dry mat area, organic carbon is likely to be limiting at times, as lower levels of 24,9 to 65,0 mg g dry mass<sup>-1</sup> have been recorded (Sections 5.2.2 and 6.3) and other factors must be responsible for the lack of stimulation of ARA by sucrose.

The enhancement of ARA by organic carbon amendment is generally regarded as evidence of heterotrophic nitrogen fixation in carbon-limited habitats (Jones, 1974; Marsho *et al.*, 1975; Pearson & Taylor, 1978; Talbot, 1982), although Fay (1976) reported stimulation of ARA in blue-green algae by glucose amendment. The lack of stimulation of ARA in the wet and dry mat areas is more likely to be related to the fact that blue-green algae are the predominant fixers in these areas and utilize carbon manufactured during photosynthesis. This has

been presumed by a number of workers (Capone *et al.*, 1977; Capone & Taylor, 1977; Jones, 1977a; Talbot, 1982). The stimulation of ARA by pneumatophore organisms possibly indicates that bacteria are contributing to pneumatophore-associated fixation and that carbon is limiting to organisms in this habitat. It would appear that blue-green algae and bacteria are important nitrogen fixers on the pneumatophores.

#### 4.3.6 INORGANIC NITROGEN

It has been suggested that the high concentrations of ammonium generally associated with mangrove soils may inhibit nitrogen fixation (Kimball & Teas, 1975; Zuberer & Silver, 1975). Van der Valk & Attiwill (1984a) partly attributed low rates of ARA associated with mangrove roots to high ammonium levels in interstitial waters.

The results indicate that high ammonium and nitrate concentrations were limiting to ARA. The pneumatophore organisms fix nitrogen optimally under submerged conditions at high tide, and at this time, *in situ* inorganic nitrogen levels in the creek water were relatively low (Section 6.4). High levels were recorded in the creek at low tide, but during this period, the pneumatophores are exposed and dehydration becomes the prime limiting factor.

In the wet and dry mat areas, it is difficult to relate the results of laboratory experiments to *in situ* levels, as the

concentrations of nitrogen were expressed differently i.e. per litre and per g wet mass respectively. Also, the interstitial levels of inorganic nitrogen were not taken into account, which would result in an increase in the actual concentrations of nitrogen in each experiment. *In situ* levels of ammonium are extremely high (155 to 7820  $\mu\text{g g w mass}^{-1}$ ) (Sections 5.2.2 and 6.3) when compared to inhibitory levels reported from salt marshes of 20 to 160  $\mu\text{g g}^{-1}$  (Dicker & Smith, 1980b; Casselman *et al.*, 1981) and it is likely that such levels were inhibitory to ARA in this study. However, Kimball & Teas (1975) suggested that ammonium may be immobilized by adsorption onto soil particles in mangrove soils and ARA may therefore not be so severely limited.

Severe inhibition of ARA is apparent between 1 and 5  $\text{mg g w mass}^{-1}$  and this is within the range reported in some salt marshes (Carpenter *et al.*, 1978; Teal *et al.*, 1979; Capone, 1982), but much higher than in others (Patriquin & Keddy, 1978; Dicker & Smith, 1980b; Casselman *et al.*, 1981).

In conclusion, moisture content and temperature appear to be the most important environmental factors controlling ARA in the Mgeni Estuary mangrove swamp. However, difficulty arises in the individual assessment of environmental factors, as they have a cumulative effect. The state of one factor may also override the influence of others, thus masking their effects. These experiments were conducted under favourable temperatures, light intensities, salinities and moisture content in an attempt to avoid this.

## CHAPTER 5

### SEASONAL VARIATION IN ARA

#### 5.1 MATERIALS AND METHODS

A seasonal study was conducted for a year starting in October 1985. Sampling was carried out every 2 months and 2 days after new moon (corresponding to peak spring tides) at a fixed hour (between 08h30 and 09h30) on 16/10/85, 14/12/85, 11/2/86, 11/4/86, 9/6/86 and 7/8/86.

##### 5.1.1 THE ACETYLENE REDUCTION ASSAY

Samples were collected from the main sampling area of each site as described in Section 3.3.1.1. Ten replicate samples were collected at each sampling date. Serum bottles containing pneumatophore segments were incubated under submerged and exposed conditions in order to simulate conditions of high and low tides to which the pneumatophores are exposed on a daily basis. The submerged pneumatophore samples contained 2 ml water collected from near the mouth of the creek. The exposed pneumatophore samples contained no water. Samples from the wet and dry mat areas were submerged by 2 ml of site water when the main sampling areas were flooded, otherwise samples were incubated under exposed conditions. Samples were prepared as described in Section 3.3.1.1 and incubated in the shade near the site of collection. The bottles containing the submerged pneumatophore segments were tied to pneumatophores in the creek in order to simulate submerged conditions. Termination of the reaction and analysis of ethylene were conducted as described in

Sections 3.3.1.3 and 3.3.1.4 respectively.

#### 5.1.2 MONITORING OF PHYSICO-CHEMICAL PARAMETERS

In order to understand trends in ARA, environmental parameters were monitored simultaneously. Samples were collected and analysed for salinity and inorganic nitrogen as described in Sections 3.3.2.4 and 3.3.2.6 respectively. Percentage moisture and organic carbon of the samples were determined after ARA had been carried out as described in Sections 3.3.2.3 and 3.3.2.5 respectively. Light intensity and temperature at all 3 sites were recorded at midday for comparative purposes as described in Sections 3.3.2.2 and 3.3.2.1 respectively.

#### 5.1.3 IDENTIFICATION AND ENUMERATION OF NITROGEN-FIXING COMMUNITIES

Blue-green algae were identified as described in Section 3.3.3. Samples for enumeration of bacteria and blue-green algae were collected and analysed as described in Section 3.3.4 in order to aid in the understanding of possible trends in ARA.

#### 5.1.4 CONTRIBUTION OF BLUE-GREEN ALGAL COMMUNITIES TO THE ANNUAL NITROGEN BUDGET

The total nitrogen fixed annually in each study site was calculated from the monthly rates of ARA. For the wet and dry mat areas this was calculated as: the rate determined over 24 h every 2 months x the number of days in that month and the

succeeding month (i.e. each rate determined was assumed to represent daily rates over 2 months). For the pneumatophore area, the pneumatophores were assumed to be submerged for 50% of the time. The rates were therefore calculated as: 50% of the exposed rate + 50% of the submerged rate determined over 24 h every 2 months x the number of days in that month and the succeeding month. These figures were totalled to give annual rates of ARA. A conversion factor of 4:1 was applied to convert rates of ARA to amounts of nitrogen fixed (Peterson & Burris, 1976; Turner & Gibson, 1980; Jensen & Cox, 1983) and rates were expressed as  $\text{g N m}^{-2} \text{ yr}^{-1}$ .

In this study, rates of ARA of the pneumatophore organisms were expressed per unit surface area of pneumatophore. In order to express rates per unit surface area of ground sediment, it was necessary to calculate the mean surface area of pneumatophores per unit ground area. In order to achieve this, pneumatophores were collected from 10 random 20 x 20 cm quadrats in the study area, the total surface area of pneumatophores was calculated for each quadrat and converted to  $\text{m}^2$  pneumatophore/ $\text{m}^2$  ground area. Annual rates of nitrogen fixed per  $\text{m}^2$  pneumatophore were then multiplied by this value.

The total surface area of the 3 study sites examined in this project was calculated from aerial photographs and vegetation maps (Raiman, 1986). The wet and dry mat areas were measured directly from the photographs and maps. The area occupied by intertidal pneumatophores was calculated as follows: the mean

width of intertidal pneumatophores on either side of the creek was determined from the mean of 10 transects along the creek. This value was multiplied by the creek distance along which pneumatophores occur (calculated from aerial photographs and maps).

The contribution of nitrogen-fixing organisms to the annual nitrogen budget in the 3 study sites was then calculated as: annual nitrogen fixed per  $m^2$  x total surface area of each site.

## 5.2 RESULTS

### 5.2.1 IDENTIFICATION OF BLUE-GREEN ALGAE

The classification scheme followed is that of Desikachary (1959).

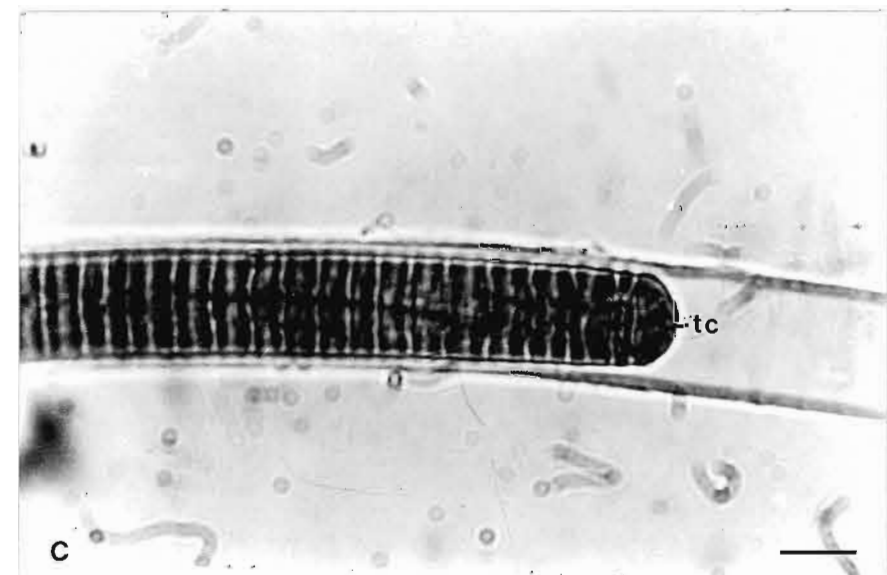
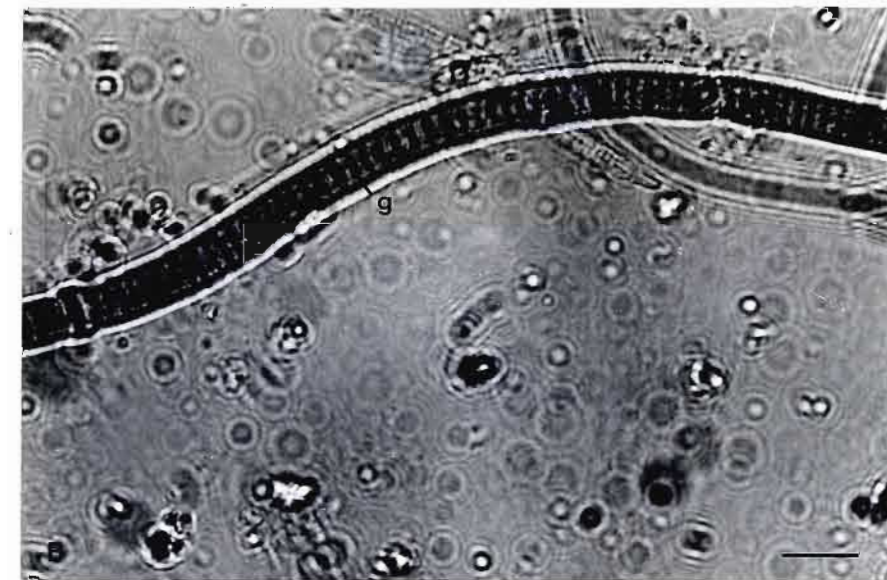
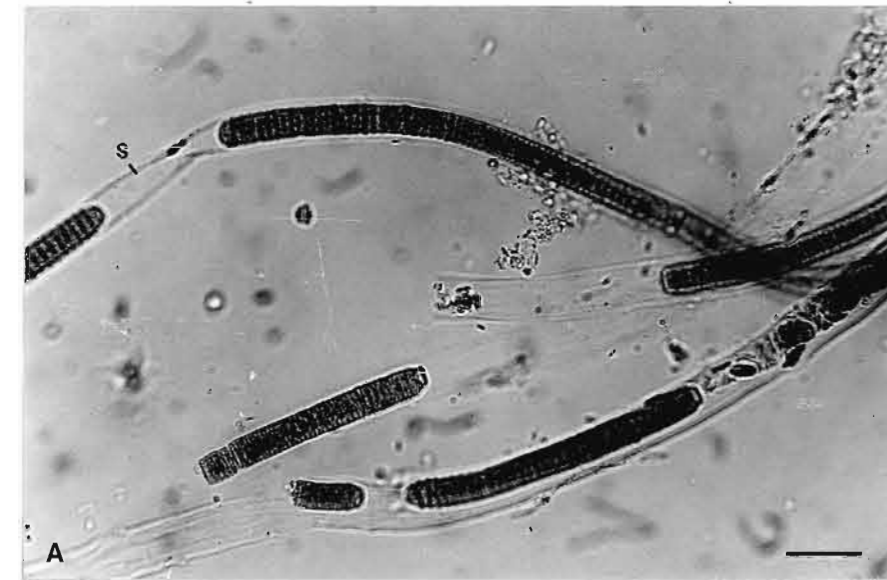
|                  |                        |
|------------------|------------------------|
| <u>Division:</u> | Cyanophyta             |
| <u>Order:</u>    | Nostocales             |
| <u>Family:</u>   | Oscillatoriaceae       |
| <u>Genus:</u>    | Lyngbya Ag. 1824 : 25. |



Figure 5.1 Filament of *L. confervoides* showing:

- (A) the prominent sheath (S),
- (B) cross-wall granules (g) and
- (C) the rounded terminal cell (tc).

Scale line = 11,9  $\mu\text{m}$  (5.1 A)  
= 6,5  $\mu\text{m}$  (5.1 B)  
= 3,1  $\mu\text{m}$  (5.1 C)





*Lyngbya confervoides* C. Ag. ex Gomont 1892 : 156; Geitler, 1932 : 314; Desikachary, 1959 : 314; Humm & Wicks, 1980 : 33,81 (= *Microcoleus lyngbyaceus* (Kütz.) Crouan) (Figure 5.1).

#### DESCRIPTION

Thallus dull green in colour forming a thick mat; trichome cylindrical, straight or slightly curved; trichomes single within a prominent sheath; sheath firm, exterior may be rough, colourless but may become yellow with age, stains blue with methylene blue, 10,5 to 21,0  $\mu\text{m}$  wide, not coloured by chlor-zinc-iodide, trichome sometimes slightly constricted at the cross-walls, cross-walls may be granulated, staining with grams iodine shows granules more clearly; terminal cell rounded, not attenuated, no calyptra present; protoplasm lightly granular; cells 5,3 to 7,9  $\mu\text{m}$  broad and 1,3 to 2,6  $\mu\text{m}$  long, cells  $\frac{1}{6}$  to  $\frac{1}{3}$  as long as broad.

#### DISTRIBUTION

Desikachary (1959 : 314) records *L. confervoides* from marine, estuarine and freshwater habitats in India. Humm & Wicks (1959 : 81) records *M. lyngbyaceus* from fresh, brackish and seawater habitats.

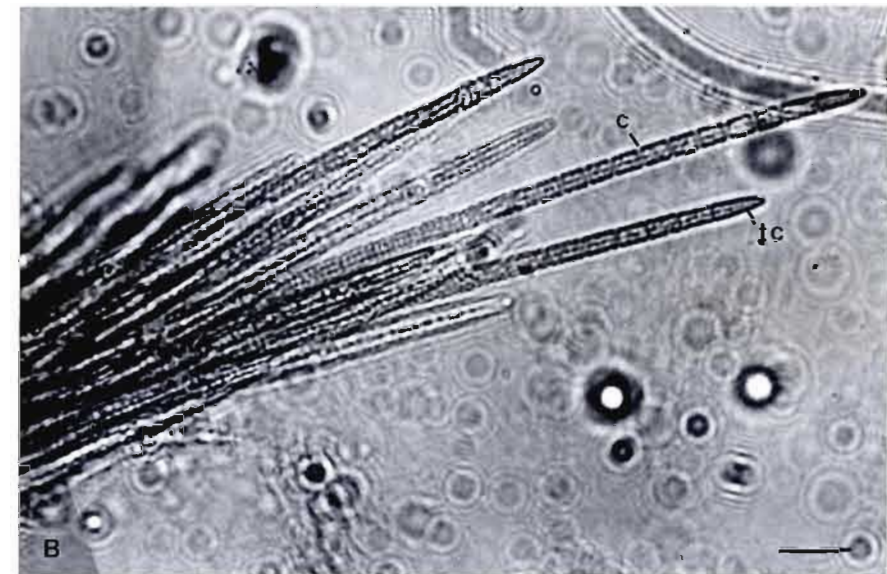
GENUS: *Microcoleus* Desmazieres 1823 : 7.

*Microcoleus chthonoplastes* Thuret ex Gomont 1892 : 353; Geitler, 1932 : 1133; Desikachary, 1959 : 343; Humm & Wicks

Figure 5.2 Filament of *M. chthonoplastes* showing:

- (A) the prominent sheath (s) and twisted trichomes (t),  
 (B) constrictions (c) at the cross-walls and the elongated conical terminal cell (tc).

Scale line = 31,3  $\mu\text{m}$  (5.2 A)  
 = 6,3  $\mu\text{m}$  (5.2 B)



1980 : 73, 145 (= *Schizothrix arenaria* (Berk.) Gomont)  
(Figure 5.2).

#### DESCRIPTION

Thallus bright green in colour, seldom branched, forming a thick mat; trichomes cylindrical, twisted around each other, many in a sheath, densely arranged; sheath distinct, mucilaginous, colourless, stains blue with methylene blue, 34,2 to 97,3  $\mu\text{m}$  wide; not coloured by chlor-zinc-iodide; trichome slightly constricted at the cross-walls, not granulated at cross-walls; terminal cell elongated, pointed conical, end wall thin; cells 1 to 2 times as long as broad, 2,6 to 3,9  $\mu\text{m}$  broad and 3,3 to 5,3  $\mu\text{m}$  long.

#### DISTRIBUTION

Desikachary (1959 : 343) records *M. chthonoplastes* from coastal areas, soil and salt- and freshwater areas in India. Humm & Wicks (1980 : 73) records *S. arenaria* from salt- and freshwater and often mixed with *M. lyngbyaceus* in salt marshes and on tidal flats.

GENUS: *Oscillatoria* Vaucher 1803 : 165.

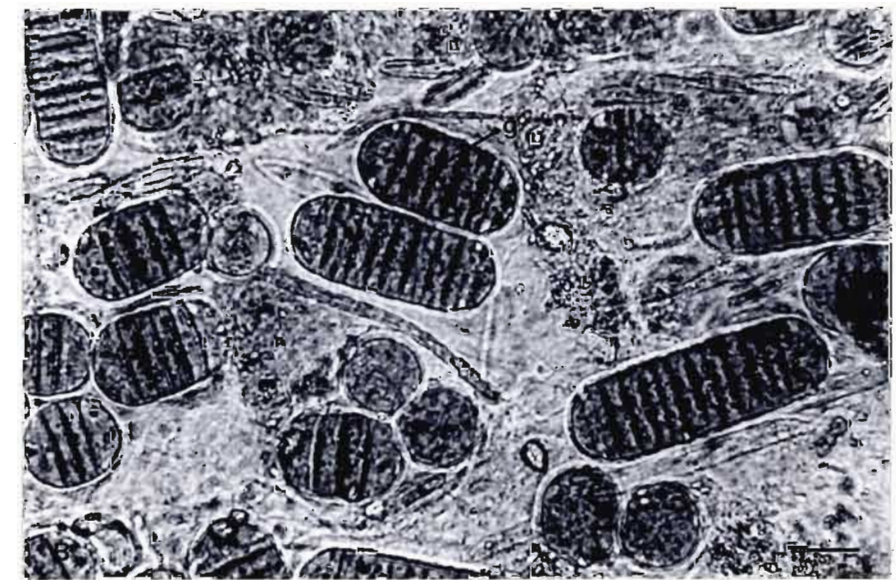
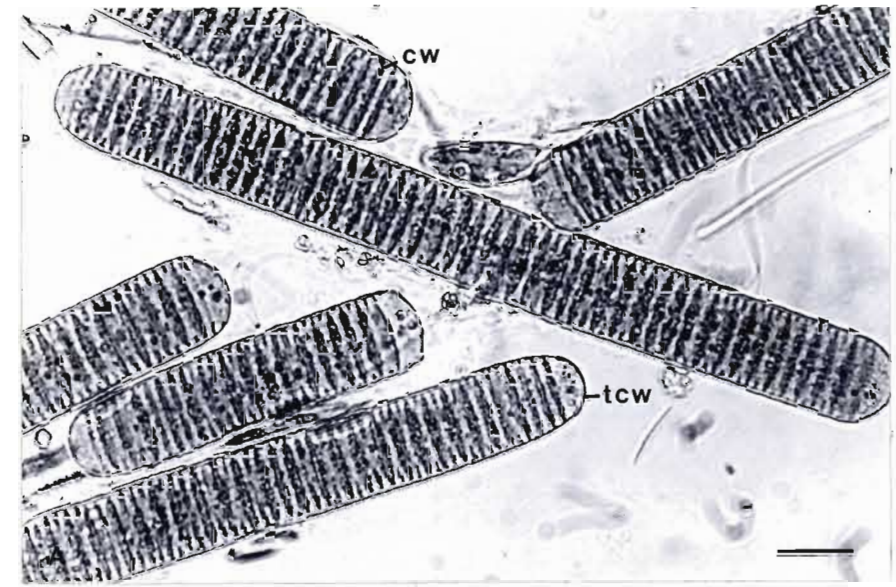
*Oscillatoria limosa* Ag. ex Gomont 1892 : 210; Geitler 1932 : 944; Desikachary 1959 : 206 (Figure 5.3).



Figure 5.3 Filament of *O. limosa* showing:

- (A) cross-walls (cw) and the thickened terminal cell wall (tcw) and
- (B) cross-wall granules (g).

Scale line = 7,8  $\mu\text{m}$  (5.3 A & 5.3 B)



DESCRIPTION

Thallus yellow-brown forming a thick mat or filaments single; trichomes cylindrical, more or less straight, without a sheath; fresh material not constricted at cross walls, becomes slightly constricted on fixing with formaldehyde; cross-walls frequently granulated, granules more apparent on staining with Gram's iodine; terminal cell rounded, may possess slightly thickened end wall which shows up on staining with Gram's iodine; cells  $\frac{1}{3}$  to  $\frac{1}{6}$  as long as broad, 10,5 to 15,8  $\mu\text{m}$  broad and 2,6 to 3,8  $\mu\text{m}$  long; trichomes short (8  $\mu\text{m}$ ) to very long (3525  $\mu\text{m}$ ).

DISTRIBUTION

Desikachary (1959 : 206) records *O. limosa* from fresh- and saltwaters in India.

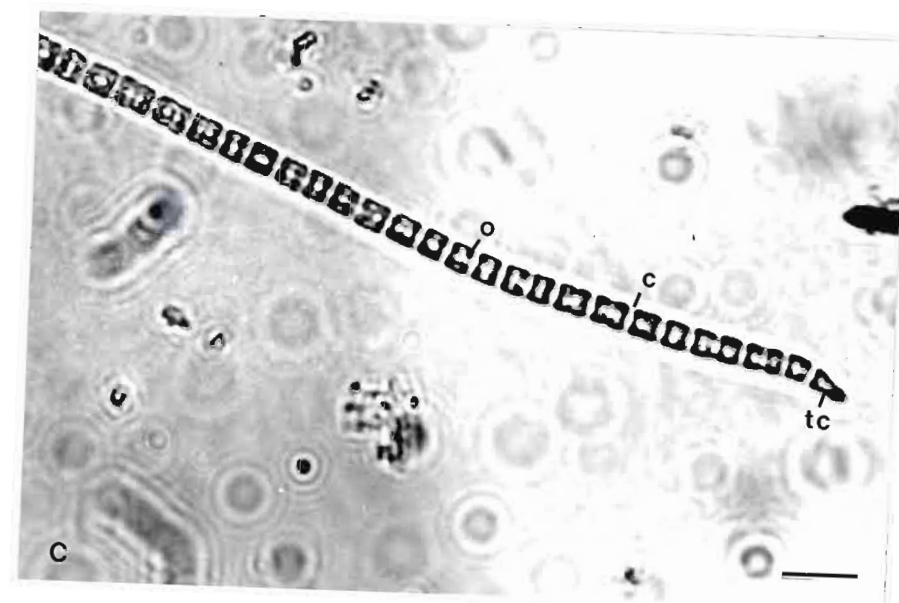
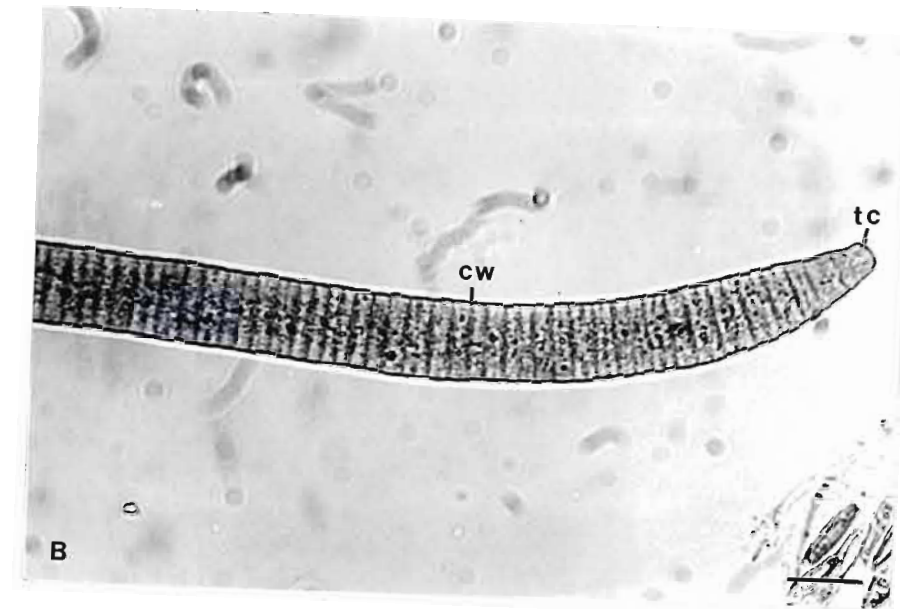
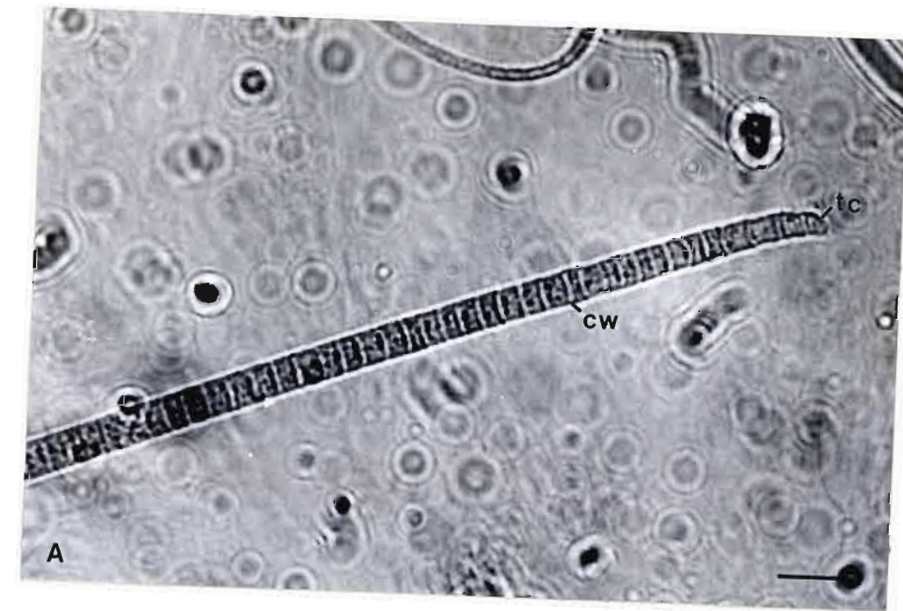
*Oscillatoria okeni* Ag. ex Gomont 1892 : 232; Geitler 1932 : 969; Desikachary 1959 : 231 (Figure 5.4a).

DESCRIPTION

Thallus light blue-green in colour; trichomes cylindrical, straight to slightly curved, without a sheath; not constricted at the cross-walls, no granules at the cross-walls; terminal cell gradually attenuated, slightly bent; cells slightly broader than long, 3,9 to 5,3  $\mu\text{m}$  broad and 2,9 to 4,4  $\mu\text{m}$  long.

- Figure 5.4 (A) Filament of *O. okeni* showing the bent terminal cell (tc) and cross-walls (cw).
- (B) Filament of *O. proboscidea* showing the capitate terminal cell (tc) and cross-walls (cw).
- (C) Filament of *O. schultzei* showing constrictions (c) at the cross-walls, the hooked, attenuated terminal cell (tc) and optically dense regions (o).

Scale line = 5,3  $\mu\text{m}$  (5.4 A)  
 = 7,8  $\mu\text{m}$  (5.4 B)  
 = 4,0  $\mu\text{m}$  (5.4 C)



DISTRIBUTION

Desikachary (1959 : 231) records *O. okeni* from ponds, drains and moist soils in India.

*Oscillatoria proboscidea* Gomont 1892 : 209; Geitler 1932 : 948; Desikachary 1959 : 211 (Figure 5.4b).

DESCRIPTION

Thallus dull green; trichomes cylindrical, straight to curved, without a sheath, not constricted at the cross-walls; cross walls not granulated; trichome attenuates through a number of cells; end cell flatly rounded, capitate; cells  $\frac{1}{4}$  as long as broad, 11,6  $\mu\text{m}$  broad and 2,6  $\mu\text{m}$  long.

DISTRIBUTION

Desikachary (1959 : 211) records *O. proboscidea* from standing water, drains or moist soil in India.

*Oscillatoria schultzi* Lemm. 1905 : 145; Geitler 1932 : 970; Desikachary 1959 : 232 (Figure 5.4c)

DESCRIPTION

Thallus light-green; trichomes cylindrical, straight or slightly curved, without a sheath; distinctly constricted at



the cross-walls, not granulated at the cross-walls; terminal cell distinctly attenuated to a point, hooked, longer than other cells; cells as long as broad or slightly longer, 2,6  $\mu\text{m}$  broad and 2,6 to 2,9  $\mu\text{m}$  long; distinct optically dense region in each cell corner.

#### DISTRIBUTION

Desikachary (1959 : 232) records *O. schultzei* from mud or paddy field soils in India.

#### 5.2.2 ARA AND PHYSICO-CHEMICAL PARAMETERS

Seasonal variation in ARA and physico-chemical factors for the pneumatophore, wet mat and dry mat areas are represented in Figures 5.5 to 5.10. A marked seasonal trend in ARA was apparent in all sites, with highest rates being achieved in the summer months of December and February. Rates decreased significantly for the rest of the year ( $P < 0,05$ ), especially in association with the pneumatophores, where little to no ARA was recorded in the winter months of June and August. At all sampling periods, the pneumatophore algae achieved significantly higher rates of ARA under submerged conditions in comparison to exposed conditions



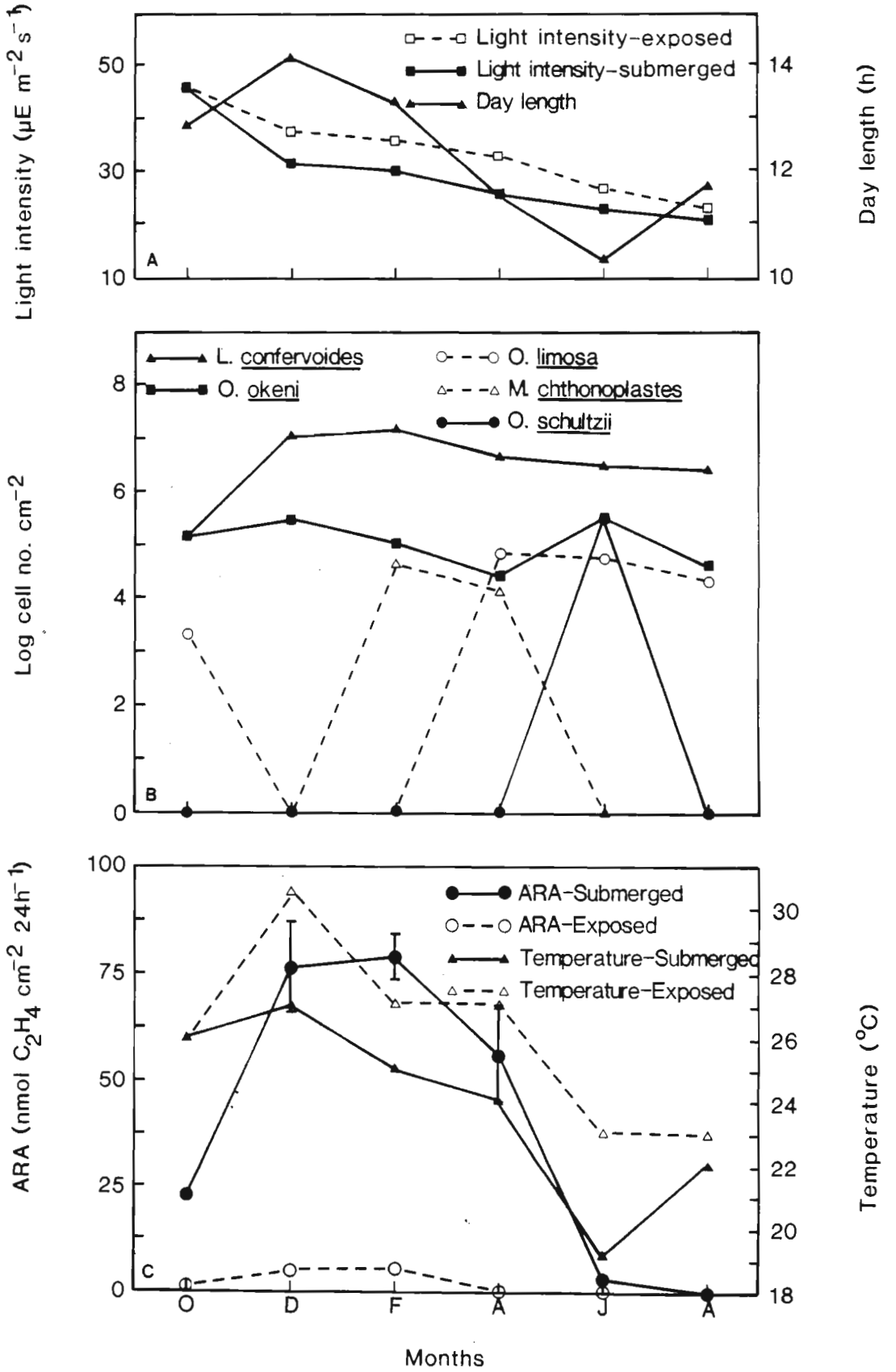


Figure 5.5 Seasonal variation in:  
 (A) light intensity and day length,  
 (B) blue-green algal numbers and  
 (C) acetylene reduction activity and temperature associated with the pneumatophore organisms. The vertical bars represent one standard error.

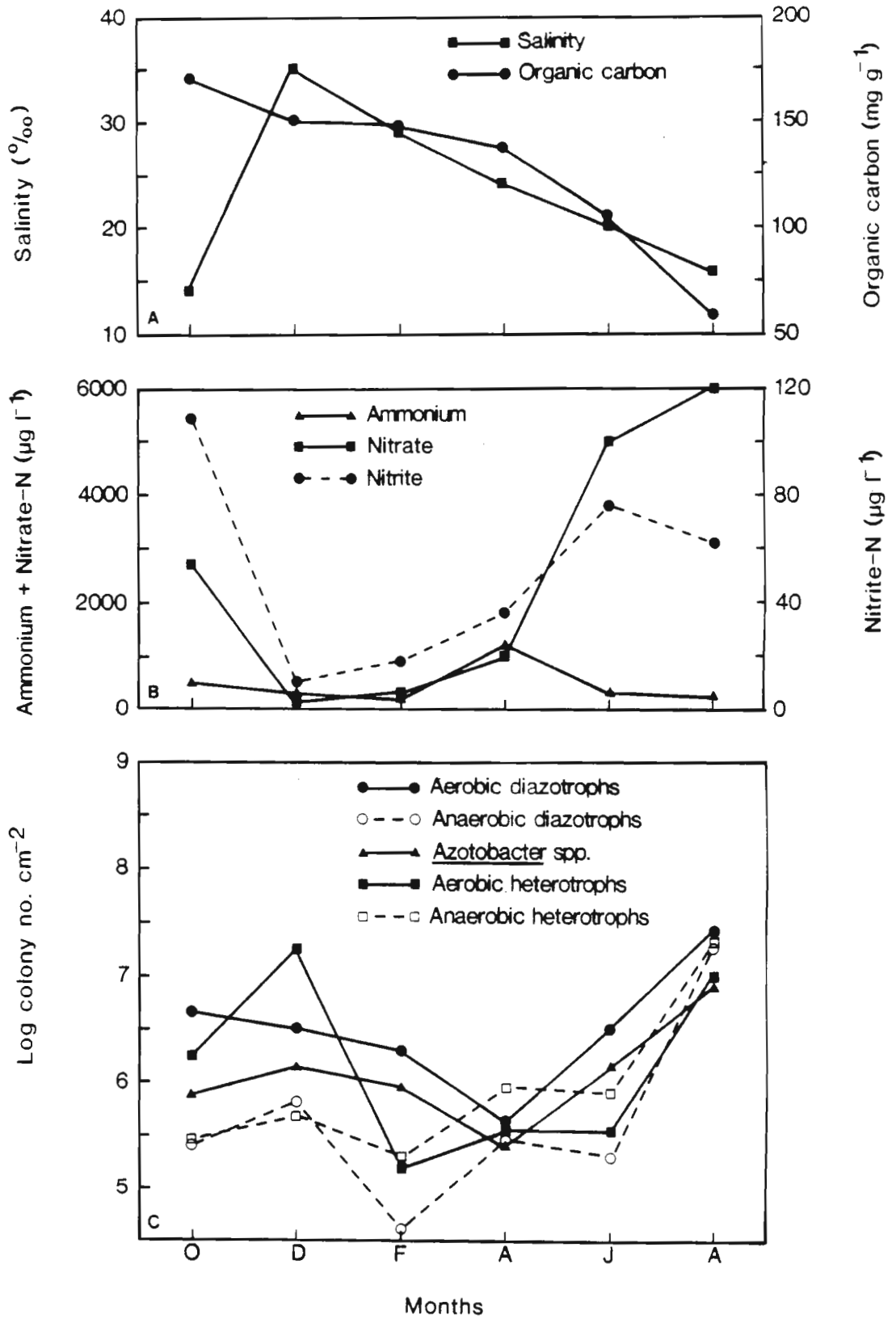


Figure 5.6 Seasonal variation in:  
 (A) salinity and organic carbon,  
 (B) inorganic nitrogen and  
 (C) bacterial numbers associated with the  
 pneumatophore organisms.

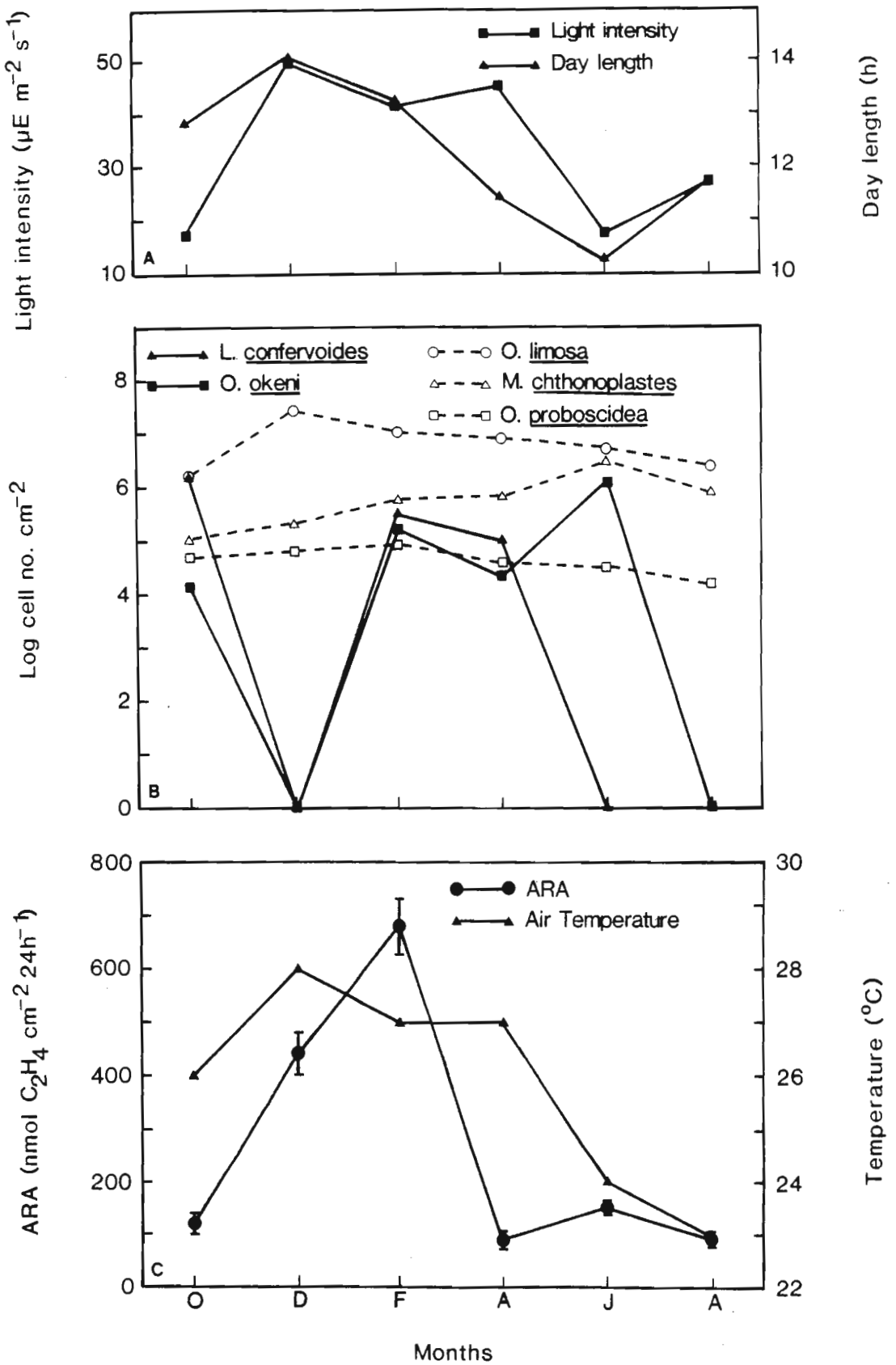


Figure 5.7 Seasonal variation in:  
 (A) light intensity and day length,  
 (B) blue-green algal numbers and  
 (C) acetylene reduction activity and temperature  
 in the wet mat area. The vertical bars  
 represent one standard error.

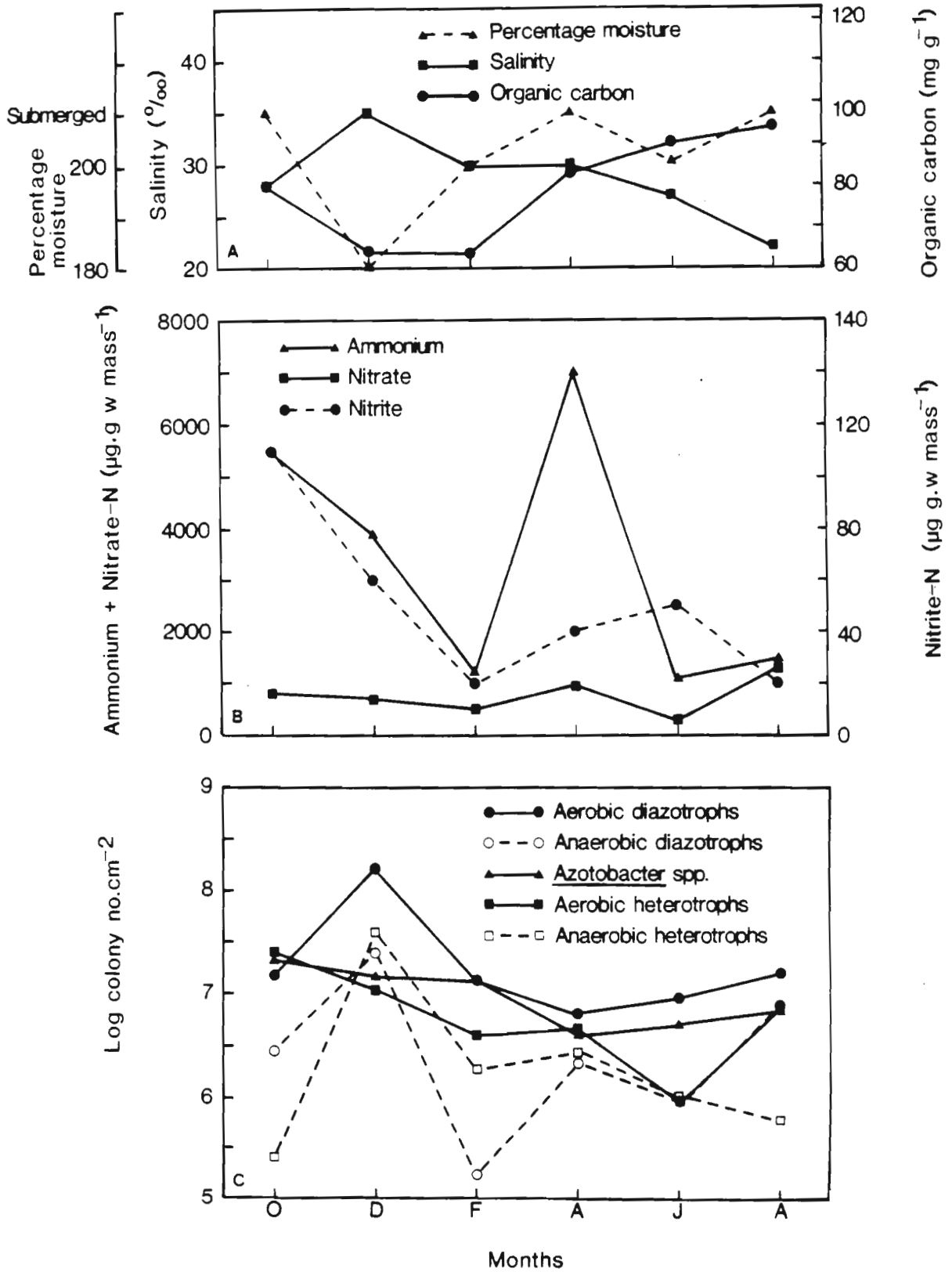


Figure 5.8 Seasonal variation in:

- (A) percentage moisture, salinity and organic carbon,
- (B) inorganic nitrogen and
- (C) bacterial numbers in the wet mat area.

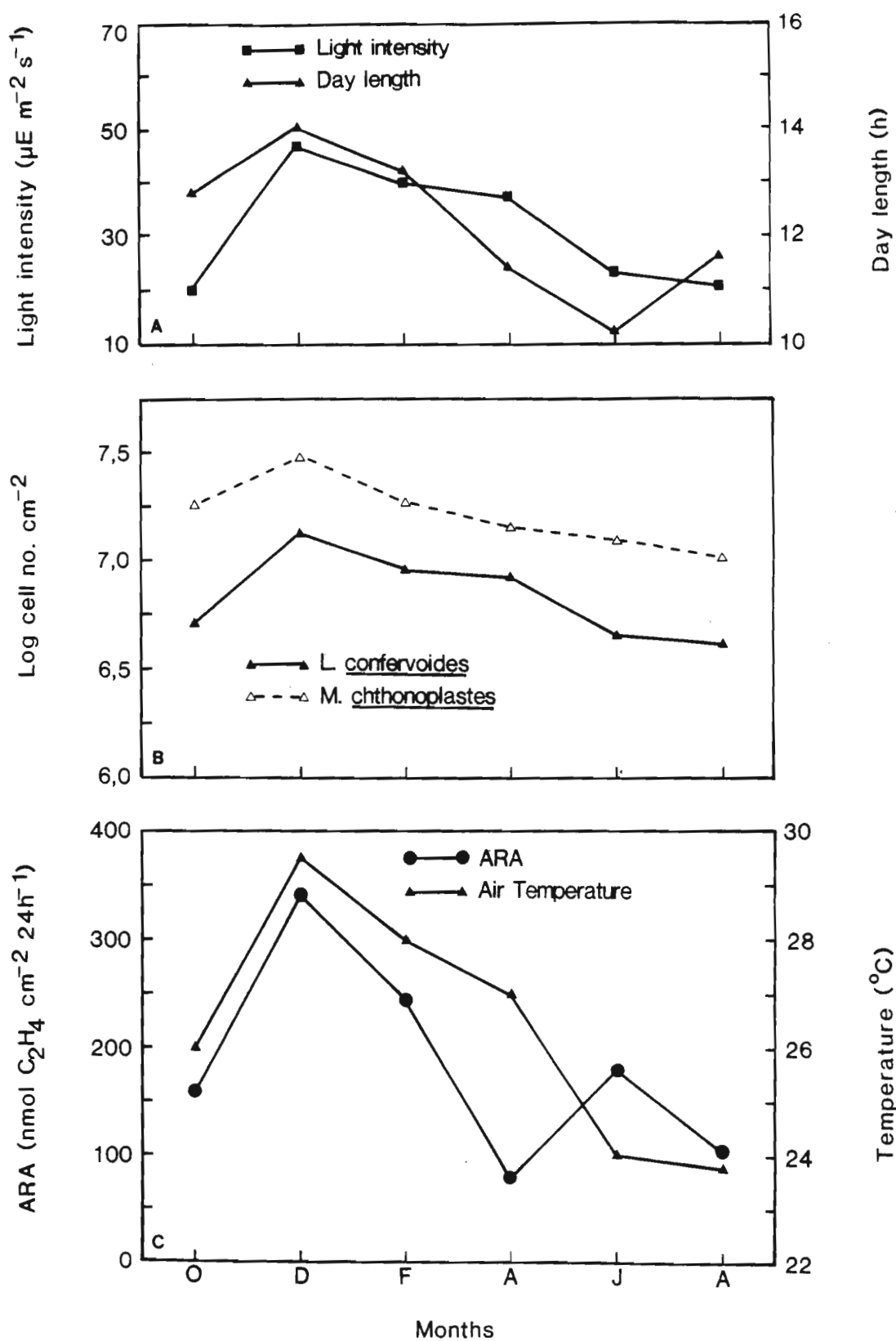


Figure 5.9 Seasonal variation in:  
 (A) light intensity and day length,  
 (B) blue-green algal numbers and  
 (C) acetylene reduction activity and temperature  
 in the dry mat area. The vertical bars  
 represent one standard error.

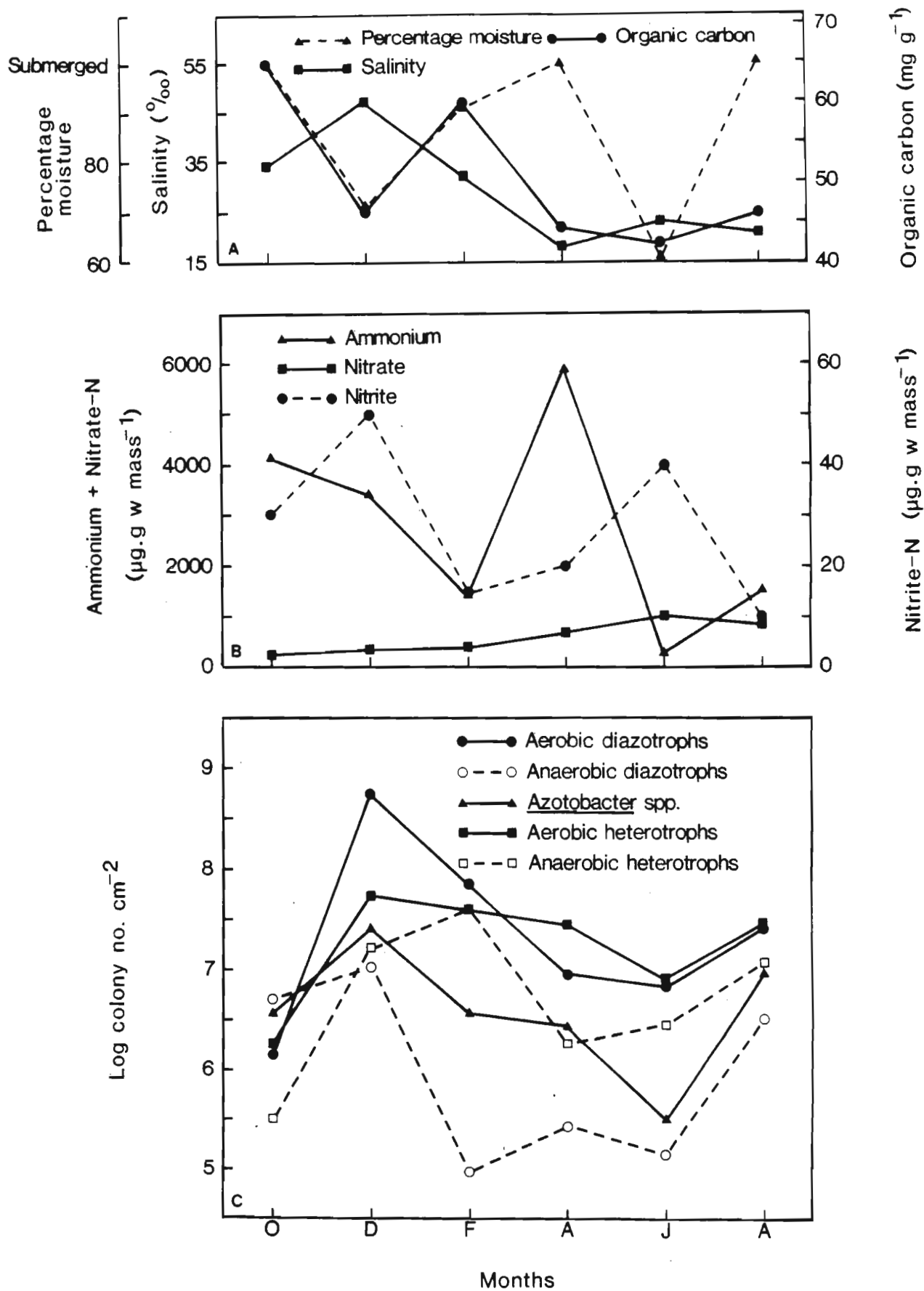


Figure 5.10 Seasonal variation in:

- (A) percentage moisture, salinity and organic carbon,  
 (B) inorganic nitrogen and  
 (C) bacterial numbers in the dry mat area.

( $P < 0,01$ ). In order to compare rates of ARA with studies conducted in other areas, results are also expressed per g dry mass (Table 5.1).

Recorded midday temperatures and light intensities at the incubation sites revealed expected seasonal trends, with summer maxima and winter minima. Day length recorded by Louis Botha weather station revealed similar trends. Mean, maximum and minimum air temperatures recorded from Botanic Gardens weather station gave a better indication of the range of temperatures experienced (Table 3.1).

Ammonium was generally the most abundant form of inorganic nitrogen in the wet and dry mat areas. In the creek, levels of nitrate and ammonium were variable. Low nitrite levels were generally recorded from all 3 sites. Seasonal levels of inorganic nitrogen were variable in the wet and dry mat areas. Low levels of nitrate and ammonium were recorded in the summer in the creek water in which pneumatophore segments were submerged. Levels were highest during autumn (April) and winter months in this site. No apparent seasonal trends were observed in organic carbon levels, percentage moisture and salinity at all 3 sites.

TABLE 5.1 Seasonal variation in acetylene reduction activity associated with blue-green algal communities.

|                                     | ARA   | Oct  | Dec  | Feb  | Apr  | June | Aug |
|-------------------------------------|---|------|------|------|------|------|-----|
| Pneumatophore organisms - submerged | $\text{nmol C}_2\text{H}_4 \text{ cm}^{-2} \text{ 24 h}^{-1}$             | 22   | 76   | 78   | 56   | 3    | 0   |
|                                     | $\text{nmol C}_2\text{H}_4 \cdot \text{g dry wt.}^{-1} \text{ 24 h}^{-1}$ | 1367 | 3749 | 4359 | 3518 | 186  | 0   |
| Pneumatophore organisms - exposed   | $\text{nmol C}_2\text{H}_4 \text{ cm}^{-2} \text{ 24 h}^{-1}$             | 1    | 5    | 6    | 0,5  | 0    | 0   |
|                                     | $\text{nmol C}_2\text{H}_4 \cdot \text{g dry wt.}^{-1} \text{ 24 h}^{-1}$ | 105  | 504  | 872  | 94   | 0    | 0   |
| Wet mat                             | $\text{nmol C}_2\text{H}_4 \text{ cm}^{-2} \text{ 24 h}^{-1}$             | 126  | 444  | 678  | 88   | 153  | 89  |
|                                     | $\text{nmol C}_2\text{H}_4 \cdot \text{g dry wt.}^{-1} \text{ 24 h}^{-1}$ | 617  | 1718 | 3451 | 658  | 1443 | 678 |
| Dry mat                             | $\text{nmol C}_2\text{H}_4 \text{ cm}^{-2} \text{ 24 h}^{-1}$             | 158  | 341  | 243  | 79   | 179  | 105 |
|                                     | $\text{nmol C}_2\text{H}_4 \cdot \text{g dry wt.}^{-1} \text{ 24 h}^{-1}$ | 785  | 1163 | 1142 | 357  | 704  | 408 |



### 5.2.3 ENUMERATION OF NITROGEN-FIXING COMMUNITIES

Blue-green algal and bacterial counts of the 3 sites are represented in Figures 5.5 to 5.10. The most abundant blue-green alga on the pneumatophores was *L. confervoides* and high numbers were evident in the summer months. *O. okeni*, *O. limosa* and *M. chthonoplastes* were also recorded at most sampling periods, but maintained low numbers throughout the study period. *O. schultzei* was recorded only once, in June, when significant numbers were recorded.

*M. chthonoplastes* was dominant in the dry mat area and maintained a high population throughout the year, with a maximum number being recorded in the summer months. *L. confervoides* was also present in smaller numbers and exhibited similar seasonal trends.

The dominant alga in the wet mat area was *O. limosa*. Numbers of this alga were highest in the summer months. Significant decreases in numbers occurred the rest of the year. Low numbers of *O. okeni*, *M. chthonoplastes*, *L. confervoides* and *O. proboscidea* were recorded at most sampling periods throughout the study period.

Bacterial counts were variable and no discernible seasonal patterns of any of the groups enumerated were apparent. Abnormally high numbers of all groups enumerated on the pneumatophores occurred in August. Numbers of aerobic diazotrophs were higher than anaerobic diazotrophs at all sampling periods. Aerobic heterotroph numbers were generally greater than anaerobic heterotroph numbers.

#### 5.2.4 CONTRIBUTION OF BLUE-GREEN ALGAL COMMUNITIES TO THE ANNUAL NITROGEN BUDGET

The mean surface area of pneumatophore per  $m^2$  was calculated as  $1,54 m^2$  pneumatophore/ $m^2$  ground area.

The annual rates of nitrogen fixation, the total area of each site and the contribution of each site to the nitrogen budget of the Mgeni Estuary mangrove swamp is represented in Table 5.2.

TABLE 5.2 Contribution of blue-green algal communities to the annual nitrogen budget.

|  | Pneumatophore study site | Wet mat study site | Dry mat study site |
|--|--------------------------|--------------------|--------------------|
| Nitrogen fixed<br>(g N m <sup>-2</sup> yr <sup>-1</sup> )      | 0,20                     | 3,35               | 2,36               |
| Surface area<br>(m <sup>2</sup> )                              | 7689,10                  | 13931,75           | 21315,36           |
| Contribution to the nitrogen budget<br>(g N yr <sup>-1</sup> ) | 2076,06                  | 46671,36           | 50304,35           |
| Total<br>(g N yr <sup>-1</sup> )                               | 98768,87                 |                    |                    |

### 5.3 DISCUSSION

#### 5.3.1 IDENTIFICATION OF BLUE-GREEN ALGAE

The references available for identification of blue-green algae are not entirely satisfactory as they are based on algae from other parts of the world and often on freshwater species. The identifications must therefore be regarded as tentative. The difficulties experienced in the identification of certain species are outlined below.

The specimen identified as *L. confervoides* may be *L. aesturii* Liebm. ex Gomont. According to Desikachary (1959), *L. aesturii* possess a yellow sheath when mature and a flattened terminal cell with a thickened end wall, whereas *L. confervoides* always possess a colourless sheath and the terminal cell is rounded with a thin end wall. Specimens collected from the dry mat area revealed the presence of yellow sheaths on rare occasions, but difficulty was encountered in the identification of a thickened end wall. Lee (1980) regards yellow and brown sheaths as common in specimens from habitats of high salinity and low moisture content. This suggests that sheath colour may be environmentally induced and therefore not relevant taxonomically. Only one recording of *L. confervoides* from mangroves was located (Potts & Whitton, 1980), whereas *L. aesturii* has been recorded frequently from mangrove swamps (Saenger *et al.*, 1977; Potts, 1979; Potts & Whitton, 1980; Dor, 1984; Potts, 1984) and salt marshes (Stewart & Pugh, 1963; Green & Edmisten, 1974; Jones, 1974; Van Raalte *et al.*, 1974; Carpenter *et al.*, 1978). Nevertheless,

it was felt that the specimen fitted the description of *L. confervoides* more closely and was thus identified as such.

Most of the specimens identified as *O. limosa* possessed thin end walls and because of this character, key out in Desikachary as *O. subbrevis* Schmidle. In all other respects, including cell dimensions and the presence of cross-wall granules (*O. subbrevis* does not have granules), the specimen resembled *O. limosa* and thus has been identified as such. No records of *O. subbrevis* could be found in saline conditions, whereas *O. limosa* has been recorded from Sinai mangrove swamps (Dor, 1984).

The identification of *O. okeni* is not entirely satisfactory as the species identified as such by Desikachary possess distinct constrictions, whereas our specimen possessed no constrictions. In other respects, however, the description of *O. okeni* resembled our specimen more closely than any other in Desikachary's key and was therefore identified as such.

*O. schultzi*, *O. proboscidea* and *M. chthonoplastes* bear a close resemblance to descriptions in Desikachary (1959).

### 5.3.2 DISTRIBUTION OF BLUE-GREEN ALGAE

A general non-heterocystous blue-green algal community, including species of *Oscillatoria*, *Phormidium*, *Lyngbya* and *Microcoleus* is found in many salt marshes and is also widespread in mangrove swamps around the world (Chapman, 1977a). Heterocystous

blue-green algae, including species of *Scytonema*, *Rivularia*, *Calothrix*, *Nostoc* and *Anabaena* are also reported worldwide from salt marshes (Stewart & Pugh, 1963; Green & Edmisten, 1974; Jones, 1974; Van Raalte *et al.*, 1974; Hanson, 1977b; Carpenter *et al.*, 1978; Sage & Sullivan, 1978) and mangroves (Dor, 1975; Potts & Whitton, 1977; Saenger *et al.*, 1977; Potts, 1979; Aleem, 1980; Potts, 1980; Potts & Whitton, 1980; Hicks & Silvester, 1985). In mangrove swamps heterocystous forms are commonly reported on the upper region of the pneumatophores, whereas non-heterocystous forms occur more commonly on the lower region (Dor, 1975; Potts, 1979; Potts, 1980; Hicks & Silvester, 1985). Heterocystous forms of blue-green algae are very rare in the Mgeni Estuary mangrove swamp and *Scytonema hofmannii* Ag. ex Born. et Flah. has been recorded only once on pneumatophores and mud (Lambert *et al.*, in prep.). Heterocystous forms including species of *Scytonema* and *Calothrix* have a widespread distribution along the southern African coast and have been recorded from mangrove swamps from Zululand to the Transkei. *Rivularia* sp. has been recorded only from one Zululand mangrove swamp (Lambert *et al.*, in prep.).

*M. chthonoplastes* was common in drier, exposed areas of the Mgeni Estuary mangrove swamp (the dry mat area), although this species was also recorded from the wet mat and pneumatophores in this study. This species has a very thick sheath, which possibly prevents excessive dehydration during long periods of exposure. *M. chthonoplastes* has been reported in the literature as ubiquitous in salt marsh habitats and has been recorded from

mud surfaces at most tidal levels in British and Massachusetts salt marshes (Stewart & Pugh, 1963; Carpenter *et al.*, 1978) and mangrove swamps of Australia, the Sinai, the Aldabra Atoll and West Africa (Saenger *et al.*, 1977; Aleem, 1980; Potts, 1980; Potts & Whitton, 1980). Lambert *et al.* (in prep.) and Strong (pers. comm.) have recorded this species commonly on *A. marina* pneumatophores in the Mgeni Estuary mangrove swamp.

High numbers of *L. confervoides* occurred on the pneumatophores and in the dry mat areas. This species is also bounded by a mucilaginous sheath, preventing dehydration during exposure at low tide on the pneumatophores and during longer periods of exposure in the dry mat area. In the latter area, it occurs in close association with *M. chthonoplastes*, which possibly further prevents dehydration of this species. The occurrence of these two species in the dry mat area indicates that these algae are well adapted to dry conditions. The close association of these two algae has been reported as common in salt marshes by Humm & Wicks (1980). *L. confervoides* has been reported in abundance from all tidal levels of mud flats and mangrove swamps of the Aldabra Atoll (Potts & Whitton, 1980). Lam *et al.* (1979) reported species of *Microcoleus* and *Lyngbya* to be common in environments experiencing severe fluctuations in water supply.



The species of *Oscillatoria* recorded in this project all lack mucilaginous sheaths and are possibly less resistant to exposure and dehydration. This may explain the absence of this genus from the dry mat area. Limited numbers were recorded on the pneumatophores where they occurred in close association with *L. confervoides*, which may have aided moisture retention during exposure at low tide. *O. limosa* occurred abundantly in the wet mat area, where a high moisture content is maintained at most times. This species has been recorded previously from the constantly submerged lower pneumatophore region in the Sinai mangrove swamps (Dor, 1984). Dor *et al.* (1977) and Aleem (1980) reported *Oscillatoria* spp. as abundant on mangrove mud in West Africa and the Sinai. In contrast, Stewart & Pugh (1963) regarded certain *Oscillatoria* spp. as robust and well adapted to environmental changes such as salinity and moisture in a British salt marsh. Species of *Oscillatoria* have also been reported from North America and New Zealand salt marshes (Hanson, 1977b; Bohlool & Wiebe, 1978; Carpenter *et al.*, 1978).

### 5.3.3 NITROGEN-FIXING BLUE-GREEN ALGAE

Amongst the heterocystous forms of blue-green algae, the ability to fix nitrogen appears to be universal (Sprenst, 1979). The situation with respect to non-heterocystous forms is much less clear. The ability of this group to fix nitrogen is of interest in this study as only non-heterocystous species have been identified from the various study sites. It is reported that non-heterocystous forms require reduced oxygen tensions in

order to fix nitrogen (Kenyon *et al.*, 1972; Stewart, 1977) and reports of aerobic nitrogen fixation are rare (Carpenter & Price, 1976; Potts, 1984; Stal & Krumbein, 1985b). Potts (1984) reported aerobic ARA by a strain producing significant amounts of sheath material which, when disrupted, resulted in a decrease in ARA. This indicated a possible maintenance of micro-aerophilic conditions within the colony structure. It is suggested that the mucilaginous sheaths of *M. chthonoplastes* and *L. confervoides* may provide a micro-aerophilic atmosphere, enabling nitrogen-fixation to take place. Reduced oxygen tensions may also result from a close association with anaerobic mud of many salt marshes and mangrove swamps (Jones, 1974) or clustering together of filaments (Carpenter & Price, 1976). These situations may explain nitrogen fixation in filamentous blue-green algae lacking sheaths.

Many assumptions of ARA by non-heterocystous blue-green algae have been reported in natural populations of these organisms. In salt marshes, populations of *L. aesturii* (Jones, 1974; Van Raalte *et al.*, 1974; Carpenter *et al.*, 1978), *M. chthonoplastes* (Carpenter *et al.*, 1978) and *Oscillatoria* spp. (Hanson, 1977b, Bohlool & Wiebe, 1978; Carpenter *et al.*, 1978) have been reported to fix nitrogen. In mangrove swamps, ARA has been reported in populations of *L. aesturii* (Potts, 1979) and *M. chthonoplastes* (Potts, 1980). In order to ascertain that blue-green algae and not associated bacteria are responsible for the observed nitrogen fixation, it is necessary to confirm ARA in pure culture. Such confirmation has been obtained for

*M. chthonoplastes* (Pearson *et al.*, 1981), *L. aesturii* (Green & Edmisten, 1974; Potts, 1979), *Lyngbya* spp. (Kenyon *et al.*, 1972; Stewart *et al.*, 1978) and *Oscillatoria* spp. (Kenyon *et al.*, 1972; Hanson, 1977b; Stewart *et al.*, 1978; Stal & Krumbein, 1985a,b). The above references provide evidence that all the species identified in this study are probably nitrogen fixers. The dominant nitrogen fixers in each site were therefore regarded as *M. chthonoplastes* in the dry mat area, *L. confervoides* on the pneumatophores and *O. limosa* in the wet mat area.

#### 5.3.4 SEASONAL VARIATION IN ARA AND PHYSICO-CHEMICAL PARAMETERS

Results showed a strong seasonality associated with ARA in blue-green algal communities of the mangrove swamp. The general pattern that emerged was one of low activity in winter, with peak rates of ARA occurring in summer. Seasonality of ARA seemed strongly related to favourable temperatures and to a lesser extent light conditions (light intensities and day length). Maximum ARA coincided with higher temperatures, light intensities and longest day length. This pattern is in agreement with several other workers in salt marsh systems where peaks and troughs of ARA have been correlated with the seasons (Green & Edmisten, 1974; Jones, 1974; Carpenter *et al.*, 1978; Teal *et al.*, 1979; Dicker & Smith, 1980a; Casselman *et al.*, 1981). Similar results were recorded by Hicks & Silvester (1985) in sediment cores of a New Zealand mangrove swamp. Talbot (1982),

working in the Swartkops Estuary, recorded high autumn values of ARA in the surface sediment.

No discernible seasonal patterns in inorganic nitrogen levels were evident in the wet and dry mat areas supporting findings of Dicker & Smith (1980a) in a salt marsh and Wojceichowski & Heimbrook (1984) in the Tundra regions. Other research conducted in salt marshes revealed a general decrease in inorganic nitrogen levels during the growing season, suggesting a link between high summer levels of ARA and low levels of nitrogen (Teal *et al.*, 1979; Casselman *et al.*, 1981; Talbot, 1982). Lower summer levels of ammonium and nitrate in the creek are possibly coincidental as nitrogen levels have been shown to be tidally influenced (Section 6.4).

The high ammonium levels measured in the wet and dry mat areas are typical of mangrove sediments (Stephens, 1979 in Van der Valk & Attiwill, 1984a). Low nitrite levels have also been reported from mangrove swamps (Potts, 1979). Day (1981) reported high ammonium levels from the Mgeni Estuary and regarded high inorganic nitrogen levels as indicative of pollution. Levels of inorganic nitrogen recorded in this study appear to be limiting to ARA (Section 4.2.6). Talbot (1982) recorded nitrate as generally the most abundant form of inorganic nitrogen in surface sediments of the Swartkops Estuary, but overall levels of inorganic nitrogen were much lower than levels recorded in this study.

No seasonal trends in percentage moisture or salinity were

observed, supporting the findings of Dicker & Smith (1980a), Wojciechowski & Heimbrook (1984) and Talbot (1982). Dicker and Smith (1980a) and Wojciechowski & Heimbrook (1984) also reported no seasonal trends in organic carbon levels. Talbot (1982) related low spring and summer values of ARA in the Swartkops Estuary to low availability of organic carbon in these seasons, causing a 2 to 3 month lag in ARA.

Rates of ARA measured in this study are within the range of rates reported from other salt marshes and mangrove swamps. Significantly higher rates ( $24\ 492\ \text{nmol C}_2\text{H}_4 \cdot \text{g dry mass}^{-1} \cdot 24\ \text{h}^{-1}$ ) were reported by Jones (1974) in salt marsh blue-green algal mats, but generally, rates of between 257 and  $2\ 140\ \text{nmol C}_2\text{H}_4\ \text{cm}^{-2} \cdot 24\ \text{h}^{-1}$  have been reported in such communities (Van Raalte *et al.*, 1974; Carpenter *et al.*, 1978). Lower rates of between 5 and  $330\ \text{nmol C}_2\text{H}_4 \cdot \text{g dry mass}^{-1} \cdot 24\ \text{h}^{-1}$  were reported by Talbot (1982) in Swartkops Estuary surface sediments. Rates of ARA associated with mangrove surface sediments are generally significantly lower and range from 0,24 to  $44\ \text{nmol C}_2\text{H}_4 \cdot \text{g dry mass}^{-1} \cdot 24\ \text{h}^{-1}$  (Kimball & Teas, 1974; Zuberer & Silver, 1978). Bacteria were reported to be the main nitrogen fixers in these areas. Hicks & Silvester (1985) reported rates of between 2400 and  $12000\ \text{nmol C}_2\text{H}_4\ \text{pneumatophore}^{-1} \cdot 24\ \text{h}^{-1}$  and these are comparable to rates associated with pneumatophores in the present study, assuming a mean pneumatophore surface area of  $22,4\ \text{cm}^2$  (measured in Section 5.1.4).

### 5.3.5 SEASONAL VARIATION IN BLUE-GREEN ALGAL AND BACTERIAL NUMBERS

A marked seasonal variation was also apparent in blue-green algal numbers, with maxima being recorded in the summer months. This was particularly evident in the dominant blue-green algal communities in each site. The observed seasonal variations in blue-green algal numbers may be temperature-related, as low minimum winter temperatures appear to be unfavourable to blue-green algal development. Sage & Sullivan (1978) related the winter decrease in blue-green algal biomass to temperature. Moisture may also be an important influencing factor in the wet and dry mat areas as maximal blue-green algal development coincided with periods of highest monthly rainfall.

Peaks in blue-green algal numbers coincided with high rates of ARA in all sites, supporting the findings of Lean *et al.* (1978) and Lindahl & Wallström (1985). Low rates of ARA in April in the wet and dry mat areas were possibly related to high levels of inorganic nitrogen at the time of sampling. *M. chthonoplastes* in the dry mat area exhibited only a minor decrease in cell numbers in the winter months. This species seemed least affected by lower temperatures and rainfall in the winter months. The activity of this alga exhibited a significant decrease in winter, though, as environmental conditions depressed rates of ARA. Significant decreases in the dominant species from the wet mat and pneumatophore areas i.e. *O. limosa* and *L. confervoides* respectively, occurred in the winter months. Spatial variation in blue-green algal numbers has also been related to differences in rates of ARA (Bohlool & Wiebe, 1978; Carpenter *et al.*, 1978;

Smith, 1984). Smith (1984) related differences in rates of ARA of different parts of a moss stem to densities of colonising blue-green-algae and not to variations in temperature, pH or moisture.

Changes in bacterial populations did not coincide with seasonal changes in ARA. High numbers of bacteria recorded from the pneumatophores in August were possibly related to masses of decomposing *Eichhornia crassipes* (Mart.) clogging the channel at this time. Dicker & Smith (1980c) also showed no discernible patterns in any bacterial groups enumerated in a Delaware salt marsh (species of *Azotobacter*, *Clostridium* and *Desulfovibrio*) and indicated that seasonal patterns in ARA were not due to a change in numbers of organisms, but rather to a change in their activity. Talbot (1982) come to similar conclusions.

Bacterial counts were generally higher than those reported in the literature for mangrove swamps (Rodina, 1964; Zuberer & Silver, 1975; 1978) and salt marshes (Patriquin & Knowles, 1972; Dicker & Smith, 1980c). Most of these studies showed low rates of ARA prior to carbon amendment in comparison to rates reported in this study, giving a further indication that blue-green algae are important nitrogen fixers in surface sediments and on pneumatophores of the Mgeni Estuary mangrove swamp.

The presence of high numbers of diazotrophs does not imply that optimal ARA is taking place. Jensen (1981) reported high activity of nitrogen fixing bacteria only under conditions of



an almost neutral pH, high moisture levels, low oxygen tensions, rich carbon supply and energy sources and low concentration of available combined nitrogen. Conditions for ARA appear less than favourable for diazotrophic bacteria in the mangrove swamp. Soil pH measured by Naidoo (1980) and Raiman (1986) in the Mgeni Estuary mangrove swamp was acidic with pH ranging from 4,7 to 7,2. In the dry mat area, moisture levels were often low. Oxygen tensions are likely to be high in the surface sediments. The high levels of inorganic nitrogen in the soil and channel water has already been mentioned. Organic carbon levels measured were high, especially in the surface sediment (Raiman, 1986) and appeared to be one of the few factors favourable for ARA by bacteria.

The generally higher numbers of aerobic bacteria (diazotrophs and heterotrophs) was expected as aerobic conditions are likely to prevail in the surface sediments and pneumatophore surfaces under study. High numbers of *Azotobacter* spp. cells were found in the upper soil horizons from mangrove swamps of the Gulf of Tonkin (Rodina, 1964). However, underlying sediments, consisting of black layers in the mangrove swamps (Rodina, 1964) would provide an ideal anaerobic environment for the development of anaerobic nitrogen fixers. *Clostridium* sp. and *Desulfovibrio* sp. have been isolated from sub-surface mangrove sediments (Rodina, 1964; Zuberer & Silver, 1975; 1978).

### 5.3.6 CONTRIBUTION OF BLUE-GREEN ALGAL COMMUNITIES TO THE ANNUAL NITROGEN BUDGET

The calculated annual rates of nitrogen fixation must be treated with caution, as they are based on only 10 samples taken in a small area over 24 h every 2 months. The total surface area of each of the 3 sites must also be regarded as a rough estimate.

Annual nitrogen budgets for areas dominated by blue-green algal mats have been calculated for only a few salt marshes. Estimates of Jones (1974) are high (between 31,57 and 46,17 g N m<sup>-2</sup> yr<sup>-1</sup>), whereas those of Carpenter *et al.* (1978) are comparable to results obtained in this study (2,2 g N m<sup>-2</sup> yr<sup>-1</sup>). Nitrogen fixation by intertidal blue-green algal mats contributed 4,6 g N m<sup>-2</sup> yr<sup>-1</sup> (Gotto *et al.*, 1981), which is only slightly higher than results obtained in the wet and dry mat areas in this study.

The only nitrogen budget calculated in mangrove swamps was by Zuberer & Silver (1978). They calculated that sediment-associated nitrogen fixation contributed 2,8 g N m<sup>-2</sup> yr<sup>-1</sup> and that this would supply 30% of the requisite nitrogen and that root-associated activity could supply substantially more, assuming that 9,49 g m<sup>-2</sup> yr<sup>-1</sup> was required. As no information is available on nitrogen requirements of the Mgeni Estuary mangrove organisms, this figure was also used here. It was thus calculated that blue-green algal populations provide 23,8% of the annual nitrogen requirements in the 3 study areas combined. This is a considerable amount, considering that sub-surface

sediment, root-associated activity and increase in nitrogen due to decomposition has not been taken into account. It would appear that nitrogen fixation by blue-green algal communities contributes significantly to the nitrogen budget of the Mgeni Estuary mangrove swamp. The contribution of other sources of nitrogen fixation, namely sub-surface sediment and root-associated activity, is in need of determination.

## CHAPTER 6

### TEMPORAL VARIATION IN ARA AND PHYSICO-CHEMICAL PARAMETERS

#### 6.1 INTRODUCTION

The recording of certain environmental parameters and ARA at only one time in the lunar cycle i.e. 2 days after new moon, as described in Sections 5.1.1 and 5.1.2 did not give a good indication of the range of environmental conditions that the experimental sites are subjected to over a complete lunar cycle i.e. from one new moon to the next, or tidal cycle i.e. from one high tide to the next. More intensive sampling was therefore carried out in order to achieve this.

#### 6.2 MATERIALS AND METHODS

##### 6.2.1 THE WET AND DRY MAT AREAS

The wet and dry mat areas are submerged only during spring high tides associated with full or new moon and it could be expected that environmental conditions would show considerable variation from one spring tide to the next. It was therefore decided to monitor these areas over an entire lunar cycle, sampling at regular intervals for a month, starting at new moon and ending at the following new moon. ARA, organic carbon and inorganic nitrogen were determined every 6 days. Temperature, light intensity, salinity and percentage moisture were determined every 3 days.

Sampling took place during the warm month of March (starting on 28/2/87) and the cold month of July (starting on 26/6/87) in order to compare summer and winter conditions respectively. Samples were collected for analysis of ARA as described in Section 3.3.1.1 and incubated in a growth cabinet at 22°C, a light intensity of  $200 \mu\text{E m}^{-2} \text{s}^{-1}$  and a L/D cycle of 12:12 h. These samples were analysed as described in Section 3.3.1.3 and 3.3.1.4. Percentage moisture and organic carbon of the samples were determined after ARA had been carried out, as described in Sections 3.3.2.3 and 3.3.2.5 respectively. On the days when ARA was not being recorded, a separate soil sample was collected for analysis of percentage moisture. Samples for determination of salinity and inorganic nitrogen were collected and analysed as described in Sections 3.3.2.4 and 3.3.2.6 respectively. Temperatures (mud and air) and light intensity were recorded in the sun for the dry mat area and in the shade for the wet mat area as described in Sections 3.3.2.2 and 3.3.2.1 respectively. All sampling and recordings took place between 08h30 and 09h30.

#### 6.2.2 THE BEACHWOOD CREEK

Conditions in the creek, to which the pneumatophores are exposed, change on a daily basis, as they are subjected to submergence and exposure by the tides. In order to determine the range of environmental conditions to which intertidal pneumatophores are subjected, environmental conditions were to be monitored over a tidal cycle i.e. from high tide to low tide, at neap and spring tides. This study was planned at neap tide on 30/9/87 and spring tide on 7/10/87.

Water samples were to be taken for analysis of salinity and inorganic nitrogen levels at hourly intervals as described in Sections 3.3.2.4 and 3.3.2.6 respectively. Temperature and light intensity of air and water were to be recorded simultaneously as described in Sections 3.3.2.1 and 3.3.2.2 respectively.

However, this study could not be carried out, as the floods on 28-29/9/87 caused a blockage of the mouth of the Beachwood Creek, resulting in an accumulation of fresh water in the swamp and the retardation of tidal exchange of water for about 6 weeks. Similar research conducted by Steyn (1977) and Raiman (1986) in this swamp is therefore quoted in the discussion.

### 6.3 RESULTS

Significantly higher rates of ARA ( $P \leq 0,01$ ) and percentage moisture levels and lower salinities were evident in March than in July in the wet (Figure 6.1) and dry mat areas (Figure 6.2). Increases in ARA over the study periods coincided with increases in percentage moisture levels, which were increased by submergence at spring high tides and rainfall. However, these increases were not always significant. Percentage moisture levels were generally higher in the wet mat area than the dry mat area. Salinity generally decreased with increase in percentage moisture.

Variation in other physico-chemical factors (air temperatures, mud or water temperatures, organic carbon, light intensity and inorganic nitrogen) during March and July for the wet and dry mat areas are

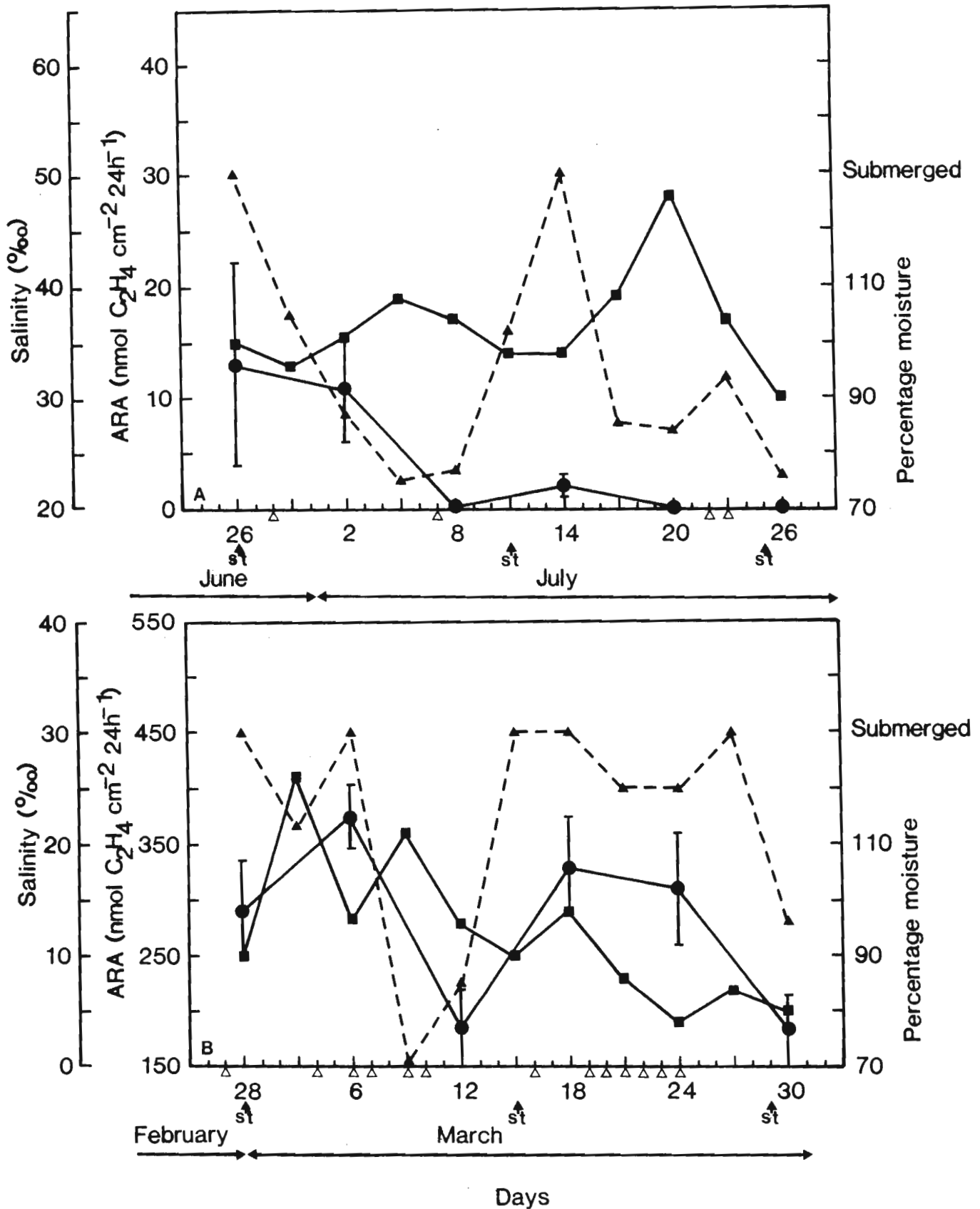


Figure 6.1 Temporal variation in ARA (●—●), percentage moisture (▲---▲) and salinity (■—■) in the wet mat area during (A) July and (B) March. The vertical bars represent one standard error. S.T. indicates spring tide and Δ represents days when rain was recorded.



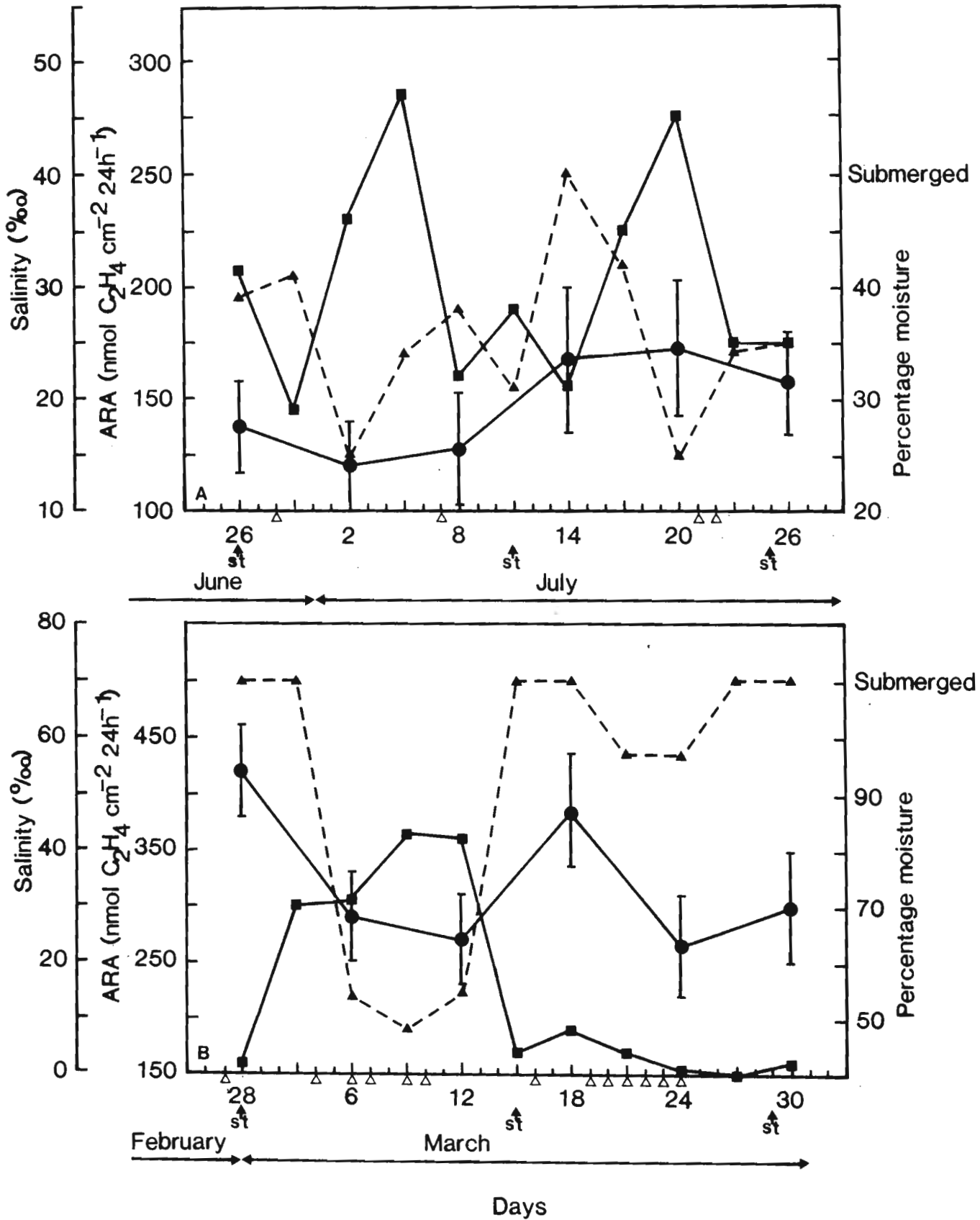


Figure 6.2 Temporal variation in ARA (●—●), percentage moisture (▲---▲) and salinity (■—■) in the dry mat area during (A) July and (B) March. The vertical bars represent one standard error. S.T. indicates spring tide and Δ represents days when rain was recorded.

TABLE 6.1 Variation in physico-chemical parameters in the wet mat area during March.

|   | 28/2              | 3/3   | 6/3               | 9/3               | 12/3   | 15/3              | 18/3              | 21/3  | 24/3   | 27/3              | 30/3   |
|---|-------------------|-------|-------------------|-------------------|--------|-------------------|-------------------|-------|--------|-------------------|--------|
| Air temperature<br>(°C)                                   | 29,0              | 27,0  | 27,0              | 23,0              | 29,0   | 27,5              | 29,5              | 27,0  | 24,0   | 25,5              | 27,0   |
| Mud/water <sup>□</sup><br>temperature<br>(°C)             | 25,0 <sup>□</sup> | 25,0  | 28,0 <sup>□</sup> | 22,5              | 24,0   | 26,0 <sup>□</sup> | 26,5 <sup>□</sup> | 25,0  | 23,0   | 23,0 <sup>□</sup> | 26,0   |
| Organic carbon<br>(mg g dry mass <sup>-1</sup> )          | 54,9              |       | 58,2              |                   | 69,0   |                   | 60,2              |       | 51,4   |                   | 55,4   |
| Light intensity<br>( $\mu\text{E m}^{-2} \text{s}^{-1}$ ) | 265,0             | 110,0 | 95,0              | 62,5 <sup>△</sup> | 125,0  | 100,0             | 75,5 <sup>△</sup> | 150,0 | 95,0   | 250,0             | 137,5  |
| Nitrate<br>( $\mu\text{g. g w mass}^{-1}$ )               | 2565,0            |       | 300,0             |                   | 1480,0 |                   | 2670,0            |       | 3300,0 |                   | 2780,0 |
| Nitrite<br>( $\mu\text{g. g w mass}^{-1}$ )               | 330,0             |       | 35,0              |                   | 95,0   |                   | 270,0             |       | 325,0  |                   | 165,0  |
| Ammonium<br>( $\mu\text{g.g w mass}^{-1}$ )               | 7820,0            |       | 3425,0            |                   | 6048,0 |                   | 3460,0            |       | 3310,0 |                   | 2640,0 |

□ submerged

△ overcast

TABLE 6.2 Variation in physico-chemical parameters in the wet mat area during July.

|   | 26/6              | 29/6 | 2/7    | 5/7  | 8/7    | 11/7 | 14/7              | 17/7 | 20/7              | 23/7              | 26/7              |
|---|-------------------|------|--------|------|--------|------|-------------------|------|-------------------|-------------------|-------------------|
| Air temperature<br>(°C)                                   | 17,2              | 19,0 | 15,2   | 19,0 | 17,5   | 19,5 | 14,0              | 17,0 | 20,0              | 18,0              | 17,0              |
| Mud/water <sup>□</sup><br>temperature<br>(°C)             | 16,0 <sup>□</sup> | 17,0 | 13,0   | 16,0 | 16,0   | 15,5 | 15,5 <sup>□</sup> | 16,0 | 17,5              | 16,0              | 17,0              |
| Organic carbon<br>(mg g dry mass <sup>-1</sup> )          | 50,8              |      | 54,7   |      | 69,3   |      | 65,6              |      | 61,1              |                   | 58,4              |
| Light intensity<br>( $\mu\text{E m}^{-2} \text{s}^{-1}$ ) | 25,0              | 23,0 | 20,0   | 30,0 | 30,0   | 35,0 | 16,0              | 20,0 | 17,0 <sup>△</sup> | 22,5 <sup>△</sup> | 23,0 <sup>△</sup> |
| Nitrate<br>( $\mu\text{g.g w mass}^{-1}$ )                | 1010,0            |      | 1145,0 |      | 955,0  |      | 940,0             |      | 940,0             |                   | 360,0             |
| Nitrite<br>( $\mu\text{g.g w mass}^{-1}$ )                | 30,0              |      | 20,0   |      | 105,0  |      | 35,0              |      | 35,0              |                   | 45,0              |
| Ammonium<br>( $\mu\text{g.g w mass}^{-1}$ )               | 3280,0            |      | 3385,0 |      | 3125,0 |      | 1840,0            |      | 3125,0            |                   | 2410,0            |

□ submerged

△ overcast

TABLE 6.3 Variation in physico-chemical parameters in the dry mat area during March.

|   | 28/2              | 3/3               | 6/3    | 9/3                | 12/3   | 15/3              | 18/3               | 21/3  | 24/3   | 27/3              | 30/3              |
|---|-------------------|-------------------|--------|--------------------|--------|-------------------|--------------------|-------|--------|-------------------|-------------------|
| Air temperature<br>(°C)                                   | 27,0              | 30,0              | 27,0   | 23,0               | 27,0   | 28,0              | 31,0               | 28,0  | 23,5   | 25,5              | 29,0              |
| Mud/water <sup>□</sup><br>temperature<br>(°C)             | 27,5 <sup>□</sup> | 22,5 <sup>□</sup> | 28,0   | 23,5               | 30,0   | 27,0 <sup>□</sup> | 26,5 <sup>□</sup>  | 29,0  | 24,0   | 23,0 <sup>□</sup> | 26,0 <sup>□</sup> |
| Organic carbon<br>(mg g dry wt <sup>-1</sup> )            | 28,5              |                   | 26,9   |                    | 36,1   |                   | 54,7               |       | 26,8   |                   | 27,8              |
| Light intensity<br>( $\mu\text{E m}^{-2} \text{s}^{-1}$ ) | 860,00            | 600,0             | 450,0  | 150,0 <sup>△</sup> | 690,0  | 560,0             | 260,0 <sup>△</sup> | 600,0 | 530,0  | 580,0             | 570,0             |
| Nitrate<br>( $\mu\text{g.g w mass}^{-1}$ )                | 2750,0            |                   | 390,0  |                    | 3565,0 |                   | 1225,0             |       | 2545,0 |                   | 1885,0            |
| Nitrite<br>( $\mu\text{g.g w mass}^{-1}$ )                | 325,0             |                   | 45,0   |                    | 270,0  |                   | 20,0               |       | 140,0  |                   | 100,0             |
| Ammonium<br>( $\mu\text{g.g w mass}^{-1}$ )               | 4435,0            |                   | 3500,0 |                    | 3230,0 |                   | 1850,0             |       | 1940,0 |                   | 2505,0            |

□ submerged

△ overcast

TABLE 6.4 Variation in physico-chemical parameters in the dry mat area during July.

|   | 26/6               | 29/6  | 2/7    | 5/7   | 8/7                | 11/7  | 14/7              | 17/7  | 20/7   | 23/7  | 26/7              |
|---|--------------------|-------|--------|-------|--------------------|-------|-------------------|-------|--------|-------|-------------------|
| Air temperature<br>(°C)                                   | 18,5               | 18,0  | 15,5   | 19,5  | 17,5               | 19,0  | 16,0              | 18,0  | 19,5   | 19,0  | 18,0              |
| Mud/water <sup>□</sup><br>temperature<br>(°C)             | 14,5               | 17,0  | 12,0   | 16,0  | 16,0               | 15,0  | 14,5 <sup>□</sup> | 17,0  | 17,0   | 17,0  | 16,0              |
| Organic carbon<br>(mg g dry mass <sup>-1</sup> )          | 31,7               |       | 24,9   |       | 30,6               |       | 41,7              |       | 35,3   |       | 30,4              |
| Light intensity<br>( $\mu\text{E m}^{-2} \text{s}^{-1}$ ) | 150,0 <sup>△</sup> | 275,0 | 250,0  | 450,0 | 150,0 <sup>△</sup> | 300,0 | 250,0             | 275,0 | 300,0  | 275,0 | 75,0 <sup>△</sup> |
| Nitrate<br>( $\mu\text{g.g w mass}^{-1}$ )                | 750,0              |       | 600,0  |       | 1100,0             |       | 4585,0            |       | 780,0  |       | 775,0             |
| Nitrite<br>( $\mu\text{g.g w mass}^{-1}$ )                | 20,0               |       | 20,0   |       | 30,0               |       | 10,0              |       | 20,0   |       | 10,0              |
| Anmonium<br>( $\mu\text{g.g w mass}^{-1}$ )               | 3800,0             |       | 3520,0 |       | 4340,0             |       | 5000,0            |       | 3225,0 |       | 2745,0            |

□ submerged

△ overcast

represented in Tables 6.1 to 6.4. Higher temperatures were recorded during March than during July. In the dry mat area, mud temperatures were higher than air temperatures under exposed conditions in March. This trend was reversed in July. Under submerged conditions, water temperatures were generally lower than air temperatures. In the wet mat area, mud or water temperatures were generally lower than air temperatures. Small variations in organic carbon levels occurred during the study period at both sites. Light intensities were highest during March, except on the occasional overcast day, and were highly variable.

Levels of inorganic nitrogen varied greatly during this study.

Ammonium was generally the most abundant form of inorganic nitrogen in both sites and higher levels of all forms generally occurred in March. Nitrite levels were low at all sampling periods.

#### 6.4 DISCUSSION

Higher rates of ARA recorded in the warmer months correspond to results obtained in the seasonal study (Section 5.2.2). Rates of ARA in the wet mat area were very low in July and may have been attributed to a visible absence of blue-green algae at this time. Variations within the two sampling periods of approximately one month each appeared to be primarily linked to percentage moisture levels in both sites and highest rates of ARA generally occurred under submerged conditions. Percentage moisture levels were increased largely by submergence at spring high tides and a progressive dehydration as a result of evaporation was expected in the interim period. This trend was not entirely

evident, as rain fell frequently, especially during March, resulting in increases in percentage moisture at these times. Much higher spring high tides (equinoctial) in March resulted in conditions of submergence on more than half of the sampling days. Percentage moisture levels were lower in July as a result of lower rainfall and less frequent inundation at spring high tides.

Salinity of the mud was determined by salinity of the submerging water at spring high tides. An increase in salinity was evident in the period between successive spring tides (neap tides) as a result of evaporation. This trend was interrupted by rain, which noticeably decreased the salinity by diluting the salts.

Temperatures were recorded between 08h30 and 09h30 and therefore do not represent the daily range. Mud temperatures were low as the sun had not yet heated it up.

Light intensities were also low, especially during July. This was partly as a result of the type of sensor used to measure light intensity, which only recorded light from one direction. For uniformity, the sensor was always held in an upright position. During July, the sun's angle was low between 08h30 and 09h30, resulting in low light intensities being recorded. Expected decreases in light intensity occurred on overcast days.

Organic carbon showed little variation during both study periods. Levels of ammonium, nitrate and nitrite were highly variable during the two study periods in the wet and dry mat areas. The reasons for this

are not clear, although inorganic nitrogen levels in the water inundating these sites at spring high tides must have exerted an effect.

In the Beachwood Creek, conditions of salinity, temperature and inorganic nitrogen levels varied on a daily basis i.e. from one high tide to the next. Higher salinities at high tide have been reported in the Beachwood Creek by Steyn (1977) and Raiman (1986). Salinity differences between high and low tide were most marked at spring high tides (35 to 18<sup>0</sup>/oo and 34 to 13<sup>0</sup>/oo) as opposed to neap tides (16 to 6<sup>0</sup>/oo and 17 to 11<sup>0</sup>/oo) (Steyn, 1977).

Water temperature in the creek was higher at spring low tides than spring high tides. At neap tides, the situation was reversed (Steyn, 1977). At spring low tides, a smaller volume of water is present in the creek and this heats up quickly as a result of heating by direct sunlight. At spring high tide, cooler seawater is pushed up the creek, whereas at neap high tides, warmer estuarine water enters the creek.

Inorganic nitrogen levels were also studied by Steyn (1977) in the Beachwood Creek. Nitrate concentrations were higher at low tide, indicating that water from upstream has higher nitrate levels. This trend was more marked at spring tides (low tide - 2000  $\mu\text{g l}^{-1}$ ; high tide - 0,5  $\mu\text{g l}^{-1}$ ) than neap tides (low tide - 2000  $\mu\text{g l}^{-1}$ ; high tide - 1041  $\mu\text{g l}^{-1}$ ). Nitrite levels were higher at spring low tides than spring high tides (39  $\mu\text{g l}^{-1}$  - low tide; 1  $\mu\text{g l}^{-1}$  - high tide), but the trend was reversed at neap tides (74  $\mu\text{g l}^{-1}$  - high tide; 28  $\mu\text{g l}^{-1}$  - low tide). Ammonium levels were not measured in this study. Moisture



levels associated with pneumatophores would also vary on a daily basis, being exposed at low tide and submerged at high tide. The period of exposure would be more marked at spring tides than neap tides.

It would appear that short term variations in rates of ARA i.e. from one spring tide to the next, in the wet and dry mat areas can mainly be attributed to percentage moisture levels in the soil. A progressive change in percentage moisture levels and salinity levels was apparent from one spring tide to the next, interrupted only by rainfall. Conditions in the creek vary on a daily basis and variations are more marked at spring than neap tides. ARA associated with pneumatophores is therefore more likely to vary on a daily basis in response to these factors.

## CHAPTER 7

### ARA ASSOCIATED WITH DECOMPOSING LEAVES

#### 7.1 INTRODUCTION

Decomposing mangrove leaves have been identified as important sites of nitrogen fixation (Section 2.5). The possibility that blue-green algae may be important nitrogen-fixers associated with decomposing mangrove leaves and that observed increases in total nitrogen of decomposing leaves with time may result from nitrogen fixation, was suggested by Steinke *et al.* (1983) (Chapter 1). It has already been mentioned (Section 3.2) that blue-green algae are associated with decomposing leaves on the wet mud sediment. Many of the samples taken from the wet mat area included decomposing leaf sections. It was therefore felt that this study would not be complete without investigating the ARA of blue-green algae associated with decomposing leaves.

Contrary to previous work by Steinke *et al.* (1983) (Section 1), no blue-green algae were observed on the experimental decomposing leaves during the course of this study (Section 7.3). As a result, this section does not really fit in with the title of this thesis.

Nevertheless, a report of findings has been included as it was felt that nitrogen fixation associated with decomposing leaves contributes significantly to the nitrogen budget of the Mgeni Estuary mangrove swamp.

#### 7.2 MATERIALS AND METHODS

Senescent, yellowing leaves of *A. marina* were collected from the trees

in April, 1987 and transported to the laboratory within 1 h. Sets of 15 leaves were weighed out to equal masses (between 5,35 and 5,45 g fresh mass) and placed into 60 nylon mesh bags (mesh size 2,5 x 5 mm). Five such samples were prepared and dried immediately to a constant mass at 80°C in order to determine the initial dry mass of the leaves. Fresh senescent leaves were also incubated for ARA at day 0. One leaf was placed into each of 20 serum bottles. Of these, 10 were submerged by seawater made up to 25‰ and a pH of 7,8, while the other 10 were left exposed. Bottles were incubated in a growth cabinet at 22°C,  $200 \mu\text{E m}^{-2} \text{s}^{-1}$  and 12:12 h L:D cycle for 24 h, following an incubation period of 3 h. A  $p\text{C}_2\text{H}_2$  of 0,15 atm. was established in each bottle. Samples were analysed for ARA as described in Section 3.3.1.4.

Thirty bags containing weighed leaves were submerged in the creek and attached to a stake. This site is indicated in Figure 3.1. The remaining 30 bags were deposited on the wet mud surface and tied to mangrove trees. This site was immediately adjacent to the submerged bags. These leaves remained exposed for most of the study period, but were submerged periodically at spring high tides.

From 8/4/87 to 22/7/87, 4 bags were collected at random from each site after 1, 2, 3, 4, 8, 12 and 16 weeks and transported to the laboratory in a bucket at ambient temperatures.

At each harvest, 10 leaves from each site were gently washed in seawater to remove adhering sediment (Van der Valk & Attiwill, 1984a, b; Hicks & Silvester, 1985) and incubated for ARA as described above. The area of each leaf incubated for ARA was calculated by tracing

the leaf outline on to graph paper and calculating the area by counting the number of squares occupied by the leaf. Each square represented  $4 \text{ mm}^2$ . These leaves were subsequently dried to determine their dry mass. Initially, results were expressed on a per unit area basis, in order to facilitate comparison with other results. Towards the end of the experiment, the leaves were too fragmented to record their areas accurately. Results were therefore expressed as  $\text{nmol C}_2\text{H}_4 \cdot \text{g dry mass}^{-1} \cdot 24 \text{ h}^{-1}$ .

The leaves from the remaining 3 litter bags were washed and dried at  $80^\circ\text{C}$  until a constant mass was obtained. The total dry mass of leaves from each bag was recorded. The percentage leaf material remaining in each bag at each sampling period was calculated as:

$$\frac{\text{mean dry mass of washed leaves}}{\text{mean dry mass of fresh leaves}} \times \frac{100}{1} .$$

Samples were then ground in a SPEY 5100 mill and the total nitrogen content of leaves determined using the micro-Kjeldahl method (Section 3.3.2.7).

### 7.3 RESULTS

Rates of ARA associated with exposed litter were significantly higher than rates associated with submerged litter ( $P < 0,01$ ) (Figure 7.1). Relatively high rates of ARA were apparent after 1 week in association with the submerged litter and a decrease occurred after 2 and 3 weeks. Maximum rates of ARA of exposed and submerged litter occurred after 3 and 4 weeks of decomposition respectively, after which a steady

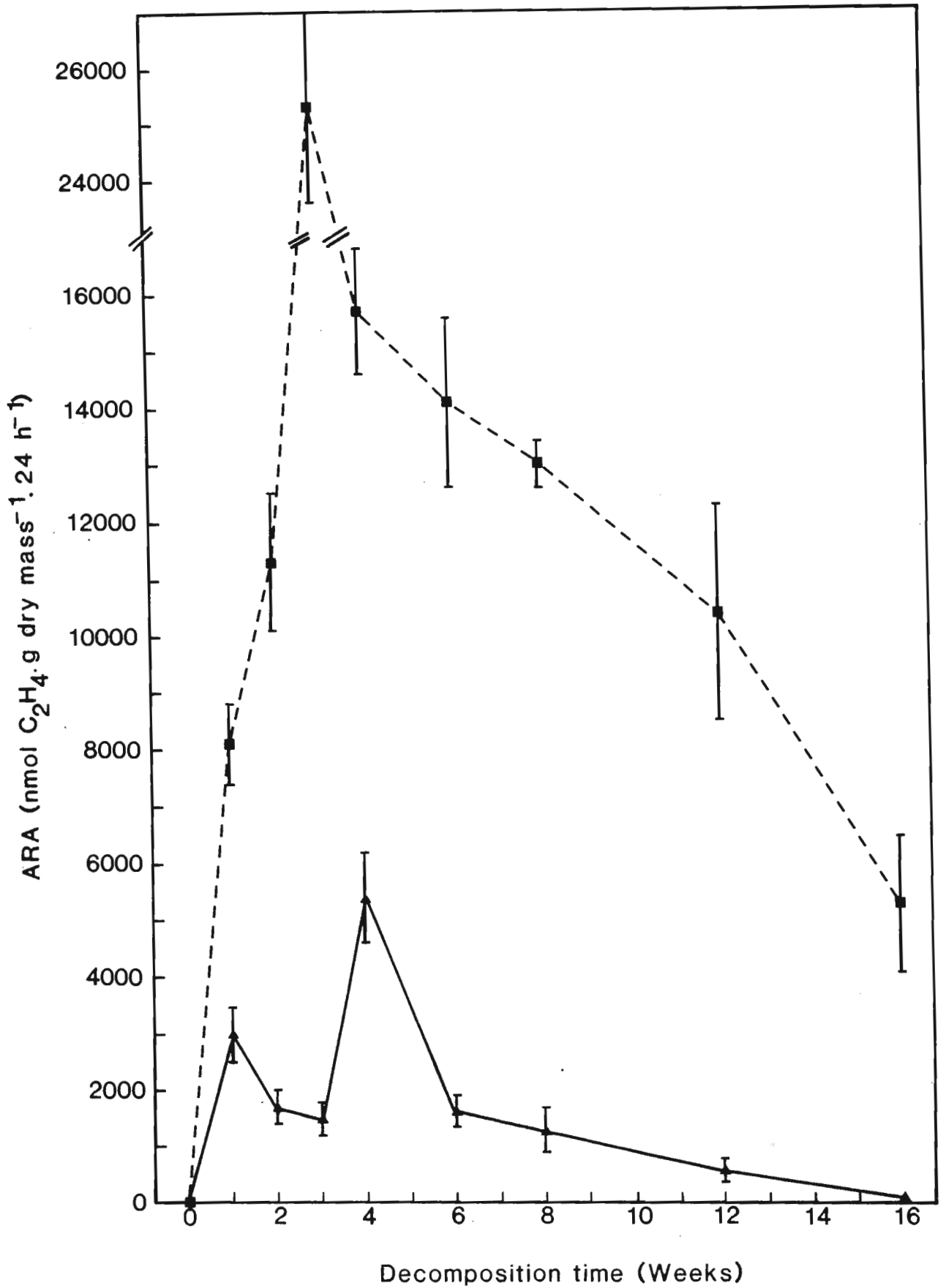


Figure 7.1 Acetylene reduction activity associated with decomposing *A. marina* litter under exposed ( ■ --- ■ ) and submerged ( ▲ — ▲ ) conditions. The vertical bars represent one standard error.

decrease was apparent up to 12 weeks. Analysis of variance showed that this decrease was not significant. A significant decrease in ARA occurred after 16 weeks in exposed and submerged litter ( $P < 0,05$ ). The decrease in mass of litter with decomposition under exposed and submerged conditions is represented in Figure 7.2. A rapid decrease in mass of 36 and 46% occurred within a week under exposed and submerged conditions respectively, with significantly greater losses occurring under submerged conditions ( $P < 0,05$ ). Thereafter a slower steady decrease in mass occurred with losses significantly greater under exposed conditions ( $P < 0,05$ ), except after 6 weeks where no significant difference occurred. After 16 weeks, losses of 89 and 82% had occurred under exposed and submerged conditions respectively.

Air and water temperatures recorded at each harvest are represented in Figure 7.3a. Salinity ranged from 4 to 25<sup>0</sup>/oo during the study.

An initial, rapid increase in percentage nitrogen occurred during the first week of decomposition (Figure 7.3b). Thereafter, a steady increase in percentage nitrogen occurred in the exposed litter for the duration of the experiment. Percentage nitrogen levels of the submerged litter decreased significantly during the second and third weeks of decomposition ( $P < 0,01$ ), followed by a steady increase thereafter. Percentage nitrogen levels of the submerged litter were significantly higher at all sampling periods ( $P < 0,05$ ), except after 16 weeks, when no significant differences were apparent.

Microscopic examination of scrapings from the decomposing leaf surfaces failed to reveal the presence of blue-green algae at any stages of decomposition.

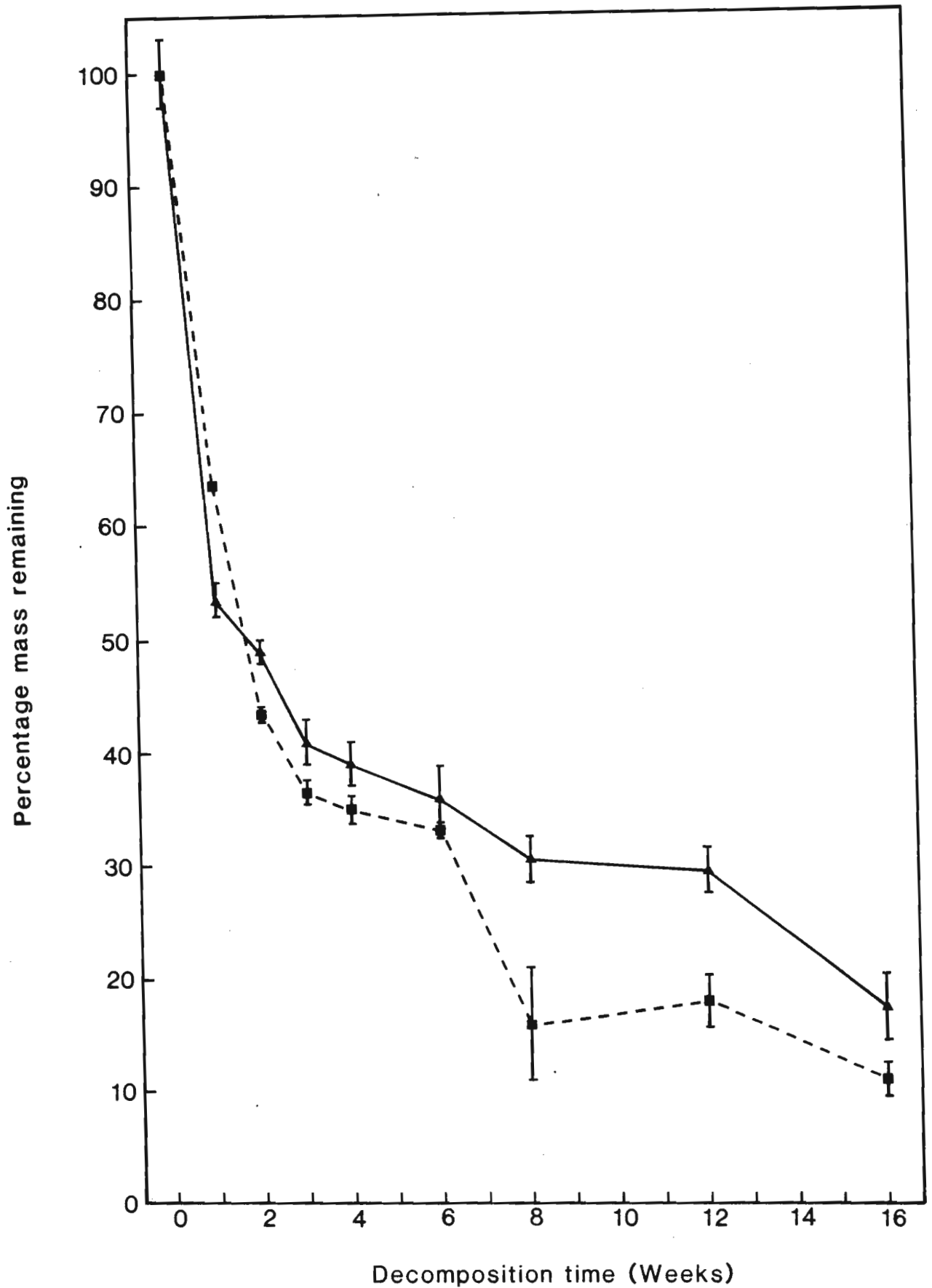


Figure 7.2 Dry matter losses of *A. marina* litter during decomposition under exposed ( ■ --- ■ ) and submerged ( ▲ —▲ ) conditions. The vertical bars represent one standard error.

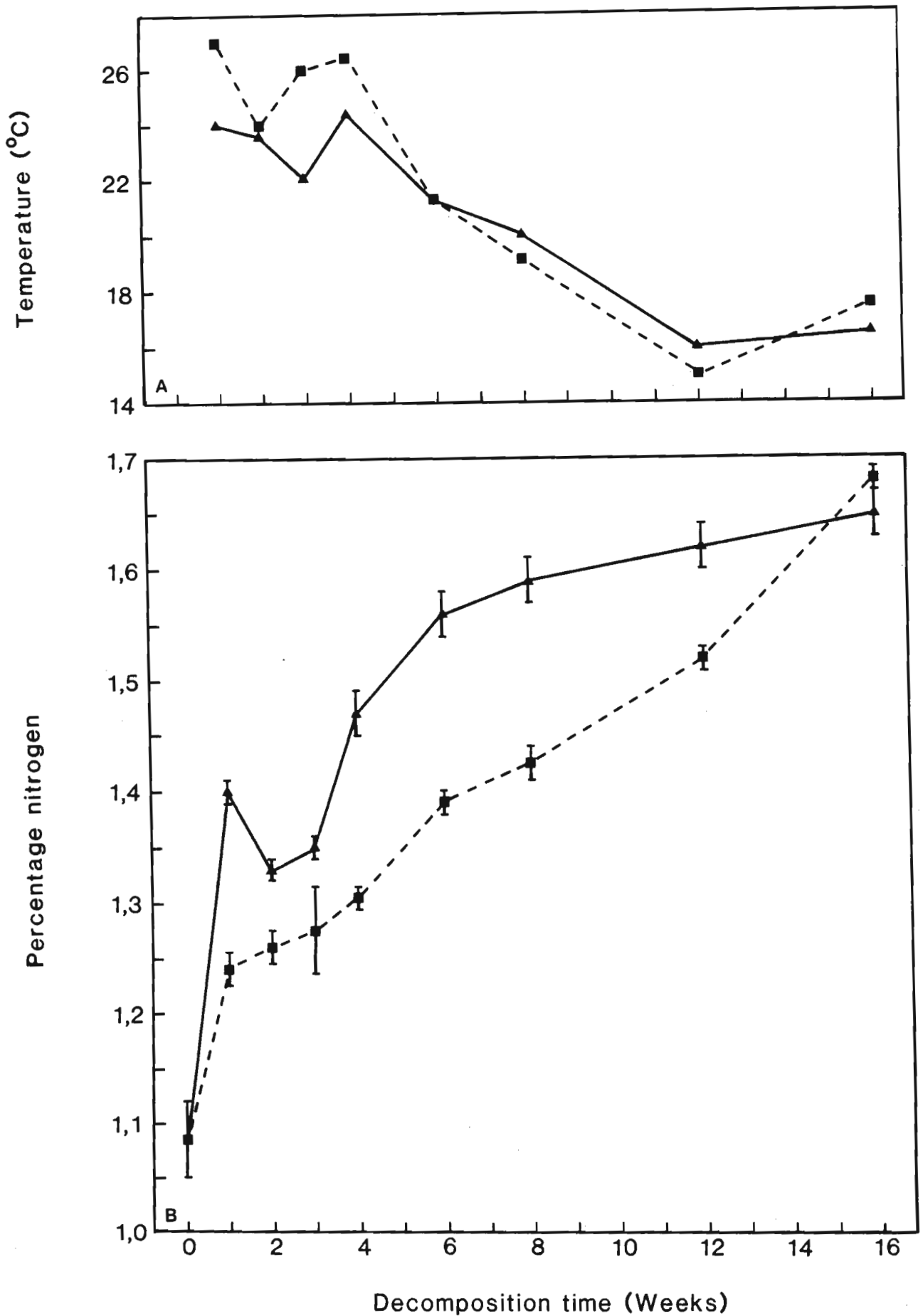


Figure 7.3 A. *In situ* temperatures recorded at time of sampling under exposed ( ■ --- ■ ) and submerged ( ▲ — ▲ ) conditions.  
 B. Increase in percentage nitrogen of *A. marina* litter during decomposition under exposed ( ■ --- ■ ) and submerged ( ▲ — ▲ ) conditions. The vertical bars represent one standard error



#### 7.4 DISCUSSION

High rates of ARA were associated with decomposing leaf litter of *A. marina*. Higher rates of ARA under exposed conditions as opposed to submerged conditions supported results of Van der Valk & Attiwill (1984a) on decomposing *A. marina* leaves in Australia. This indicates that daily tidal inundation may have a marked effect on ARA, causing a decrease during submergence.

The assays were conducted under aerobic conditions. Although the effects of anaerobic conditions on this system need to be determined, Hicks & Silvester (1985) showed such conditions to depress ARA significantly in decaying *A. marina* leaves. Zuberer & Silver (1978) found no significant differences in ARA of decomposing *R. mangle* leaves under exposed and submerged conditions.

Most reports in the literature associate ARA of decomposing mangrove leaves with bacteria (Zuberer & Silver, 1978; Van der Valk & Attiwill, 1984a; Hicks & Silvester, 1985), in support of present findings. Contrary to these findings, Gotto & Taylor (1976) attributed  $\frac{2}{3}$  of ARA associated with decomposing *R. mangle* leaves to photosynthetic bacteria and non-heterocystous blue-green algae. Steinke *et al.* (1983) and Newell *et al.* (1984) observed significant numbers of blue-green algae associated with decomposing *A. marina* and *R. mangle* leaves respectively.

Maximum rates of ARA occurred after 3 to 4 weeks of decomposition and thereafter rates decreased significantly. Similar studies by Van der Valk & Attiwill (1984a) revealed highest rates of ARA over a longer

time period, i.e. from 1 to 8 weeks of decomposition. This difference may be temperature-related, as temperatures decreased significantly towards the latter part of the study, possibly resulting in decreased rates of ARA. *In situ* temperatures were not reported by Van der Valk & Attiwill (1984a). Hicks & Silvester (1985) reported maximum rates of ARA in decomposing *A. marina* litter at 30°C, indicating that temperature does affect ARA of decomposing mangrove leaves.

Loss of mass associated with decomposition was highest under exposed conditions at most harvest periods. This is in contrast to reports by Gopal (1984), Qualls (1984) and Steinke & Ward (1987) in which submerged litter decomposed more rapidly than exposed litter. The fairly large mesh size of the litter bags allowed entry of small crabs, which may have eaten leaf fragments, thus causing accelerated mass loss from the exposed litter bags. In a study of unbagged mangrove leaf litter, Van der Valk & Attiwill (1984b) indicated that crabs ate about 50% of the litter. The total exclusion of such small invertebrates using a smaller mesh size would have been unrepresentative of the natural situation.

The initial rapid decrease in mass of litter up to the first and second weeks of decomposition of the exposed and submerged litter respectively may be related to leaching of soluble organic (Van der Valk & Attiwill, 1984a, b; Wilson *et al.*, 1986) and inorganic compounds (Steinke *et al.*, 1983). A slower, more constant loss of mass was apparent during a second phase, when more resistant material was possibly lost. Slower rates of decomposition towards the end of the study period may also have been temperature related.

An increase in percentage nitrogen during decomposition of leaf litter is commonly reported in the literature in association with mangrove (Fell *et al.*, 1972; Steinke *et al.*, 1983; Newell *et al.*, 1984; Van der Valk & Attiwill, 1984a; Steinke & Ward, 1987), pond cypress (Dierberg & Brezonik, 1981) and *S. alterniflora* (Lee *et al.*, 1980; Wilson *et al.*, 1986) litter. The reasons for such increases are not clear from the literature and conflicting explanations have been put forward in this respect. Such increases in percentage nitrogen have been attributed to increase in microbial and fungal biomass (Odum *et al.*, 1979).

although Lee *et al.* (1980) demonstrated that living cells of these microbes accounted for only a minor part of the nitrogen increases in decomposing *S. alterniflora* leaves. Other possibilities include increases in microbial protein during decay (Odum & de la Cruz, 1967), production of resistant microbial extracellular products (Lee *et al.*, 1980) and nitrogen fixation by diazotrophic micro-organisms (Gotto & Taylor, 1976; Dierberg & Brezonik, 1981; Van der Valk & Attiwill, 1984a). Lee *et al.* (1980) also suggested a concentration rather than a loss of protein during decomposition, but studies by de la Cruz & Poe (1975) have shown that this could only account for a certain amount of the relative increase in detrital nitrogen. Clearly, more research in this field is necessary.

The higher nitrogen levels in submerged litter were unexpected, as significantly lower rates of ARA were associated with submerged litter. This suggests that nitrogen fixation is not the primary cause of increased nitrogen levels in decomposing leaf litter, especially with respect to submerged litter. Other factors, such as colonisation of leaves by fungi and bacteria may well be more important in

increasing nitrogen levels, especially under submerged conditions.

Nevertheless, rates of ARA in association with decomposing leaf litter were high in comparison to rates from other mangrove systems, where rates of between 122 and 11 256  $\text{nmol C}_2\text{H}_4 \cdot \text{g dry wt}^{-1} \cdot 24 \text{ h}^{-1}$  have been reported (Zuberer & Silver, 1978; Van der Valk & Attiwill, 1984; Hicks & Silvester, 1985). Higher rates have been reported by Gotto & Taylor (1976) ( $51\,428 \text{ nmol C}_2\text{H}_4 \cdot \text{g dry wt}^{-1} \cdot 24 \text{ h}^{-1}$ ), where ARA was mainly associated with photosynthetic micro-organisms. This indicates that although nitrogen fixation may not be the prime contributor to increased nitrogen levels on decomposing leaves, it is an important source of nitrogen to the mangrove ecosystem.

CHAPTER 8CONCLUSION

Percentage moisture appeared to be the most important environmental parameter controlling ARA in the Mgeni Estuary mangrove swamp and highest rates were recorded under submerged conditions. Short-term variations in ARA, recorded every 6 days in the wet and dry mat areas, were related to this parameter. Temperature also had a major influence on ARA and seasonal variations in ARA appeared to be largely influenced by this parameter, with high summer rates of ARA coinciding with warmer temperatures. High *in situ* inorganic nitrogen levels recorded during the study may well be inhibitory to ARA, to the extent that the full nitrogen-fixing potential of the swamp is seldom realised. Salinity appeared to have no effect on ARA over the *in situ* range of salinities. The effects of light intensity and organic carbon are inseparably linked and results indicated that blue-green algae were the most important nitrogen fixers in the wet and dry mat areas, where dark rates of ARA were negligible and organic carbon amendment had no significant effect on ARA. On the pneumatophores, results suggested the importance of bacteria and blue-green algae as nitrogen fixers, as relatively high dark rates occurred, and sucrose amendment had a significant effect. Preliminary time-course experiments also indicated the importance of bacteria as nitrogen-fixers on the pneumatophores as ARA increased exponentially in this habitat, indicating a rapid increase in the population of nitrogen-fixing organisms.

Distinct seasonal variations in ARA, with summer maxima, were linked to high populations of nitrogen-fixing blue-green algae, favourable temperature and to a lesser extent, light intensity and day length. Other factors measured in the field such as organic carbon, inorganic nitrogen, salinity, percentage moisture and bacterial numbers showed no seasonal trends.

Bacteria were largely responsible for high rates of ARA associated with decomposing leaves of *A. marina* .

Estimates of the contribution of nitrogen fixation to the annual nitrogen budget indicate that blue-green algal communities contribute significantly to the nitrogen budget of the Mgeni Estuary mangrove swamp, with the wet and dry mat areas contributing by far the major portion of nitrogen.

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