NITROGEN ENVIRONMENT, ECOPHYSIOLOGY AND GROWTH OF *GRACILARIA GRACILIS* IN SALDANHA BAY, SOUTH AFRICA

BY

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DECLARATION

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Material presented here is all original work by the author and has not been submitted in this or any other form to another university. Where use has been made of research of others, it has been duly acknowledged in the text.

ABSTRACT

The growth of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham was examined by studying the effect of organismic determinants such as thallus length, position along the thallus and branching in a series of *in situ* and laboratory-based experiments. Knowledge of these factors is essential in order to maximise production from suspended seaweed rafts seeded with vegetative G. *gracilis* fragments. Seeding netlons with freshly collected material provided up to 30 % higher relative growth rates than seaweed maintained on the netlons for three successive months. Initial seedstock length greatly affected growth rate and yield such that 30 cm thalli fragments resulted in growth rates 14 % higher than for 10 cm fragments. This difference is suggested to be due to higher contribution of growth by lateral branches to overall biomass. Comparisons of the growth of apical and basal fragments suggested that growth takes place over the entire length of the thallus but that the apex contributes more to overall elongation than does the proximal part. The removal of apical meristems resulted in an enhanced branching frequency with production of four times as many branches as intact fragments. Evidence is also provided for severe morphological differentiation following long periods of rapid growth. These thalli have very high frequency of branching, are hollow due to the disintegration of medullary cells and are considered to be completely senescent.

A large-scale study carried out in Small Bay, the northern part of Saldanha Bay, during the summer of 1996/1997 used the natural abundance of $^{15}N/^{14}N$ ratios to determine the extent of fish-factory pollution in the bay and the uptake of fish-derived nitrogen by *Gracilaria gracilis*. Each year, two fish processing factories release approximately 3.0×10^6 m^3 effluent containing about 650 tons of nitrogen into the north-western part of Small Bay. Sediment $\delta^{15}N$ analysis indicates that this nitrogen is distributed over most of Small Bay, making up 55 - 75 % of the total sedimentary nitrogen, the balance being normal marine detritus. $^{15}N/^{14}N$ analysis of *Gracilaria* shows that dissolved inorganic nitrogen (DIN) is transported in a clockwise direction with surface currents into the eastern corner of Small Bay. This is indicated by high $\delta^{15}N$ values of *Gracilaria* cultivated on a raft in this area relative to seaweed obtained from another raft closer to the mouth of Small Bay towards the west. Furthermore, fish-derived DIN is maintained in the warm surface layer and is prevented from mixing across the thermocline into the cold bottom layer. Results from this part of the study clearly indicate that the turbulent diffusion and entrainment models normally used to model the nitrogen flux into Saldanha Bay should be revised to allow for high anthropogenic nitrogen inputs. These findings also have important implications for seaweed mariculture site selection.

Nitrogen uptake rates and kinetic parameters were determined for *Gracilaria* in a series of perturbation experiments. The kinetics of uptake were affected by nutrient history, ammonium-nitrate interactions, temperature, water motion and thallus morphology. Nitrate uptake showed the presence of a rate-saturating mechanism described by the Michaelis-Menten equation. Here, temperature had no effect on V_{max} in N-replete *Gracilaria*, but K_s determined for specimens acclimatised at 20 °C was lower than at 15 °C. N-limitation did not affect K_s-values for nitrate uptake and were

similar to those measured in N-replete *Gracilaria* at 15 and 20 °C respectively. V_{max}-values were lower than those determined for N-replete material, with a higher value in the 20 °C treatment. Rates of ammonium uptake were always higher than that of nitrate at the same concentration and there was some evidence of suppression of nitrate uptake in the presence of ammonium. Ammonium uptake rates showed a linear response to substrate concentration with the greatest slope measured in N-limited *Gracilaria* at 20 °C, decreasing in N-replete material at 15 °C. The uptake response of N-replete *Gracilaria* could be explained by biphasic kinetics with a dominant diffusive component. A change in thallus morphology from normal type to a highly branched form is accompanied by a change in the normal linear V vs. S response to a rate-saturated relationship with very low V_{max} and high K_s. Water motion was shown to affect the rate of nitrogen uptake, with an 445 % increase in response to doubling of turbulent water motion. Results clearly showed that this species is well adapted to long- and short-term fluctuations in ambient-N concentrations. Despite the efficiency of nitrogen uptake, however, findings suggest that the seaweed would be largely ineffective as a biofilter due to the large amount of fish-derived nitrogen released into Saldanha Bay annually.

Table of Contents

1 General Introduction

1.1 Introduction

Saldanha Bay is considered to be the most important coastal water body along the South African coastline where open-water cultivation of seaweed and shellfish is possible. The Iron and Steel Industrial Corporation's (ISCOR) announcement of the development of an iron smelter in the area however, has resulted in a debate involving the various users of the water space on the future of Saldanha Bay as both an ecosystem and industrial harbour. The importance of Saldanha Bay has long been stressed. For example, during the "Symposium on Research in the Natural Sciences at Saldanha Bay and Langebaan Lagoon", it was described as a unique feature along the southern African coastline (see *Transactions of the Royal Society of South Africa*, 1977, Vol. 42 parts 3 and 4 for the proceedings).

The aim of Chapter 1 is to summarise the status of scientific knowledge pertinent to Saldanha Bay and to provide a brief history of events leading to the current situation regarding the environment and seaweed cultivation in Saldanha Bay. Emphasis is placed on the hydrodynamic conditions in Saldanha Bay since these are responsible for setting the limits to *Gracilaria* productivity attained on suspended rafts in the area.

1.2 The Langebaan Lagoon - Saldanha Bay system

Saldanha Bay is situated about 100 km north of Cape Town and is the only natural deep water port on the South African west coast (Weeks *et al.*, 1991). The Bay is part of a larger complex known as the Saldanha Bay – Langebaan Lagoon system that opens to the sea through a wide mouth (Outer Bay) and is fed by the Benguela upwelling system (Monteiro and Brundrit, in press). Langebaan Lagoon lies parallel to the shore and is approximately 15 km in length with a maximum width of 4 km (Christie and Moldan, 1977a). It is separated from Saldanha Bay by Schapen and Meeuw Islands. Two other islands, Malgas and Jutten Islands, are situated in the mouth of Outer Bay.

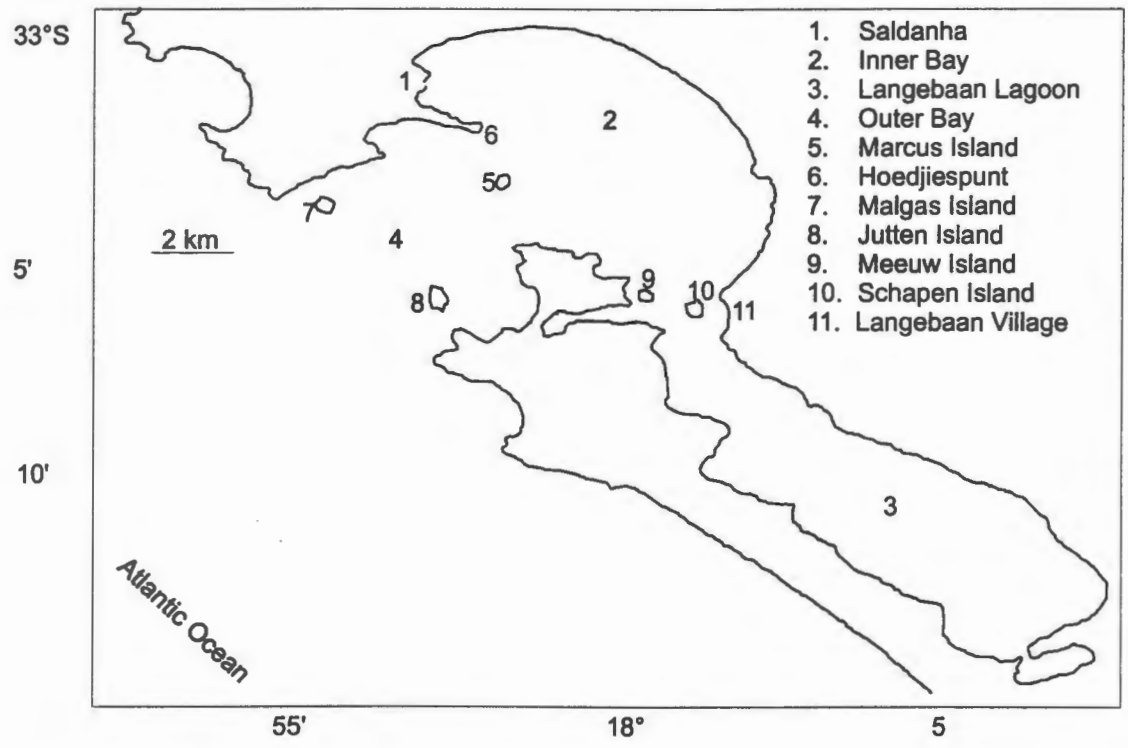

Figure 1. Map showing Saldanha Bay before the construction of the breakwater and ore jetty in 1974.

The lagoon is under protection of the National Parks Board and is the only part of the Langebaan Lagoon - Saldanha Bay system that has remained relatively undeveloped and unpolluted. Since the lagoon has been set aside for conservation and recreation it will not be discussed in the following sections dealing with development, utilisation and mariculture. More detailed discussions on Langebaan Lagoon can be found in Christie and Moldan (1977a, macrofauna), Flemming (1977, recent sediments), Grindley (1977), Henrey *et al.* (1977), Puttick (1977), Shannon and Stander (1977), Summers (1977) and Willis *et al.*, 1977.

1.2.1 Development and utilisation

Prior to 1974, Saldanha Bay functioned as a single system (refer to Figure 1) with open access to ocean waters fed by the Benguela current. The construction of a 2.5 km long ore jetty and breakwater linking Marcus Island to Hoedjiespunt in 1974 changed the circulation pattern and physiography of the bay so that Inner Bay was separated into two water bodies (Weeks *et al.*, 1991; Anderson *et al.*, 1993). The section between the breakwater and the ore jetty became known as Small Bay, while the part south of the ore

jetty was named Big Bay (Figure 2). Big Bay connects with Langebaan Lagoon to the south and the open Atlantic Ocean to the west, while Small Bay has limited access to the adjacent upwelling system through Outer Bay.

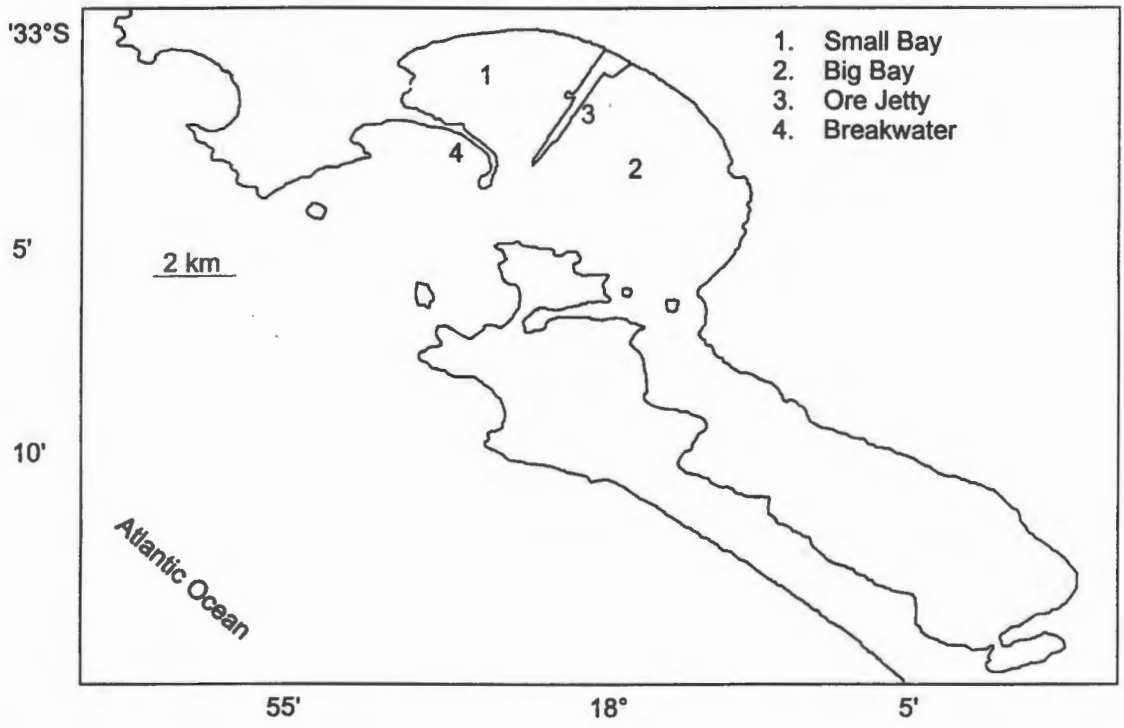

Figure 2. Map showing Saldanha Bay after harbour construction in 1974, but prior to 1997. New development initiated during 1997 is indicated in Figure 3.

Saldanha Bay is important for oil- and ore-landing and is significant in terms of future mariculture development (Hecht and Britz, 1992). With the development of the Saldanha ISCOR plant, renewed interest in the use of Saldanha Bay for the export of iron ore has led to conflict between the mariculture industry and the harbour authorities.

The present ore jetty handles approximately 2.1 million tons of iron ore each year (Bilski, 1996). With the recent development of the ISCOR plant, plans to extend the current ore jetty and build additional jetties parallel to the existing one for multipurpose-cargo and oil handling have been accepted (Figure 3). Expansion of the jetties would result in the decrease of water space available to mariculture, recreational fishing and sailing. Of this remaining space, the Saldanha Bay Sea Water Management Committee has earmarked 750 – 1 000 ha for mariculture in Big Bay by the year 2010. In addition, the Mussel Farmers Cooperative will have access to two smaller areas in Small Bay, including the one

currently occupied by commercial mussel and seaweed rafts owned by Sea Harvest. The area south of the breakwater linking Marcus Island to Hoedjiespunt, currently occupied by the military, will also be zoned for mariculture when vacated. A 200 m radius buffer zone around Marcus, Malgas and Jutten Islands will exclude all forms of mariculture. The remainder of the water space will be set aside for recreation (angling and sailing), conservation and marina development (Figure 3).

Mussel farming in Saldanha Bay has a current turnover of R20 – 30 million per annum (Bilski, 1996). In November 1995 one of the companies responsible for mussel farming (Sea Harvest) established the first pilot scale suspended seaweed rafts in South Africa. The rafts covered a total area of 1 ha at sites in Small and Big Bay (Figure 3), but placement of the raft close to the mussel farm resulted in exceptional settlement of mussel spat. This together with an unusually poor summer for growth the next year led to crop failure. Although this company failed to produce *Gracilaria* commercially, other companies are currently expressing interest in farming the seaweed in Saldanha Bay.

Saldanha Bay also houses an important fishing industry located on the western shore of Small Bay which lands catches of around 30 000 tons per annum (Bilski, 1996). Waste resulting from fish processing at stock and pelagic fish factories is pumped back into the Bay, a means of disposal that has been reported to cause localised eutrophication during summer months (Anderson *et al.*, 1996b; Monteiro *et al.*, in press). In at least one instance, high nutrient levels resulted in a localised bloom of the nuisance alga, *Ulva lactuca* L. (summer, 1993/1994). Such blooms are not only unsightly but contaminate natural *Gracilaria* beach-casts that are collected commercially, rendering the wash-up useless. Fish waste, together with industrial waste resulting from shipping and harbour works, also affects the quality of water available to the shellfish farming industry. The implications of fish-factory waste disposal are discussed in detail in Chapters 3 and 4.

Maintenance of good water quality is essential for a wide range of activities in coastal waters, especially in sheltered bays such as Saldanha Bay. Long-term water quality monitoring is therefore fundamental for the coexistence of recreation, mariculture and industry.

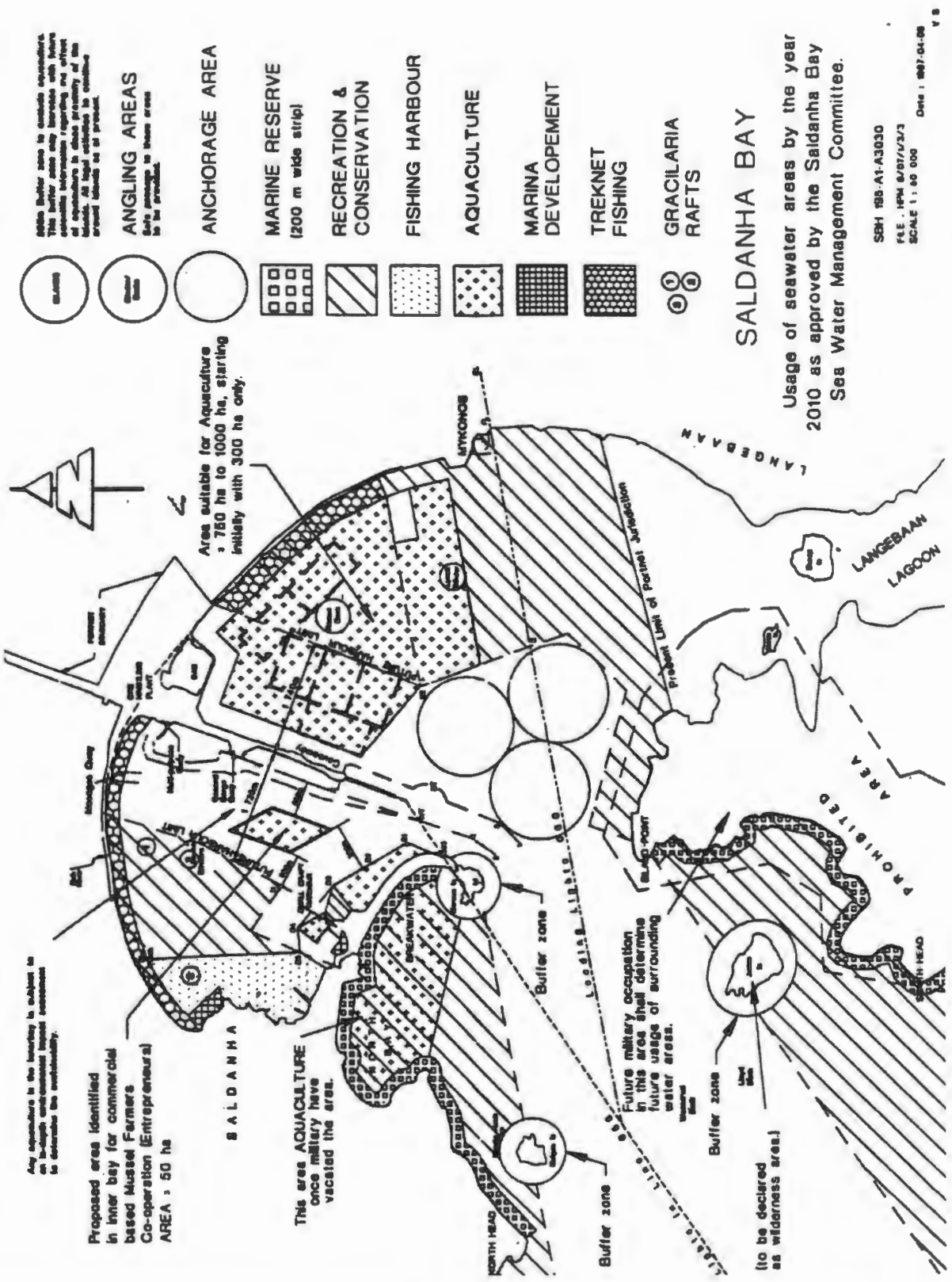

Figure 3. Allocation of water space by the year 2010 proposed by the Saldanha Bay Sea Water Management Committee.

1.2.2 Physical and chemical characteristics

Meteorologically, Saldanha Bay is characterised by strong seasonal winds with south to south-westerlies dominant in summer and northerlies in winter. It has a semi-arid, mediterranean type climate with annual rainfall rarely exceeding 300 mm (Flemming, 1977). Since the area has a small catchment resulting in very little terrestrial sediment input, the sediments of the Saldanha Bay – Langebaan Lagoon system are predominantly of marine origin and are produced by wave abrasion and tidal current scouring (Flemming, 1977). Since the Langebaan Lagoon – Saldanha Bay system does not receive any riverine freshwater inputs, its salinity is similar to that in coastal waters (~35 ‰). However, increased evaporation in the southern end of the lagoon, and possibly the north-eastern corner of Small Bay can produce more saline water (36 – 37 ‰) during summer months.

The construction of the breakwater and iron-ore jetty has severely interfered with the water circulation and exchange in Saldanha Bay. Prior to 1974, Inner Bay functioned as a single water body that was open to the southern Benguela in the absence of the ore jetty and breakwater that now connects the headland to Marcus Island. Today the presence of these barriers largely isolates Small Bay from the southern Benguela and Big Bay, resulting in the reduction of flushing rates (Monteiro *et al.*, 1990). This is supported by the observation that the average temperature of the sun-warmed surface layer in Small Bay is 1 - 2 °C warmer than that of Big Bay, as was correctly predicted by Shannon and Stander in 1977. Apparent oxygen utilisation (AOU) levels in Small Bay, defined as the difference between saturated oxygen concentration and the observed value, also show a long-term increasing deficit while remaining constant in Big Bay. The AOU deficit results from the combination of organic rich fish factory effluent with long residence time of the water within Small Bay (Monteiro *et al.*, 1990).

Surface layer current direction in both Small and Big Bays is determined by wind-forcing (Shannon and Stander, 1977; Bilski, 1996), with winds from the south-west quarter being dominant (Figure 4). Tidal forcing is felt at depths beneath the thermocline and is more marked with increasing proximity to Langebaan Lagoon (Weeks *et al.*, 1991). The presence of the ore jetty constrains the circulation in Small Bay resulting in a usually clockwise circulation pattern (Weeks *et al.*, 1991). Since the construction of the ore jetty there has been evidence of environmental stress in Small Bay on fish, seaweed, plankton

and benthic invertebrates (Monteiro *et al.*, 1990) due to the reduction in circulation and flushing rates in the Bay.

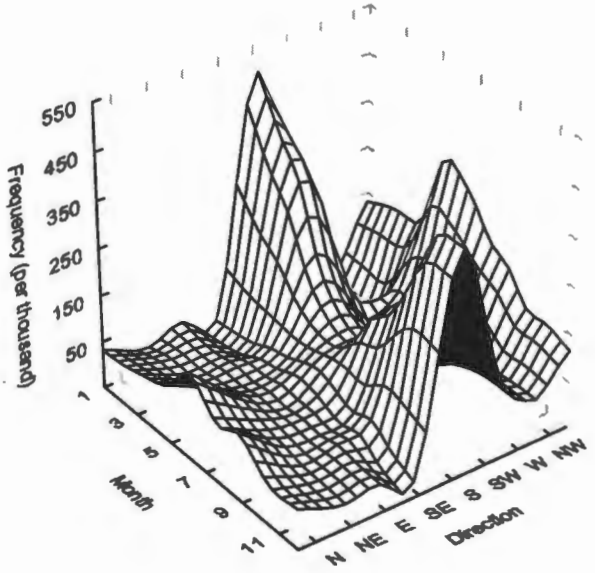

Figure 4. Average wind direction frequency measured at Langebaanweg for the period 1989 - 1995 (1 000 observations per month). Data: South African Weather Bureau.

Water column stratification in Saldanha Bay is strongly seasonal and controlled by solar irradiance, prevailing winds and upwelling from the Benguela system (Anderson *et al.*, 1996b; Monteiro and Brundrit, in press). Upwelling develops along the west coast with the onset of southerly and south-easterly winds in early spring (August - October). This causes the advection of cold bottom water into the Bay and stratification develops. The effect is strengthened during summer (November - March) with prevailing southerly winds and the increasing intensity of solar irradiance resulting in a warm (17 - 20 °C) surface layer and a colder (10 - 12 °C) bottom layer. Destratification begins in autumn (April - May) with reduced upwelling and solar heat flux. In winter (June - August), the water body is well mixed due to the effects of winds associated with cold fronts with an equilibrium temperature of 12 -13 °C throughout the water body (Monteiro and Brundrit, in press; see Figure 5). In contrast to temperature stratification, the salinity time-series indicates that the water column is predominantly isohaline with an inter-annual variability (Monteiro and Brundrit, 1990).

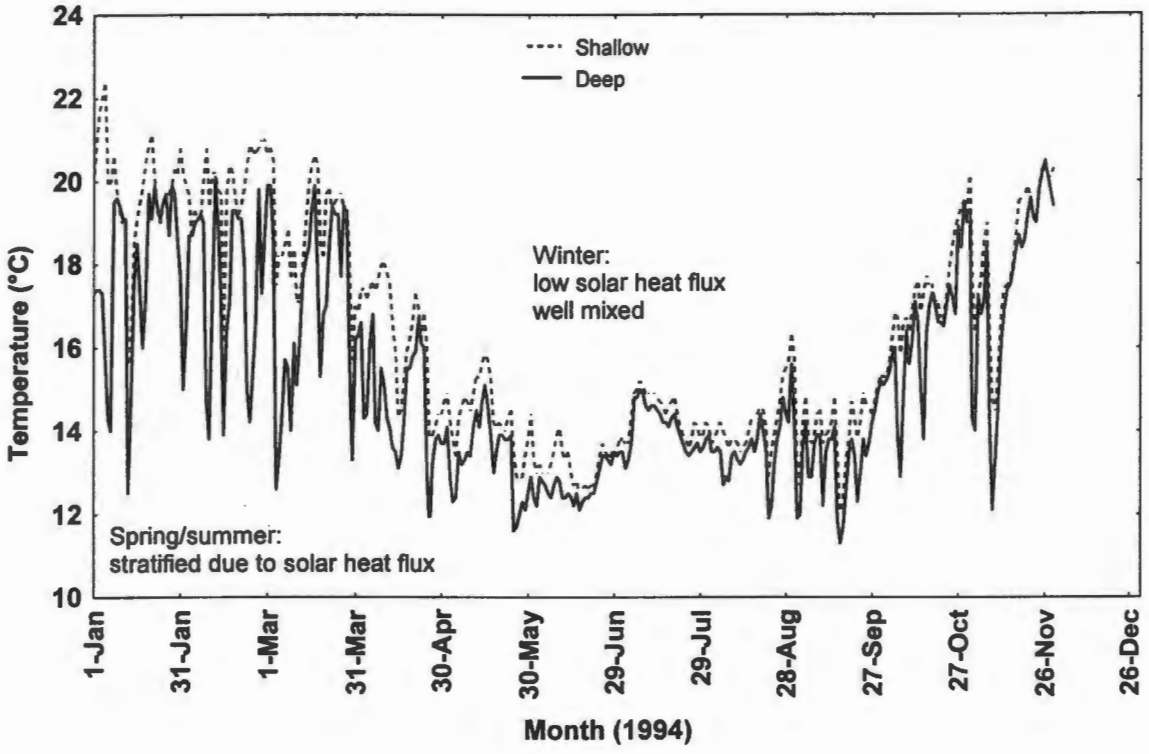

Figure 5. Seasonal water temperature characteristics of surface and deep water in Saldanha Bay (1994). Data: Seaweed Unit, SFRI.

Three types of water each with different characteristics define different spatial zones in the Saldanha Bay system during the spring and summer phases of stratification (Monteiro and Brundrit, in press). Zone A lies geographically within the Bay, but is characterised by coastal surface water (14 - 16 °C) overlying subsurface upwelled water (<11 °C). Zone B is located within Small and Big Bay and is characteristic of the deeper Saldanha Bay (5 - 25 m). It is of two-layered nature, where water heated by solar irradiance (>17 °C) overlies cold upwelled water. Zone C occurs where the bathymetry is less than the maximum depth of the thermocline. Here the whole water column structure is isothermal, consisting of only warm Bay surface layer water (the same as the surface layer in Zone B). The last zone can be subdivided into two sub-zones: the permanent Zone C and the intermediate Zone C. The former is always occupied by a warm water body and occurs in areas in the bay where the depth is less than 5 m. The intermediate Zone C fluctuates with the depth range of the thermocline.

The spatial boundaries of Zones A, B and C vary in time and can be described by the two extremes of the inflow-outflow mechanism responsible for the development of the zones

(Monteiro and Brundrit, in press). During the 'Active Phase', (see Figure 6) upwelling of cold Benguela water forces the shoaling of the thermocline to a depth of 4 - 6 m and results in the outflow of warm surface water from the Bay. The horizontal area of Zone B increases as the depth of the thermocline decreases (Zone B extends into shallower water) which causes the reduction in size of Zone C so that only the permanent Zone C remains. The location of the boundaries depends on the strength of the upwelling event.

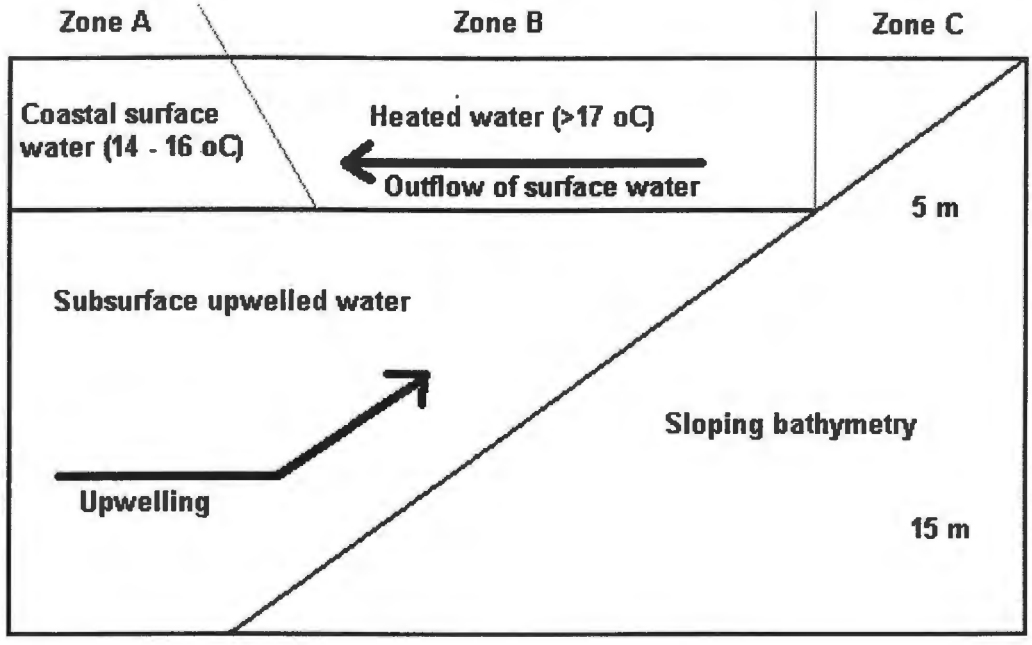

Figure 6. Schematic representation of the spatial boundaries of Zones A, B and C during the Active Phase (modified from Monteiro and Brundrit, in press).

The 'Relaxation Phase' (Figure 7) starts when upwelling ceases and the deeper, cold layer retreats, causing the inflow of warm surface water from the coast. The result is a deepening of the thermocline and an increase in the size of Zones A and C (Zone B decreases in size). The change in water stratification is rapid with a period of approximately 6 - 7 days.

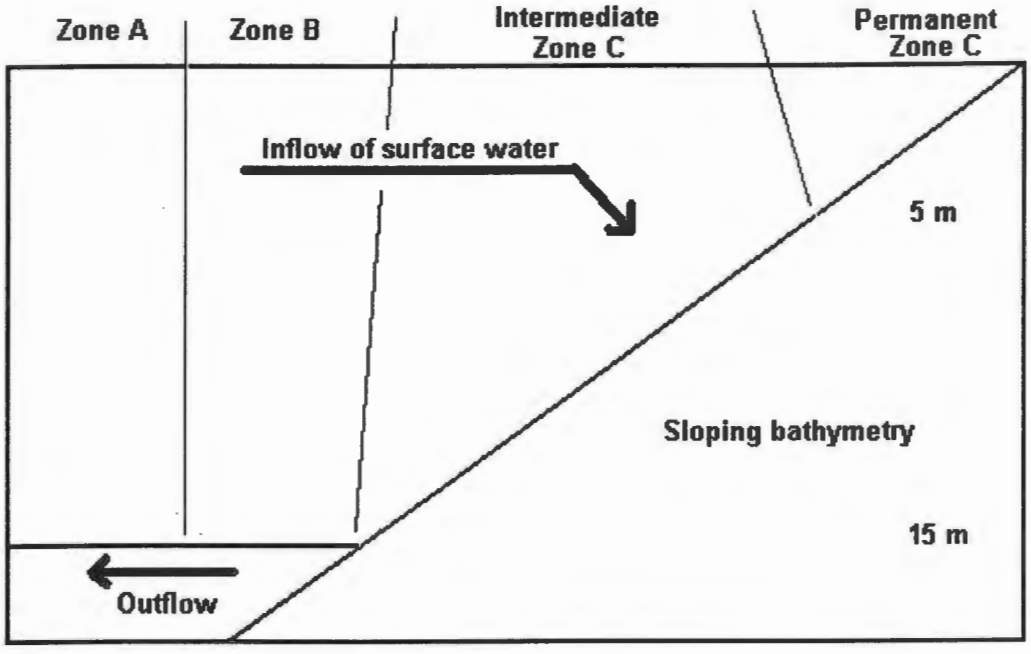

Figure 7. Schematic representation of the spatial boundaries of Zones A, B and C during the Relaxation Phase (modified from Monteiro and Brundrit, in press).

An important feature associated with the stratified water column of Zone B during summer is the inverse relationship between temperature and nitrogen concentration (Anderson *et al.*, 1996b; Monteiro and Brundrit, in press). Water with a temperature of 10 – 12 °C (upwelled water below the thermocline) has a NO_3^--N concentration of 20 – 25 μM. The concentration of NH_4^+-N in upwelled water is less than 1 μM. However, water column and sediment remineralisation processes increase this value to 4 – 8 μM. Since the surface layer in Zone B is of coastal origin and is essentially 'old upwelled water' and all available nitrogen (as upwelled NO_3^--N and remineralised NH_4^+-N) has been removed by phytoplankton blooms, it contains almost undetectable levels of nitrogen (see Bailey and Chapman (1985), Probyn (1985), Shannon and Pillar (1986) for discussions on the biogeochemical cycles in the Benguela upwelling system). The development of a strong thermocline in summer reduces the vertical flux of nitrogen across the nutricline in Zone B to approximately 890 μM-N m^{-2} h^{-1}, almost half the flux in Zone A (Monteiro and Brundrit, in press). Since Zone C is exposed only to the warm surface layer which is nutrient poor, the only source of nitrogen available is that resulting from remineralisation from the sediment (Monteiro and Brundrit, in press).

1.2.3 Eutrophication

In addition to the natural sources of nitrogen available to sustain primary production in Saldanha Bay (see descriptions of Zones B and C above), two fish processing factories on the western shore of Small Bay (Section 3.2.1, Figure 1) release effluent into the Bay. During 'normal years', waste is released only between January and June and is a combination of pelagic fish (anchovy and pilchard) and stock fish. The effluent released by the stock fish processing factory is less than 10 % that of the pelagic fish factory. During 1993, fish processing was extended into August and the total nitrogen concentration (from particulate organic material, POM, and dissolved inorganic nitrogen, DIN) at the point of discharge ranged from 1 314 ± 256 mg L^{-1} (March to mid-June 1993) to 4 375 ± 2 101 mg L^{-1} (June to mid-July 1993) (Anderson *et al.*, 1996b). From late July to August 1993, the nitrogen concentration exceeded 11 000 mg L^{-1}. This corresponds to a nitrogen flux into the Bay of about 50 000 μM-N m^{-2} h^{-1} from March to June and about 500 000 μM-N m^{-2} h^{-1} during August (Anderson *et al.*, 1996b). Most of the nitrogen was released as NH_4^+-N although the POM would rapidly be mineralised to NH_4^+-N (Anderson *et al.*, 1996b). The result of this massive eutrophication was the development of an *Ulva lactuca* bloom between August 1993 and March 1994.

Effluent released by the fish processing factories tends to flow towards the northern part of Hoedjies Bay during periods of southerly winds (Stander and Shannon, 1977) and is directed into the permanent Zone C. Due to the restricted nature of the circulation in this area and reduced mixing with the bottom layer in Zone B, the nitrogen rich effluent is kept buoyant within the otherwise oligotrophic layer. Shannon and Stander (1977) estimated the removal time of pollutants from Inner Bay (before 1974) to be in the order of 20 days. This figure however, can be expected to be higher since the introduction of barriers. For this reason the excess nitrogen (as POM, DIN and dissolved organic nitrogen, DON) released into Zone C requires the development of new biogeochemical pathways to disperse the excess nitrogen load. These pathways favour incorporation of nitrogen into biomass and are likely to lead to the development of blooms of opportunistic species, such as *U. lactuca*.

The only other major pollutants entering the Bay that can be associated with human activity are those resulting from iron ore or oil handling (Willis *et al.*, 1977) but the magnitude and effect of such pollution is as yet unknown.

1.3 *Gracilaria*

1.3.1 Taxonomy, distribution and ecology

For South African seaweed resource biologists, *Gracilaria* is synonymous with Saldanha Bay. Apart from Lüderitz Lagoon in Namibia (see Molloy, 1992) and St. Helena Bay (north of Saldanha Bay), Saldanha Bay is the only other coastal area along the southern African coastline that yields significant commercially utilisable beach-casts of *Gracilaria* (Anderson *et al.*, 1993). Natural *Gracilaria* populations are found in Small Bay where the sandy bottom slopes towards the north. The seaweed does not grow deeper than about 10 m and maximum percentage cover is normally associated with water of less than 7 m deep (Anderson *et al.*, 1993). *Gracilaria* makes up large monospecific stands of free floating or attached thalli of up to 1.7 m in length (pers. obs.). Attachment is provided by sand burial or by entanglement on the anchored ascidian *Pyura* (red bait). Large populations are also found in Langebaan Lagoon, although these remain unutilised. Gracilarioid algae have also been recorded from Table Bay, Hout Bay, the Swartkops River estuary near Port Elizabeth, the Knysna Lagoon, Keurbooms River estuary near Plettenberg Bay and the Kowie River near Port Alfred, although some of these populations may be *Gracilariopsis* sp. (RJ Anderson and JJ Bolton, pers. comm.).

Simons (1977) describes *Gracilaria* from Saldanha Bay as consisting of "ramifying, stringy streamers and looks like branching, reddish-brown, bootlaces". This description also applies to many other species of *Gracilaria* and to some species of *Gracilariopsis*. The morphological plasticity of the Gracilariaceae coupled with the fact that some populations rarely become fertile (reproductive anatomy is essential for identification) has proved a hinderance to algal taxonomists (Bird, 1995; Fredericq and Hommersand, 1989a). The confusion between *Gracilaria* and *Gracilariopsis* is described in detail by Bird (1995) and Steentoft *et al.* (1995), and is summarised below.

Gracilaria confervoides (Stackhouse) Greville, the type specimen of *Gracilaria* was originally described from a British specimen and for many years was used to describe all terete British gracilarioid material. *Gracilaria confervoides* was later renamed as *Gracilaria verrucosa* (Hudson) Papenfuss (Papenfuss, 1950). The specimen of Hudson (1762, cited Simons, 1977) was later applied as lectotype after the holotype was lost. This description is now invalid since none of the above work made any distinction between *Gracilaria* and *Gracilariopsis*. Since then, Phillips (1925) separated *Gracilariopsis*

confervoides var. *procerrima* (Esper) Greville from *Gracilaria confervoides* and Fredericq and Hommersand (1989a, 1989b) distinguished between *Gracilaria sensu stricto* (= *G. verrucosa*) and *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto et Foldvik which they recorded for the first time under that name in the British Isles.

Dawson (1949) separated *Gracilaria* from *Gracilariopsis* on the following basis:

1. *Gracilaria*: gonimoblast irregular, consisting of a few, large vacuolated cells; *Gracilariopsis*: dome-like gonimoblast consisting of many small, non-vacuolated cells;

2. *Gracilaria*: sparse protoplasm; *Gracilariopsis*: dense protoplasm;

3. *Gracilaria*: carposporangia in clusters and short chains; *Gracilariopsis*: carposporangia in well-marked radiating chains;

4. *Gracilaria*: nutritive filaments present; *Gracilariopsis*: nutritive filaments absent.

Studies by Fredericq and Hommersand (1989a, 1989b) were based on the characters identified by Dawson (1949) in addition to several others to provided evidence that the genera *Gracilaria* and *Gracilariopsis* are separate and are both found in Britain. These studies clearly indicate that terete British gracilarioids have been regularly misidentified and that there has been some confusion in the application of the genera *Gracilariopsis* and *Gracilaria*.

Steentoft *et al.* (1991) showed that the lectotype of *Gracilaria confervoides* (Stackhouse) Greville is in fact a *Gracilariopsis*. In order to prevent the renaming of a large number of species as a result of a generitype belonging to another genus, conservation of *Gracilaria compressa* (Agardh) Greville as lectotype of the genus has been proposed and accepted (Steentoft *et al.*, 1991; Silva, 1994). Further confusion was prevented by renaming *Gracilaria verrucosa* as *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham and selecting it as lectotype for the species. The specific epithet *verrucosa* was rejected with the neotypification of *Gracilariopsis longissima* (Gmelin) Steentoft, Irvine et Farnham (Steentoft *et al.*, 1995).

Gracilaria in Saldanha Bay has been identified as *Gracilaria gracilis* (as *G. confervoides*) by Isaac (1956). This identification was based solely on the presence of nutritive filaments

near the gonimoblast in the cystocarp. However, Isaac (1956) does not mention the type of spermatangia found in the Saldanha Bay seaweed, neither are there any reports of spermatangial type in subsequent literature. A fertile cystocarpic specimen collected and sectioned by RJ Anderson (pers. comm.) confirms the seaweed belongs to the genus *Gracilaria*. Bird *et al.* (1994) have shown using molecular techniques that the southern African species show definite links with the *G. verrucosa* group and subsequently Bird (1995) has suggested it be called *G. gracilis*.

Isaac (1956) studied the annual reproduction of *Gracilaria* in the Langebaan Lagoon-Saldanha Bay area. He concluded that cystocarpic plants are found throughout the year, tetrasporic plants occur for the greater part of the year and that the greatest proportion of the seaweeds was cystocarpic. Although this study is of limited value because of the non-quantitative terminology used, it does indicate that the seaweed became fertile during the 1950's. Today *Gracilaria* in the Saldanha Bay - Langebaan Lagoon area reproduces solely by vegetative means and fertile plants are rarely found. The reason for the change to an almost completely vegetative mode of reproduction is unknown. Interestingly, *Gracilaria* in Lüderitz Lagoon has never been observed to be fertile (Molloy, 1992)

1.3.2 Commercial interest

The world-wide importance of *Gracilaria* has been discussed by Armisen (1995). In southern Africa the importance of the seaweed was realised during World War II when there was a global shortage of agar and since this time large annual beach-casts have been collected. Anderson *et al.* (1989) and Rotmann (1990) reported collections of up to 2013.5 dry tonnes in the three years prior to 1974. The industry based on the collection of beach-cast material was successful until 1974 with the construction of the iron ore jetty. Since then, yields have been drastically reduced but recovered again to about 430 tons in 1988. Within this time however, several collapses (partly due to grazing by invertebrate herbivores) and recoveries have occurred, making the resource unpredictable thereby hampering further development of the seaweed industry (Anderson *et al.*, 1993; Anderson *et al.*, 1996a). No wash-ups were collected between 1988 and 1992, subsequent to which annual yields recovered to about 400 dry tonnes per annum (Anderson *et al.*, 1996b). The industry currently employs between 60 - 100 people in collecting and preparing the

seaweed for export as dried, baled material and was worth approximately US $ 453 000 in 1992 (Plate 1; Anderson *et al.*, 1996b).

The unpredictability of *Gracilaria* wash-ups since the 1970's in Saldanha Bay and the successful development of a seaweed industry based on *Gracilaria* mariculture in Namibia are responsible for the increased interest in seaweed farming in South Africa over the last decade. Taurus Chemicals initiated the first pond cultivation trials of *Gracilaria* in Namibia (Rotmann, 1987) and in 1992 installed the first commercial suspended raft in Lüderitz Lagoon (Dawes, 1995). In 1995 they had approximately 10 ha under cultivation with plans for future expansion to about 40 ha (J Fliedl, pers. comm.).

The Seaweed Unit, Sea Fisheries Research Institute, initiated *Gracilaria* growth experiments in 1989 with their early experiments concentrating on the growth of the seaweed attached with stakes to the bottom of Saldanha Bay (RJ Anderson, pers. comm.). In 1991 interest shifted to raft cultivation with the instalment of the first experimental suspended raft in the Bay in this year. Since then several other experimental rafts have been installed at various sites in Small Bay (see Section 3.2.1) and recently in St. Helena Bay. The construction of the rafts is described by Dawes (1995) and Anderson *et al.* (1996a). In November 1995, Sea Harvest started the first commercial seaweed mariculture system in South Africa based on the system developed in Lüderitz Lagoon. Production from the Saldanha Bay rafts started in April 1996, but the operation ceased soon afterwards due to mussel settlement on *Gracilaria* lines and a unusually poor summer for growth.

Plate 1. Manual collection (a) and loading (b) of beach-cast of *Gracilaria gracilis* in Saldanha Bay.

1.4 Plan of the thesis

The great importance of the Saldanha Bay - Langebaan Lagoon system has been noted by Hey (1977) who wrote '[the] complex, including the islands and riparian wetlands, is a unique natural resource judged by any standards, and particularly so in a country such as South Africa with a regular coastline and a paucity of lagoons, estuaries and enclosed bays'. Unfortunately, the Saldanha Bay part of the system no longer functions the way it did prior to 1974 due to harbour construction. Today, a large group of people with diverse interests has rights to water space in Saldanha Bay and only the wise use of the natural system within its carrying capacity will allow all users to peacefully coexist. This is especially true in the case of the now semi-enclosed water body of Small Bay where anthropogenic activities such as shipping and organic pollution from fish processing factories already result in the 'overflow' of the 'nutrient carrying capacity' (Chapters 3 and 4). Apart from the almost invisible problem of gradual eutrophication and the cumulative effect of heavy metal accumulation in sediments and organisms, there is also a real danger of 'immediate' disasters such as oil spills that can cause losses of millions of rands overnight. Conflict is inevitable between such users and particularly those of the mariculture industry who rely on good quality seawater and sufficient water space.

The management of Saldanha Bay, within its carrying capacity, will only be possible once we have obtained a thorough understanding of the physical, chemical, biological and biogeochemical processes operating in it. Industrial, urban and recreational development however, proceeds at a rapid pace leaving scientists insufficient time to study the functioning of the system. Had this knowledge been available today, or even before 1974, water space allocation would have been far more efficient and would have ensured the optimal placement of all activities in Saldanha Bay. Without proper management guidelines the Bay will no longer be a 'unique natural resource' (Hey, 1977), but just another industrial shipping port in a state of disrepair.

Shipping and effluent disposal are not the only industries linked to a decline in water quality and environmental degradation. Mariculture, which has often been made out to be the party suffering under the consequences of shipping and waste disposal, can also be implicated in causing pollution leading to water quality degradation. Pollution from shellfish or fish aquaculture or clearing of areas for aquaculture expansion has threatened the industry in certain countries (Grant *et al.*, 1995; Axler *et al.*, 1996; Neori *et al.*, 1996).

The environmental effects of shellfish farming in Saldanha Bay have been addressed in a report on the carrying capacity of Saldanha Bay (Anonymous, 1996) but further research is needed to assess the possible role of fish factory pollution to eutrophication of the bay. There are those who believe mariculture could be an indicator of environmental stress in an ecosystem by being the first to suffer under adverse conditions (Hecht and Britz, 1992). A decrease in production would suggest a decline in the environmental quality, as was the case in Thailand where mussel production dropped by 30 % due to urbanisation and pollution (Chalerm and Lutz, 1989).

A possible solution to maintaining water quality is the integration of seaweed mariculture into the management strategy for Saldanha Bay. The principle of using seaweeds as biofilters in natural systems such as semi-enclosed bays is similar to integrated seaweed cultivation described by Petrell *et al.* (1993), Subandar *et al.* (1993), Friedlander and Levy (1995), Bodvin *et al.* (1996), Buschmann (1996), Buschmann *et al.* (1996), Jiménez del Río *et al.* (1996) and Petrell and Alie (1996). In the case of Saldanha Bay, the system would employ suspended seaweed rafts seeded with *Gracilaria* placed in an area exposed to high nitrogen fluxes resulting from pollution. The cultivated *Gracilaria* would act as a sink for excess nitrogen thereby making it unavailable to nuisance algae such as *Ulva*. The feasibility of using *Gracilaria* mariculture with the secondary objective of water biofilter is examined as the overall aim of this thesis.

The first aim of this study is to gain a knowledge base of the growth and regeneration capabilities of *Gracilaria gracilis* from Saldanha Bay. This is essential for understanding how different seeding techniques can be used to improve yield from a suspended seaweed raft and is dealt with in Chapter 2.

An understanding of the properties of the Saldanha Bay system is central to the development and optimisation of seaweed cultivation in the area. The known physical and chemical characteristics of Saldanha Bay have been discussed above and other studies have focused mainly on hydrodynamic processes in Outer Bay or Langebaan Lagoon. Therefore, the second aim of this thesis is to gain an understanding of the distribution of nitrogen in Small Bay. Emphasis will be placed on nitrogen because it is the major parameter limiting the growth of *Gracilaria*, both spatially and temporally. Nitrogen originating from fish factory pollution could significantly affect seaweed growth and

production in Small Bay and so the role of fish effluent in the total nitrogen budget of the bay is assessed. Isotope ratio mass spectrometry (IRMS) involving nitrogen as a natural tracer is used as the main technique of this part of the study. The distribution of the two sources of nitrogen to Small Bay (upwelled and anthropogenic) with respect to *Gracilaria*, the water column and the sediments is discussed in Chapters 3 and 4.

Since the physico-chemico features of the seawater determine the growth performance of *Gracilaria* it is essential that we gain an understanding of the ecophysiology of the cultivated seaweed. Once the functioning of *Gracilaria* with relation to the main controlling environmental parameter is understood (here considered to be nitrogen), it becomes possible to select sites in Saldanha Bay that would best suit its ideal growth conditions. The third aim of this thesis is to gain an understanding of the nitrogen uptake kinetics of *Gracilaria* therefore enabling us to assess its effectiveness as nitrogen sink and the feasibility of using it as biofilter. The nitrogen ecophysiology of the southern African *Gracilaria gracilis* is discussed for the first time in Chapter 5 while *in situ* uptake is modelled in Chapter 6 to allow us to evaluate the possible role of *Gracilaria* as biofilter. Chapter 7 provides an integrated summary of these findings.

2 *Organismic Determinants and Their Effect on Growth and Regeneration in* Gracilaria gracilis

2.1 Introduction

Members of the genus *Gracilaria* contribute more than half the world agarophyte production (McHugh, 1991; Fletcher, 1995). The seaweed is cultivated commercially or semi-commercially (as by-product) in countries such as Chile, China, Taiwan, Namibia and South Africa (Ren *et al.*, 1984; Santelices and Ugarte, 1987; Dawes, 1995; Friedlander and Levy, 1995; Smit *et al.*, 1997). The characteristics of *Gracilaria* that make it desirable for cultivation are fast growth rate, good agar yield and quality and relative ease of growth (Buschmann *et al.*, 1995). However, the most important attribute of *Gracilaria* is that almost all the species important in the mariculture industry reproduce solely by vegetative means through fragmentation. This leads to an extremely high regenerative capacity (Hurtado-Ponce, 1990; Santelices and Varela, 1995) and eliminates the need to raise plants from spores. This, along with the fact that *Gracilaria* species are morphologically plastic (Dawes, 1993; Meneses, 1996), has initiated many studies focusing on selection of strains with good gel characteristics and fast growth rates (Santelices, 1992). Other researchers have examined ecophysiological factors affecting growth, agar quality and yield such as light intensity, temperature, salinity, water motion and nutrients (Parker, 1982; Lapointe and Duke, 1984; Engledow and Bolton, 1992; Gonen *et al.*, 1993; Dawes, 1994; Kaladharan *et al.*, 1996; Rebello *et al.*, 1996).

Several studies on *Gracilaria* have been done in southern Africa since World War II when the commercial importance of the seaweed was first realised. Most of the studies until quite recently have dealt mainly with the ecology of *Gracilaria* in Langebaan Lagoon and Saldanha Bay (Isaac, 1956; Simons, 1977; Anderson *et al.*, 1993; Anderson *et al.*, 1996b) or in Lüderitz Lagoon, Namibia (Molloy, 1992; Molloy and Bolton, 1995). Several other studies have looked at ecophysiological aspects of *Gracilaria* cultivation (Engledow and Bolton,1992; Smit, 1995; Anderson *et al.*, 1996a; Rebello *et al.*, 1996; Smit *et al.*, 1997; Anderson *et al.*, in press) or dealt with commercial aspects of its utilisation (Rotmann, 1987; Rotmann, 1990). The recent interest in suspended seaweed mariculture in South Africa however, has necessitated research into factors inherent in *Gracilaria* itself which

affect growth and regrowth performance in cultivation. Santelices and Varela (1995) termed these factors >organismic determinants=. Since productivity of *Gracilaria* is tightly coupled to vegetative regeneration, it is important to understand the processes leading to the development of new tissue and to apply this knowledge to seaweed mariculture operations in order to enhance biomass production. It has been noted that, despite the wealth of literature available on the ecophysiological aspects of seaweed growth, very little has been done in order to understand the organism itself (Santelices and Varela 1995). The evaluation of organismic determinants such as reproductive state, size of thallus fragments and the position of fragments along the thallus axis was found to be important in affecting growth (Santelices and Varela 1995). The aim of this study is to evaluate the effect of such factors on the mode of growth and regrowth in *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham from Saldanha Bay with the view of applying this knowledge to future *Gracilaria* mariculture operations in the region.

2.2 Materials and methods

2.2.1 *In situ* determination of effects of seed stock quality on growth

The hypothesis that the quality of seed stock (the material used to seed the rafts) affects growth and production of *G. gracilis* on a suspended raft was tested by comparing the growth rate of naturally occurring *Gracilaria* collected near the SFRI raft (Section 3.2.1) with that of cultivated seaweed grown on a suspended raft. The suspended seaweed raft used in these experiments in Saldanha Bay was similar to that described by Dawes (1995) and the exact specifications are given by Anderson *et al.* (1996a). The experiment was conducted during winter between the months of May and August 1996. Thallus fragments of raft-cultivated seaweed were frequently broken due to harvesting techniques and usually measured between 15 and 20 cm in length. They were often infested with *Ceramium* sp. (2.21 g (dry) *Ceramium* per 100 g (dry) *Gracilaria*), the main epiphyte of *Gracilaria*, when grown on the raft. Before seeding *Gracilaria* onto the lines most of the *Ceramium* was manually removed allthough some epiphytes remained which could later regenerate. Apart from epiphyte contamination and broken thalli the harvested *Gracilaria* was generally healthy and in good condition. *Gracilaria* collected from the natural populations was very healthy and up to 1.7 m in length and showed very little *Ceramium* sp. or other epiphyte infestation.

Netlons were seeded with cultivated or naturally occurring *Gracilaria* (four replicates per line) to an initial stocking density of 1.1 to 1.2 kg fresh per 2.5 m after removing as much as possible of the visible epiphytes. The tufts of seaweed fragments used for seeding were approximately 20 cm in length and spaced at 20 cm intervals along the netlon line. Netlons ('superope') are plastic tubular netting with a mesh size of approximately 20 mm supplied by Van Leer Plastics, Johannesburg, South Africa. After the seaweeds were threaded through the sides of the netlon using the wire hook method (Dawes, 1995) and the netlon pulled tight, the *Gracilaria* was held firmly in place on the line. The netlons were spaced 0.75 m apart on the raft to avoid abrasion against adjacent lines and to minimise self-shading. Netlons were removed, weighed and restocked once a month (May – July) with harvested and newly collected material from natural populations and relative growth rates (RGR) calculated and expressed as percent wet mass increase per day. The equation used for the calculation of relative growth rate was

$$RGR = \frac{\ln(W_2 / W_1)}{n} \cdot 100 \quad \textbf{[Eq. 1]}$$

where W_2 and W_1 are final and initial mass respectively and n is the number of days. Results were analysed using a two-way ANOVA (Statistica for Windows Release 5.1) where appropriate.

2.2.2 *In situ* determination of the growth rate of different lengths of thalli

During August 1996 the growth rate and yield of *Gracilaria* were compared among three different thallus length classes to test the hypothesis that longer thalli produce more biomass than an equal mass of shorter thalli. Whole *Gracilaria* plants were collected from benthic populations and 45 plants were selected and cut to lengths of 10, 20 and 30 cm. Each tuft was weighed on a spring balance to between 15 and 24 g (wet). The individual tufts (15 individual tufts per rope) were numbered and attached at 20 cm intervals with plastic cable ties on each of three 6 mm polypropylene ropes. No attempt was made to randomise thalli of different length classes between ropes and consequently, each rope contained tufts of the same lengths. Randomisation was not seen as necessary as it was more meaningful from a mariculture point of view to seed a standard tuft length per rope. Furthermore, previous experiments indicated that there was no significant difference in growth *of G. gracilis* on different netlons when they were placed adjacent to each other

(RJ Anderson and AJ Smit, unpublished). Ropes and cable ties were used as they allowed the use of individual tufts as replicates and could easily be labelled and recovered at the end of the experiment. The seeded ropes were installed on the raft and the growth determined a month later by recording the fresh mass and length of each tuft. Negligible epiphyte material was present eliminating the need for epiphyte correction (see above). Growth was expressed as relative growth rate as above, with calculations for both length and mass data. The statistical significance of any differences among results were determined using a one-way ANOVA, while the Pearson product-moment corellation was used the determine p-values associated with linear regressions (Statistica for Windows Release 5.1).

2.2.3 Standard conditions for laboratory growth experiments

Healthy seaweed material was collected from Saldanha Bay and placed in a laboratory holding tank (part of a 3000 L recirculating seawater system) until required, usually within a week of collection. Before each experiment, thallus fragments were rinsed in 0.45 µm filtered seawater, most of the visible epiphytes removed manually and treated with freshwater and povidone-iodine to kill any remaining macroalgal epiphytes and diatoms. The povidone-iodine treatment involved soaking fragments for 1 minute in a solution containing 0.5 % (w:v) povidone-iodine in distilled water containing a few drops of wetting agent (commercial liquid soap) followed by a thorough rinse in distilled water. The seaweed was acclimatised for four to five days to experimental conditions of 18 °C, a 16 : 8 (light : dark) photoperiod and a light intensity of 80 to 90 µmol photons m^{-2} s^{-1} (Osram cool-white fluorescent tubes). One-third strength standard Provasoli=s Enriched Seawater (PES, McLachlan, 1973) was used as culture medium in acclimation and experimental phases.

Standard growth conditions involved individual seaweed fragments (five replicates) being placed separately into 100 mL Erlenmeyer flasks and receiving aeration through disposable pipette tips attached to plastic air-tubing. The fresh mass of each fragment was determined every three days on a four decimal place balance while replacing the culture medium. Fragment lengths were measured to the nearest millimetre and total number of lateral branches counted. Growth rates were calculated from the final slope of the resulting exponential growth curve when cumulative biomass was plotted against time.

2.2.4 Growth comparison between thallus base and tip

To determine the contribution of intercalary growth to overall growth, the change in wet biomass and length of five 10 mm tips cut from lateral branches were compared to that of the same length fragments removed from the base of the branch. All thallus fragments (ramets) used in the experiment were taken from the same plant (genet).

2.2.5 Effect of removal of apical tip on growth

The growth of five 12 mm *Gracilaria* thallus tips, of which 2 mm of the apex was removed (i.e. starting length of 10 mm), was compared to that of five 10 mm fragments with intact apical meristems.

2.3 Results

2.3.1 The effect of seedstock quality

The growth rate of newly collected *Gracilaria* obtained from natural populations differs markedly from that of cultivated seaweed when grown on a suspended raft cultivation system (Figure 1). Relative growth rates obtained from netlons reseeded with naturally occurring *Gracilaria* were 19.4, 29.8 and 21.5 % higher than those of netlons reseeded with cultivated seaweed for May, June and July respectively (as determined from the mean monthly relative growth rate). A two-way ANOVA indicated these differences to be significant at $p < 0.05$ between seeding treatments (d.f. = 1, F = 14.14) and over the three month experimental treatment (d.f. = 2, F = 7.88). A Tukey HSD post-hoc test showed that the between-month difference in growth rate for netlons seeded with cultivated *Gracilaria* was insignificant at $p > 0.05$, while for May, netlons containing naturally occurring *Gracilaria* had a significantly higher growth rate compared to the other two months. The two-way ANOVA showed no significant interaction between seeding treatment and time (d.f. = 2, F = 0.27).

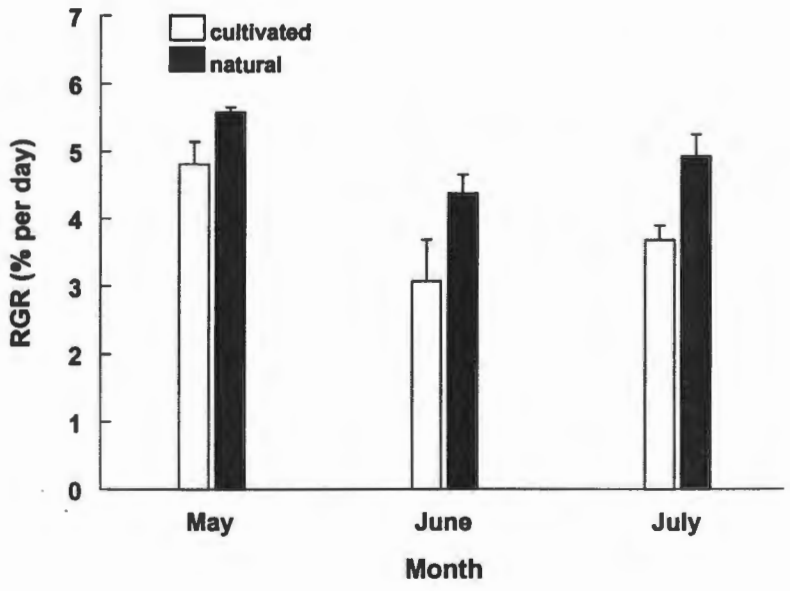

Figure 1. Relative growth rates of freshly collected and reseeded *Gracilaria gracilis* on a suspended seaweed cultivation raft (standard error bars shown, $n = 4$).

At the end of the experimental period the reseeded seaweed that had been maintained on the raft for the full three month period appeared morphologically and anatomically different from *Gracilaria* obtained from natural populations. The main axes of these plants were thick and twisted and there was evidence of a higher branching frequency (Plate 1). The morphologically differentiated specimen had a branching frequency of 14.9 ± 8.3 branches per cm with the main axis being 3.2 ± 0.3 mm in diameter. The normal undifferentiated form had a branching frequency of 1.0 ± 0.3 branches per cm and a thickness of 1.7 ± 0.1. No attempts were made to describe the anatomy in detail, but transverse and longitudinal sections through the main axis of normal and differentiated specimens are shown in Plates 2 and 3. In the differentiated form the medullary cells appear to have disintegrated resulting in a hollow interior while the cortical cells remain intact. In some laboratory culture studies *Gracilaria* with such a thick, hollow main axis floated to the surface as the interior filled with gas, presumably oxygen resulting from photosynthesis.

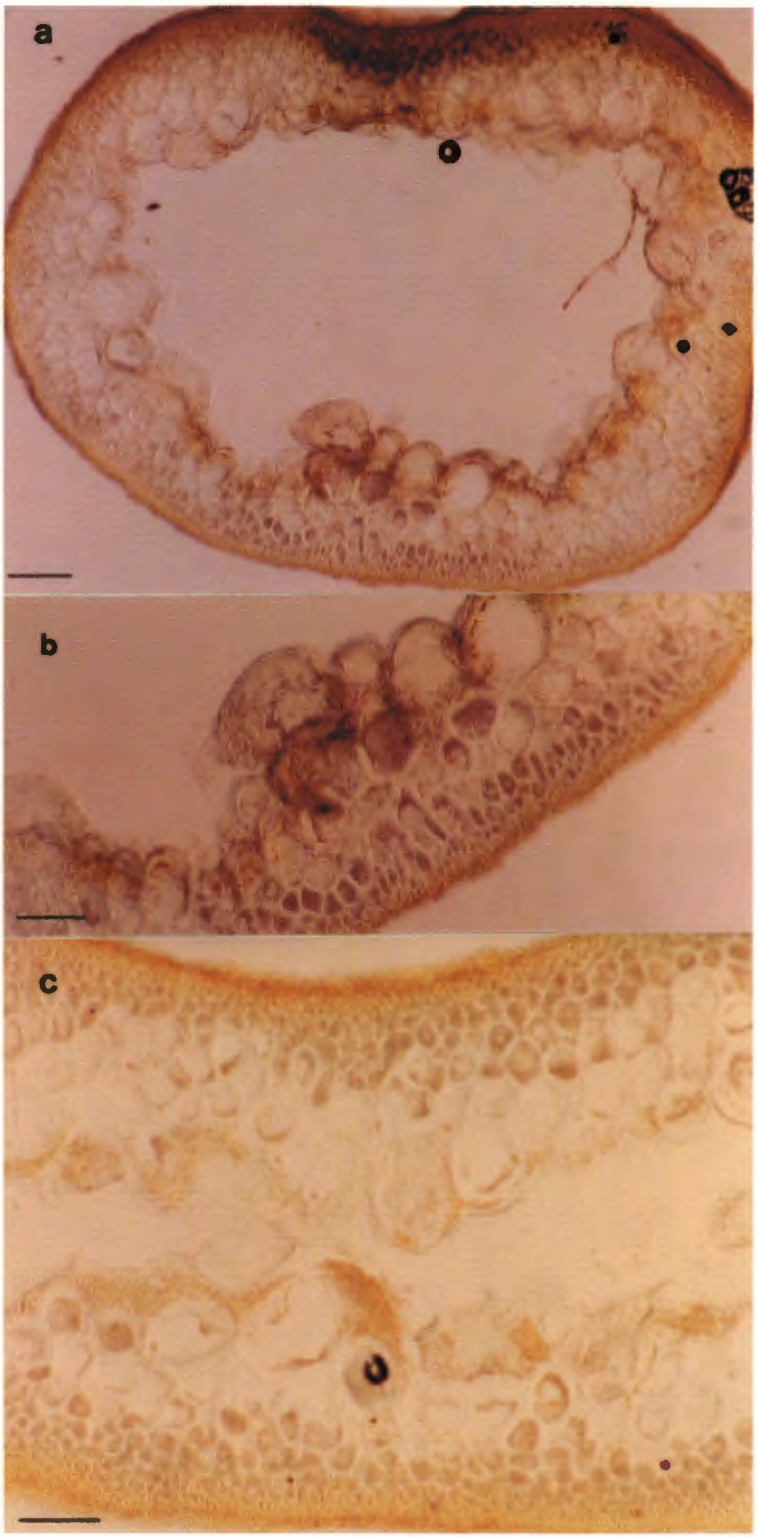

Plate 2. Transverse (a, b) and longitudinal (c) sections through a differentiated *Gracilaria gracilis* thallus with a schizogenic cavity. Scale bars: 0.29 mm (a), 0.18 mm (b) and 0.29 mm (c).

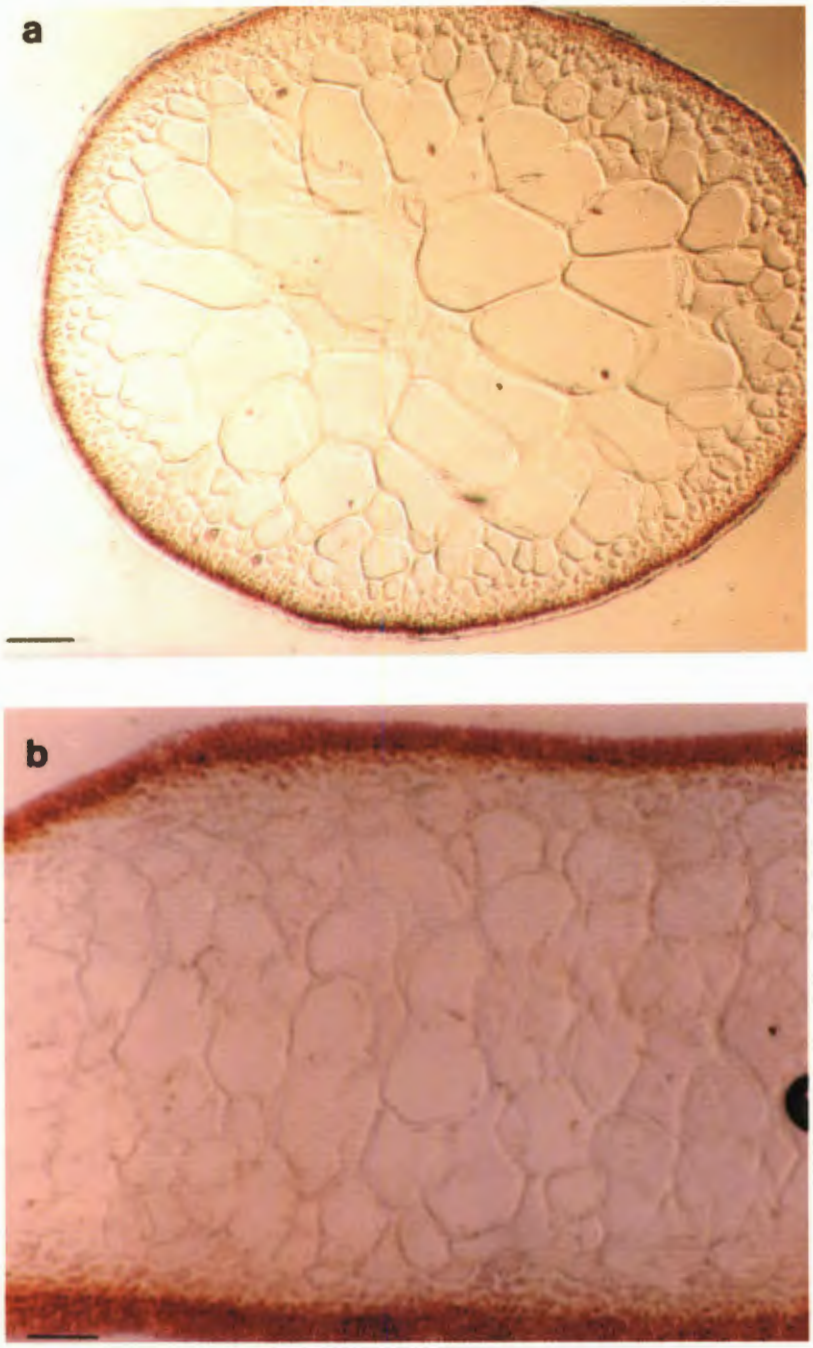

Plate 3. Transverse (a) and longitudinal (b) sections through an undifferentiated (normal) *Gracilaria gracilis* thallus. Scale bars: 0.16 mm (a) and 0.21 mm (b).

Plate 1. Laboratory cultivated thalli of *Gracilaria gracilis* showing highly branched morphology of a young specimen. This morphotype had a similar appearance to seaweeds maintained on a raft for an extended period of time (3 months). Longitudinal and transverse sections through this material are shown in Plate 2.

2.3.2 *In situ* determination of the growth rate of different lengths of thalli

Figure 2 shows the net yield of *Gracilaria* initially seeded as three different thallus lengths as calculated from change in biomass or length over a 30 day period. Initial thallus length had a significant effect on net yield per tuft expressed in terms of mass (one-way ANOVA; $p < 0.05$, d.f. = 2, F = 4.66) so that the 30 cm tufts produced 21.8 ± 5.1 % more biomass than the 10 cm tufts. Difference in yield expressed as length produced over 30 days were not significant between the three treatments (one-way ANOVA; $p > 0.05$, d.f. = 2, F = 0.44), so that the same increase in length was produced from 10, 20 and 30 cm thalli segments. Relative growth rates were significantly different between the three length groups for both the mass and length calculations (one-way ANOVA; $p < 0.001$, length: d.f. = 2, F = 9.97, mass: d.f. = 2, F = 236.06). Relative growth rate expressed in terms of length showed a positive dependency ($r = 0.567$ at $p < 0.001$) on initial seeding length so that the relative growth rate for the 30 cm treatment was 14.2 ± 2.8 % higher than for the 10 cm treatment. The slope of the regression line is negative when relative growth rate is expressed in terms of mass ($r = -0.932$; significant at $p < 0.001$; Figure 3).

2.3.3 Effect of removal of the apical meristem on growth

The final mass of the thallus fragments with and without an apical meristem was not different at the end of the experimental period of 14 days ($p > 0.05$, Student's T-test). There seemed to be some evidence of a greater increase in length for the treatment with the intact apical meristem (71.4 ± 3.4 and 62.0 ± 3.7 mm for fragments with and without meristems respectively) and although this difference was not significant ($p < 0.05$) it resulted in a significantly different relative growth rate between the treatments when calculated in terms of change in length per day (Table 1). In contrast, growth rates expressed in terms of mass were not significantly different. An examination of the branching frequency of the treatments suggests that lateral branch development is under control of the apex so that the growth and development of lateral branches are promoted when the tips are removed. *Gracilaria* fragments with the apical cells removed had 5.9 ± 0.5 lateral branches cm^{-1} of thallus compared to 1.4 ± 0.3 branches cm^{-1} for the intact thallus pieces ($p < 0.001$, Student's t-test).

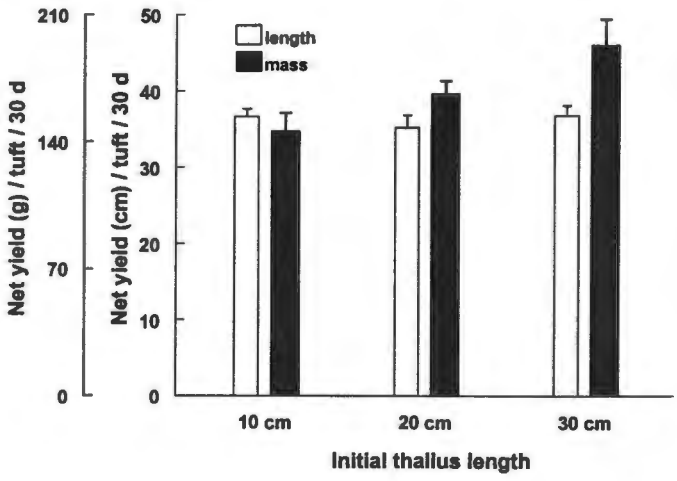

Figure 2. Net yield of *Gracilaria gracilis* initially seeded as 10, 20 and 30 cm lengths. Yield is calculated from length and mass data and normalised to a month (standard error bars shown, $n = 45$).

Table 1. Relative growth rates and final lengths and weights of *Gracilaria gracilis* fragments with the apical meristem removed and with meristem intact. T-test results are also shown.

	RGR (% length per day)	RGR (% mass per day)	Final length (mm)	Final mass (g)
with tip	14.3 ± 0.4	20.7 ± 0.8	71.4 ± 3.4	0.038 ± 0.003
no tip	11.5 ± 0.5	19.2 ± 0.7	62.0 ± 3.7	0.042 ± 0.003
p	0.001	0.191	0.098	0.356
n	5	5	5	5

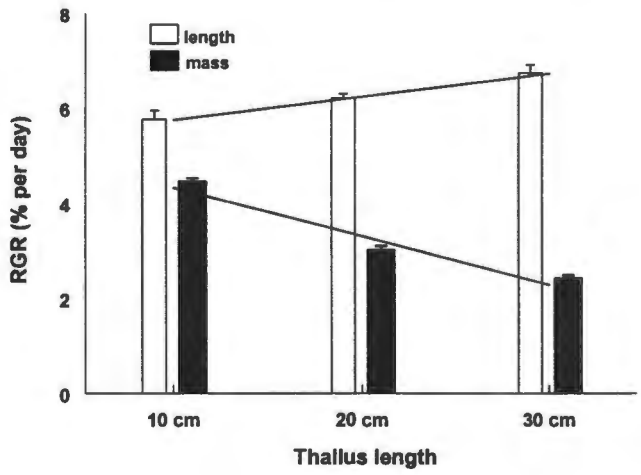

Figure 3. The effect of initial thallus length on RGR expressed as % length and wet mass per day. Vertical bars indicate treatment means and whiskers are standard errors ($n = 15$); sloping lines are linear regressions showing growth rate as a function of initial seeding length.

2.3.4 Growth comparison between thallus base and tip

Table 2 gives the growth rates for 10 mm *Gracilaria* thalli cut from either the tip or base of a thallus. Relative growth rates of the alga when expressed as percent mass and length per day were significantly higher for the apical 10 mm of the thallus compared to 10 mm fragments cut from the base of the thallus. The basal areas however, produced 42 % more lateral branches per 10 mm than did the apical region and new branch development was evident from the cut surface of proximal end of the basal fragments. Neither basal nor apical fragments had any developed or developing branches at the start of the experiment, thus this difference is not the result of the initial status of the seaweeds

Table 2. Relative growth rate and number of lateral branches per 10 mm for tips and bases of *Gracilaria gracilis* thalli. Probability values indicate the significance of the Student's t-test between tips and bases.

	RGR (% length per day)	RGR (% mass per day)	Number of lateral branches / cm
Tip	20.4 ± 1.4	41.8 ± 2.7	3.8 ± 0.5
Base	5.6 ± 1.9	25.0 ± 6.1	6.6 ± 0.9
p	0.035	0.000	0.021
n	5	5	5

2.4 Discussion

It is evident from the results that an understanding of the mode of growth in *Gracilaria* can be used to significantly enhance production from suspended rafts. Seeding with newly collected *Gracilaria* results in a higher relative growth rate than seaweed maintained on the netlons for three successive months. Similarly, Buschmann *et al.* (1995) found the productivity of *Gracilaria* cultivated on the sea-bottom in southern Chile to decrease over a period of two to three years. Two hypotheses were provided to explain this decrease. Firstly, repeated harvesting results in the removal of apical meristems leaving older, slower growing tissue behind, and secondly, harvesting results in the loss of stocking algae. For this study, the decrease in productivity can be explained by the difference in quality of seed stock used. Although seaweed harvested from netlons and natural benthic populations were both healthy, the harvested material was generally broken due to handling and fragmentation and had shorter fronds as a result. The effect of thallus length on growth is evident such that longer fronds yield more biomass per tuft than shorter thalli of the same mass. Despite a decreased growth rate (as length per day) for shorter thalli, initial thallus length did not affect the net length produced by the seaweed. This suggests that in order for longer fronds to attain a larger biomass they have to undergo a change in growth habit

in order to accommodate the extra biomass (i.e. in longer thalli the relative contribution of lateral branches to biomass production increased). This change in growth habit is most likely to be in the form of increased number of branches or more rapid growth from existing lateral branches providing greater potential for biomass production.

Reseeding netlons with harvested material is also associated with morphological and anatomical changes in the thallus, namely increased branching frequency and the development of a thick, twisted main axis with a schizogenic cavity. Meneses (1996) also found natural and artificial populations of *Gracilaria chilensis* Bird, McLachlan et Oliveira to show distinct morphological and anatomical characteristics. Examples given included differences in secondary branch lengths, differing angles of branching with respect to the main axis and variation in cortical and medullary cell size but no explanation for these effects was given. Gutierrez and Fernández (1992) found that water motion accounted for differences in thallus morphology observed in *Chondrus crispus* Stackhouse, and suggested polymorphism to be a mechanical adaptation to different hydrodynamic conditions. In the current study, the higher branching frequency observed is probably the result of cortical damage incurred during seeding (M Steentoft, pers. comm.). Alternatively, an 'apical dominance effect' due to frond breakage during successive harvests could result in axes without apical meristems (see discussion later) followed by a proliferation in lateral branch development. It is unclear as to what the ecological significance of medullary cell disintegration and the formation of a schizogenic cavity in the main axis may be. Flotation with the aid of gas-filled organs is often found in large brown seaweeds (kelps and fucoids) and has traditionally been associated with an adaptation to living in a low-light environment as it allows the fronds to float to the surface (Russell, 1978). However, it is unlikely that flotation resulting from trapping oxygen inside the axis is a response to the low light intensities experienced on the netlon since this phenomenon does not occur in natural populations which are exposed to even lower irradiances than that found on the raft. The development of a hollow main axis in *G. gracilis* has also been noted in intensive unialgal laboratory cultures where the optimal growth conditions result in relative growth rates as high as 48 % day per day (AJ Smit, unpublished data). According to M Steentoft (pers. comm.), hollow thalli have only been observed once before and are considered senescent. They are most likely to be the result of rapid growth never experienced in the wild.

Seeding *Gracilaria gracilis* onto netlons results in higher growth rates than are normally found in benthic populations suggesting that morphological differentiation could be the consequence of enhanced growth under artificial conditions. The morphology of seaweeds has also been shown to be a reflection of the degree of exposure to water movement the plants experience. For example, in *Fucus vesiculosus* L. the number of dichotomies per unit fresh mass increases with exposure while the opposite is true for the number of air-bladders (Russell, 1978). To test a similar hypothesis, the morphology and anatomy of *G. gracilis* needs to be examined when grown under a gradient of environmental conditions promoting different growth rates or degrees of exposure. It would be interesting to see whether the morphology of cultivated seaweeds would revert back to a more normal condition if they were transplanted into the original environment.

In their study on *G. chilensis*, Santelices and Varela (1995) suggested that intercalary growth is more important than apical growth in contributing to overall elongation since thallus length increment was found to be positively related to thallus length up to 20 cm, while relative growth rate was inversely related to length. This is contrary to studies on *Gracilaria confervoides* (L.) Grev. (= *G. gracilis*) and *Gracilaria debilis* (Forsskål) Børgesen in which it was suggested that elongation due to growth of the intercalary tissue does not occur (Isaac, 1956; Goldstein, 1973). According to M. Steentoft (pers. comm.), *G. gracilis* growth occurs throughout the thallus and not particularly near the apex. Data for *G. gracilis* obtained in this study seems to agree with the observation that growth (as thallus elongation) is significant over the entire thallus, however, it was found that the apex contributes more to overall elongation than does the proximal part of the thallus. During cultivation, additional increase in mass is attained through lateral branch proliferation of the basal parts of the thalli. Although increments in length of fragments with and without apical meristems were not found to be significantly different, such a difference might be obtained with greater replication. This argument is strengthened by the greater growth rate measured in apical fragments or fragments with an intact apex, compared to that of basal fragments or fragments with the apex removed. Furthermore, net length increment is not affected by the initial thallus length suggesting that most of the new length produced originates in the apical regions. In a situation where intercalary growth is more important to overall elongation (such as in the case of *G. chilensis*) one would expect to find a direct linear correlation between initial thallus length and length increment.

Gracilaria gracilis shows what appears to be an apical dominance effect as there is an increase in the degree of branching in fragments from which the apices have been excised. Apical dominance has been shown to exist in brown algae with well-developed apical cells or meristems, such as *Sargassum muticum* (Yendo) Fensholt (Chamberlain *et al.*, 1978), *Fucus vesiculosus* and *Ascophyllum nodosum* (L.) Le Jol. (Moss, 1965; Moss,1970). In higher plants, apical dominance has been associated with the action of auxin although evidence for this is less widely accepted today (Hillman, 1984). There is evidence for and against the role of plant growth substances in macroalgae (see reviews by Bradley, 1991 and Evans and Trewavas, 1991 and references therein) but there is still no consensus on their importance in algal growth and development. Branching also appears to be a function of distance from the tip of the thallus as basal fragments had a higher branching frequency than fragments from the apical region. This may be due to the control of the apex on branch development (M Steentoft, pers. comm.).

The present study clearly shows thallus length and branching frequency to be important factors in the growth of *G. gracilis*. This has important implications for cultivation since it is likely to be linked to morphological differentiation, particularly the high branching frequency of seaweed cultivated for an extended period of time. In Lüderitz Lagoon, Namibia, seaweed farmers now routinely use freshly collected seaweed from natural populations as seed stock since they experienced reduced production when harvested material which had undergone differentiation during cultivation was used (J Fliedl, pers. comm.). There is also evidence that the morphological differentiation, in particular thallus thickness, is accompanied by physiological change in nutrient uptake kinetics and photosynthesis of *G. gracilis* (Chapter 5) which could possibly contribute to reduced growth and production. Similar responses have been shown for several other macroalgae of differing thallus thickness such as *Ulva lactuca* (L.), *Chondrus crispus* Stackhouse and *Petalonia fascia* (O. F. Muller) Kuntze (Markager and Sand-Jensen, 1996).

3 *Fish Factory Pollution in Saldanha Bay: Assessment of Sedimentary Nitrogen Enrichment Using a Natural Abundance Stable Isotope Tracer Approach*

3.1 Introduction

Saldanha Bay is situated adjacent to the productive Benguela upwelling system and has been under human pressure since the early 1900's (Jackson and McGibbon, 1991). Because of the rich fishing waters supported by the Benguela current and the protected nature of Saldanha Bay, a fishing harbour, fish factories and a whaling station were developed at the beginning of this century (Burman and Levine, 1974). The most significant alteration of Saldanha Bay occurred between 1974 and 1976 with the development of the iron ore jetty and the installation of the causeway now connecting Marcus Island to Hoedjiespunt (Monteiro *et al.*, 1990; Chapter 1). Two other activities, tourism and mariculture, have also become important over the past decade (Jackson and McGibbon, 1991), and most of these activities perturb the environment in some way or another. The largest perturbation results from the combined effects of altered water circulation and the disposal of organic waste released by fish processing factories into Small Bay (see Section 3.2.1, Figure 1) which sometimes leads to eutrophication. One consequence of eutrophication is the development of blooms of unwanted opportunistic macroalgae such as *Ulva* sp. (Anderson *et al.*, 1996b; Chapter 1), and in severe cases dystrophic events due to the decomposition of algal biomass after blooms (Viaroli *et al.*, 1993). The distribution of nitrogen globally has been significantly modified directly and indirectly by human activities to the extent that more nitrogen is fixed annually by anthropogenic processes than by natural processes (Vitousek, 1994). Although the modification of the nitrogen cycle is seen both in terrestrial and marine systems, the results tend to be more profound in coastal marine systems (Nixon *et al.*, 1986). Eutrophication has been described as the oldest problem of water quality caused by human activities in lakes and coastal ecosystems (Vollenweider *et al.*, 1992) and has today become a problem of global proportions (Ackefors and Enell, 1994; Menesguen and Piriou, 1995; Peckol and Rivers, 1995; Tremp and Kohler, 1995; Castel *et al.*, 1996; Conti, 1996; Jayasekera and Rossbach, 1996).

Previous studies in Saldanha Bay on organic pollution and eutrophication dealt mainly with the effects of fish factory waste on meio- and macrobenthic communities (Christie and Moldan, 1977b; CSIR, 1991; Jackson and McGibbon, 1991), and seaweed communities (Anderson *et al.*, 1996b). Using ecological data (biomass, species composition and community structure) and *K*-dominance plots, Jackson and McGibbon (1991) found that excess stress on macrobenthic fauna is limited to within 100 m of the outfall. Similarly, Christie and Moldan (1977b) found that macrofaunal species richness and diversity increase with increasing distance from the pollution source. While ecological data is useful for studying the direct effect of pollution on benthic communities and species diversity (Pearson and Rosenberg, 1978), other consequences remain undetected, or unexplained. Organic pollution is also responsible for changes in a system's nutrient budget and biogeochemical cycles which can result in the sudden dominance of otherwise uncommon seaweed species. An example is the development of the *Ulva lactuca* L. bloom in Saldanha Bay (Anderson *et al.*, 1996b) in the summer of 1993/1994 which was brought about by the entrapment of nutrient rich effluent water in the north-western corner of Small Bay due to certain physical hydrological conditions prevailing at the time (refer to Chapter 1 and references cited therein). Even though such ecological studies pinpoint stress or disturbance normally associated with pollution, they do not provide any insight into other 'hidden' changes in the environment which might lead to undesirable events such as the *Ulva* problem. Although the above situation is not the norm, the effects of organic pollution in Saldanha Bay are continuous and widely distributed and may be more subtle than changes in the seaweed or community structure of the bay.

A powerful technique that has become popular during the last decade is the use of isotope ratio mass spectrometry (IRMS) to analyse naturally occurring light stable isotopes such as D/H, $^{18}O/^{16}O$, $^{13}C/^{12}C$ and $^{34}S/^{32}S$. These elements are present in living organisms and the environment to different degrees and the usefulness of isotope ratios stems from predictable physical and biochemical-based discrimination between living and non-living materials leading to changes in the ratio between the heavy and light isotopes (Wada and Hattori, 1978; Wada, 1980; Goericke *et al.*, 1994). For discussions of natural abundance stable isotope techniques see Bremner (1965), Handley and Raven (1992), Knowles and Blackburn (1992), Mulvaney (1992) and Lajtha and Michener (1994).

Studying natural variations in stable isotope ratios can provide valuable insight into the functioning of various physical, chemical, biogeochemical and biological systems. In recent years, the ratios of naturally occurring stable isotopes of nitrogen, ^{15}N and ^{14}N, have been applied as tracers to indicate the sources and sinks of different forms of nitrogen in ecosystems. Stable isotopes have been used successfully by various researchers to track the distribution of pollutants such as sewage in marine systems (Sweeney *et al.*, 1980; Sweeney and Kaplan, 1980a; Rau *et al.*, 1981; Farran *et al.*, 1987; Spies *et al.*, 1989; van Dover *et al.*, 1992). Furthermore, stable isotope studies can provide useful insights into the flow of nutrients or energy in and through ecosystems. These studies are successful because the isotopic signature of an organism, or a component of the environment, represents an integration of isotope ratios of different sources of nutrients (in the case of primary producers) or energy (in the case of consumers). For this reason, stable isotopes are also used in the analysis of food webs, showing the nutrient flow between primary producers and subsequent levels of the food web (Goering *et al.*, 1990; Montoya *et al.*, 1991; Michener and Schell, 1994; Keough *et al.*, 1996; Kikuchi and Wada, 1996), and the incorporation of pollutants in animal or plant tissue (van Dover *et al.*, 1992; Monteiro *et al.*, in press).

The only natural abundance stable isotope study in Saldanha Bay to date was conducted by Monteiro *et al.* (in press) who used $^{15}N/^{14}N$ ratios to show that fish-derived nitrogen in the form of DIN in effluent water can be positively linked to the development of an *Ulva lactuca* bloom in Small Bay. The premise of this study is that *Ulva* has access to two sources of DIN: 1) natural, upwelled nitrogen, and 2) effluent DIN. The study relied only on *Ulva* samples taken from two populations, one in Small Bay near the outfall and the other in Langebaan Lagoon, and therefore does not show to what extent the effluent is dispersed in Saldanha Bay. Neither does it show the extent to which this nitrogen (in the form of organic material) is incorporated into sediments. Here, a natural abundance stable isotope tracer approach was used to determine the distribution of the fish factory effluent plume in Small Bay. This chapter deals specifically with the fate of particulate fish waste and its distribution in the sediments of Small Bay. Chapter 4 uses the same approach to evaluate the effect of effluent DIN on the growth of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham.

3.2 Materials and methods

3.2.1 Study area

The physical and chemical features of Saldanha Bay are described in detail in Chapter 1. Briefly, the system can be characterised as being strongly stratified in summer and well-mixed in winter with these conditions being brought about by barotropic forcing mechanisms. In the stratified system, the presence of a nutricline accompanies the development of the thermocline, while a mixed hydrography is homogenous in terms of temperature and nutrients. This project looked mainly at the effect of pollutants originating from the Southern Sea Fishing fish-processing factory (Premier Fishing (Pty.) Ltd. situated in Small Bay [33°00'45"S, 17°57'30"E]; Figure 1). Very little freshwater runoff occurs into the system due to the semi-arid nature of the west coast, although a small amount of treated sewage is released into the north-east corner of Small Bay. Sewage disposal has been discounted as a significant source of nitrogen to Saldanha Bay (Monteiro *et al.*, in press).

This study is mainly concerned with effluents released by the Southern Sea factory. The plant consists mainly of a fishmeal plant, a cannery operation and a crayfish processing facility. Raw material for processing is pelagic fish (anchovy and other) caught by the factory's fleet. Secondary products produced from the plant include fish oil and organic fertilizer (Seagro concentrate) (Steenveld *et al.*, 1993). Effluent from the different processing operations, comprised of raw fish material not recovered as product, are combined and disposed of via the discharge pipe into Small Bay (Site P, Figure 1). During the period January 1993 to August 1993, 37 180 tons of pelagic fish together with 4 858 tons of white fish off-cuts were processed to yield 24.1 % fishmeal and raw fish, and 2.5 % oil. The remaining 73.4 % (which equates to 30 856 tons liquid fish waste) was disposed of into Saldanha Bay via two effluent release pipes (Steenveld *et al.*, 1993). The first outlet releases relatively 'clean' effluent comprised of cooling water and condensate from the fishmeal plant, while the other outfall is 'dirty' and contains wastes from the processing and canning facility. Solid waste such as crayfish shells are disposed of on land.

Both Sea Harvest and Southern Sea Fishing effluent plumes are clearly visible under certain conditions, such as during periods of prevailing south-westerly winds. These

plumes can be seen in aerial photographs such as the one published by Weeks *et al.*, (1991) as an oily slick forming a smooth area on the water surface, while high concentrations of suspended solids add turbidity and colour to the plume. Large particles such as fish scales settle out close to the outfall but smaller particles are dispersed over most of Small Bay. The saponified fats form a grey suspension in the water that often fouls *Gracilaria* lines on the rafts (pers. obs.).

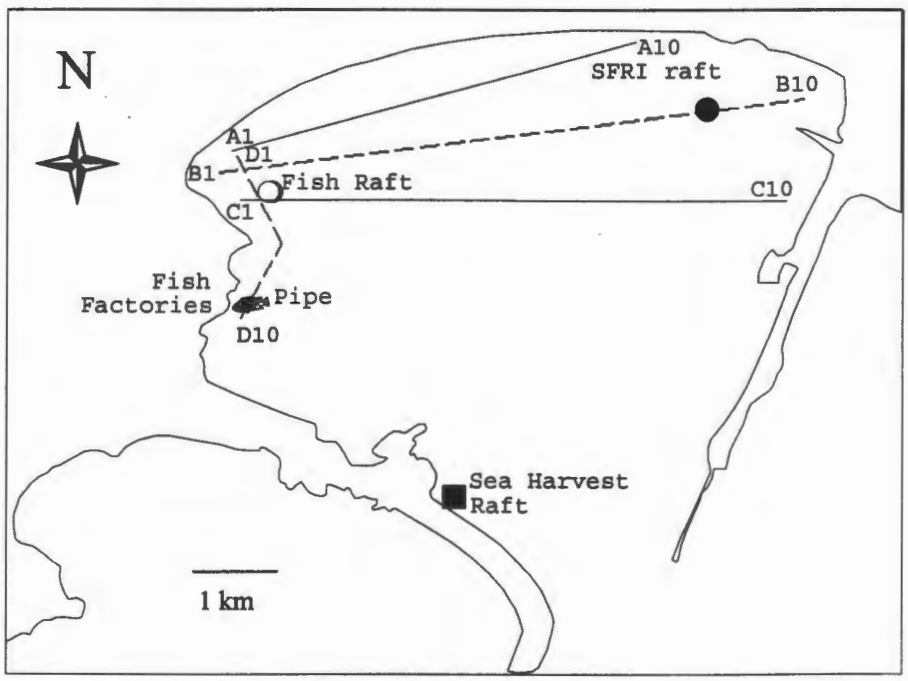

Figure 1. Map showing the location of the four transects (A, B, C and D) along which sediment and/or water samples were taken. The position of the fish factories, effluent outlet (pipe) and *Gracilaria* rafts is also indicated. The location of Schapen Island is shown in Figure 1, Section 1.2. Names of sites are explained in Table 1.

Table 1. Details of the sampling sites in Small Bay or Langebaan Lagoon. Sites B1 to B10 and D1 to D10 are stations along Transects B and D respectively. Refer to Figure 1 for locations of individual sites.

Site	Site name	Depth (m)
B1		5.0
B2		5.9
B3		6.2
B4		6.4
B5		6.1
B6		6.0
B7		5.2
B8		5.3
B9		5.3
B10		5.4
D1		4.1
D2		5.2
D3		6.0
D4		6.4
D5		6.7
D6		6.5
D8		7.7
D9		7.5
D10		7.8
DE	Deep Site	7.0
DR	Dial Rock	3.0
DRD	Dial Rock, deep	6.0
FR	Fish raft	5.0
LA	Langebaan Lagoon, control	2.0
PE	Pipe, east	4.5
PN	Pipe, north	4.5
PS	Pipe, south	4.5
PW	Pipe, west	4.5
SR	SFRI raft	5.5
WR	Wreck Site	3.0

Sampling was conducted in Small Bay along two transects, B and D, as well as several other stations in the area (Figure 1). Sites names and their abbreviations are given in Table 1, together with water depth at the site. An area about 200 m south of Schapen Island was chosen as control site, as it was shown previously to be free from any form of pollution (Monteiro *et al.*, in press).

3.2.2 Chemical and physical analysis of effluent

Available effluent quality data and pumping volumes were summarised from Sea Harvest and Southern Sea Fishing permit returns obtained from the Department of Water Affairs and Forestry (DWAF). Details of sample collection methods and analytical procedures are contained in the reports obtained from DWAF and are not cited here. The permit returns report volume of effluent released, total Kjeldahl-N, free and saline ammonium, available

(ortho-) phosphate, total suspended solids, volatile suspended solids, and fats, oils and greases. Faecal coliform and *Escherichia coli* mean probable numbers are also reported, but are not summarised here.

3.2.3 Sampling and isotope analysis

The sampling program was designed to cover the northern part of Small Bay in waters of no more than seven metres in depth and took into account both sediment and seaweed. The part of the study involving *Gracilaria gracilis* is discussed in Chapter 4. The rationale was that since effluent discharge is not continuous and is only present for part of the year, sedimentary $\delta^{15}N$ would provide information on the time-integrated effect of pollution by recording the biogeochemical processes in the water column (Holmes *et al.*, 1996). The sampling strategy was planned to test the hypothesis that the effects of effluent disposal decreased with increasing distance from the outfall. It was expected that sediment samples collected closer to the pipe would have $\delta^{15}N$ values approaching that of fish tissue.

Sampling was conducted mainly in summer (November 1996 to February 1997), the time of heaviest effluent discharge. However, after analysis of DWAF data, it became evident that fish processing takes place throughout the year. The sediment was intensively sampled during February along Transects B and D (see Figure 1) which runs either across (south to north) or away (west to east) from the effluent discharge pipe. In addition, four samples were removed about 10 m to the north, west, south and east of the effluent release pipe to obtain samples of almost undiluted effluent. During this sampling period, and on several other occasions between December 1996 and May 1997, additional sediment samples were taken from several other sites shown in Figure 1, as well as the control site (Schapen Island).

The top 10 cm of the sediment surface was sampled by a SCUBA diver using a 5 cm diameter PVC corer. One sediment sample was collected per site because of the cost involved in analysing sediments with low nitrogen contents. Samples were frozen immediately after collection and stored frozen until processing at the University of Cape Town. Samples were thawed and only the top 5 cm used in the analyses. The samples were decalcified overnight in 0.1 M HCl, rinsed in deionised water and freeze-dried. The

Plate 1. The Finnigan MAT 252 isotope ratio mass spectrometer (a) at the UCT / FRD / Goldfields Light Stable Isotope Facility showing the Faraday-collectors (b).

dried fraction containing the organic material was ground to pass through a 10 μm sieve and stored in polyethylene containers under a nitrogen atmosphere. The percentage organic material was determined in a sub-sample of the whole fraction (before decalcification) by combustion at 500 °C.

Isotopic analysis was conducted at the UCT / FRD / Goldfields Light Stable Isotope Facility. Up to 30 mg of sediment was weighed and sealed in tin capsules which were dropped from the elemental analyser (Carlo-Erba NA 1500NC) tray into the combustion column kept at 1020 °C. After flash combustion at about 1800 °C in O_2 the resulting gases were passed through the reduction column where species of nitrous oxides gave up oxygen to form N_2. The resulting CO_2, N_2 and H_20 gas mixture in a He carrier was cryogenically purified and measured with a Finnigan MAT 252 isotope ratio mass spectrometer (Plate 1). $^{15}N/^{14}N$ ratios are expressed in the usual $\delta^{15}N$ (‰, or per mil) notation, which is calculated as:

$$\delta^{15}N = \left[\frac{^{15}N/^{14}N_{smp}}{^{15}N/^{14}N_{std}} - 1 \right] \cdot 10^3 \quad \textbf{[Eq. 1]}$$

where $^{15}N/^{14}N_{smp}$ is the isotope ratio of the sample and $^{15}N/^{14}N_{std}$ the isotope ratio of the standard. $\delta^{15}N$ is reported relative to atmospheric nitrogen (Mariotti, 1984). The laboratory reference gas was high purity nitrogen (99.995 %) calibrated against atmospheric nitrogen. Analytical precision was ± 0.3 ‰ (1 SD).

3.2.4 Numerical and statistical analysis

Sediment data ($\delta^{15}N$, % N and % organic matter) were used to determine the extent of the effluent plume in Small Bay. This was done by employing the Cluster Analysis and Multidimensional Scaling (MDS) modules of Statistica for Windows release 5.1 (Statsoft, Inc., 1996). In order for the variables to be of comparable magnitude, raw data were first standardised so that each variable had a mean of 0 and standard deviation of 1. No data transformations were applied as this is mainly applicable to ecological abundance data with large discrepancies between the number of dominant and rare species (Clarke and Warwick, 1994). After standardization, cluster analysis was used to obtain a module also calculated the among-site distance (dissimilarity) matrix used in subsequent MDS ordinations. High goodness-of-fit ordinations (stress < 0.05) were obtained for two or

three dimensions by using a non-metric MDS algorithm that attempts to minimise stress through an iterative procedure (Field *et al.*, 1982; Statsoft, Inc., 1996). Statistical procedures used for data analyses include one-way ANOVA's and the non-parametric Wilcoxon matched pairs test, also performed using Statistica for Windows release 5.1.

3.3 Results

3.3.1 Factory effluents

Chemical analyses of effluent released into Small Bay by Southern Sea Fishing and Sea Harvest are presented in Figure 2a-j. Effluent is released throughout the year, but total-N, ammonium-N, available phosphate-P, fats, oils and greases (FOG's) and particulate matter concentrations are variable with highest amounts recorded during winter. Apart from the high concentrations of ammonium-N measured in the Southern Sea factory effluent relative to that in the Sea Harvest effluent, there does not appear to be a consistent difference between the concentrations of other effluent constituents between the two factories. Table 2 provides a summary of the chemical and physical properties of the effluent released by Sea Harvest and Southern Sea Fishing into Small Bay during 1996.

Table 2. Chemical parameters of effluent released into Saldanha Bay during 1996. Data for the Southern Sea factory are for the period 26/3/96 to 9/12/96 ($^{\#}$1/1/96 to 1/1/97 for effluent volume release) but exact dates for the Sea Harvest data are not given. Actual annual totals are more than indicated values since only eight months' data were available from Southern Sea Fishing (from permit returns, DWAF).

	Total-N [t]	Ammonium [t yr^{-1}]	Ortho-phosphate [t yr^{-1}]	Total suspended solids [t yr^{-1}]	Volatile suspended solids [t yr^{-1}]	Fats, oils and grease [t yr^{-1}]	Effluent volume [m^3 yr^{-1}]
Southern Sea Fishing	509	163	44	861	662	650	$^{\#}$~$1.9 \cdot 10^6$
Sea Harvest	140	53	9	610	493	132	~$1.1 \cdot 10^6$
Annual Total	>649	>216	>53	>1471	>1155	>782	>$3.0 \cdot 10^6$

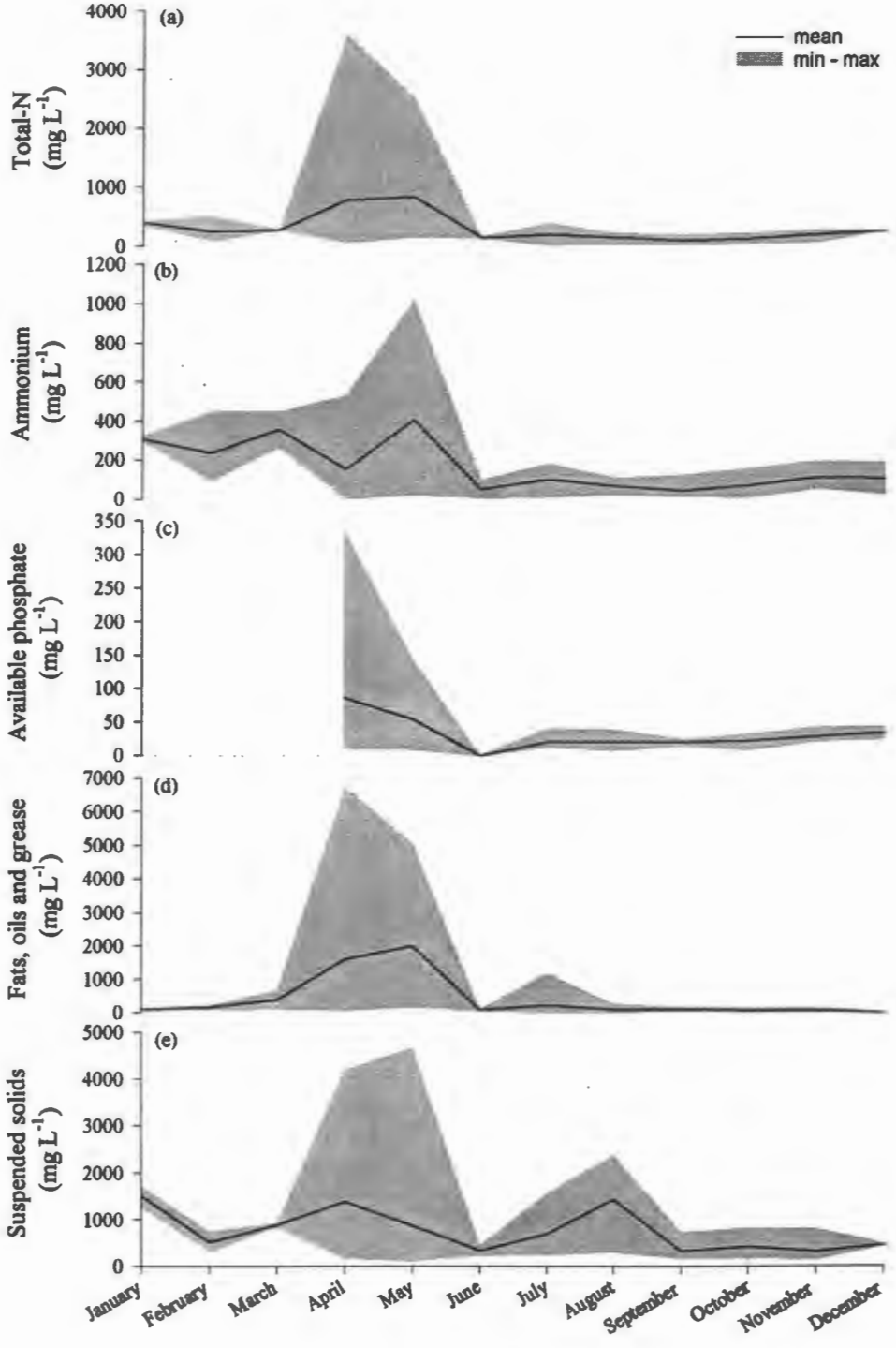

Figure 2. Monthly total-N (a), ammonium (b), available phosphate (c), fats, oil and grease (d) and suspended solids (e) concentration in the effluent released by Southern Sea Fishing into Small Bay between August 1995 to December 1996. Phosphate data before March 1997 are not available. Mean, minimum and maximum given. Data: DWAF permit returns.

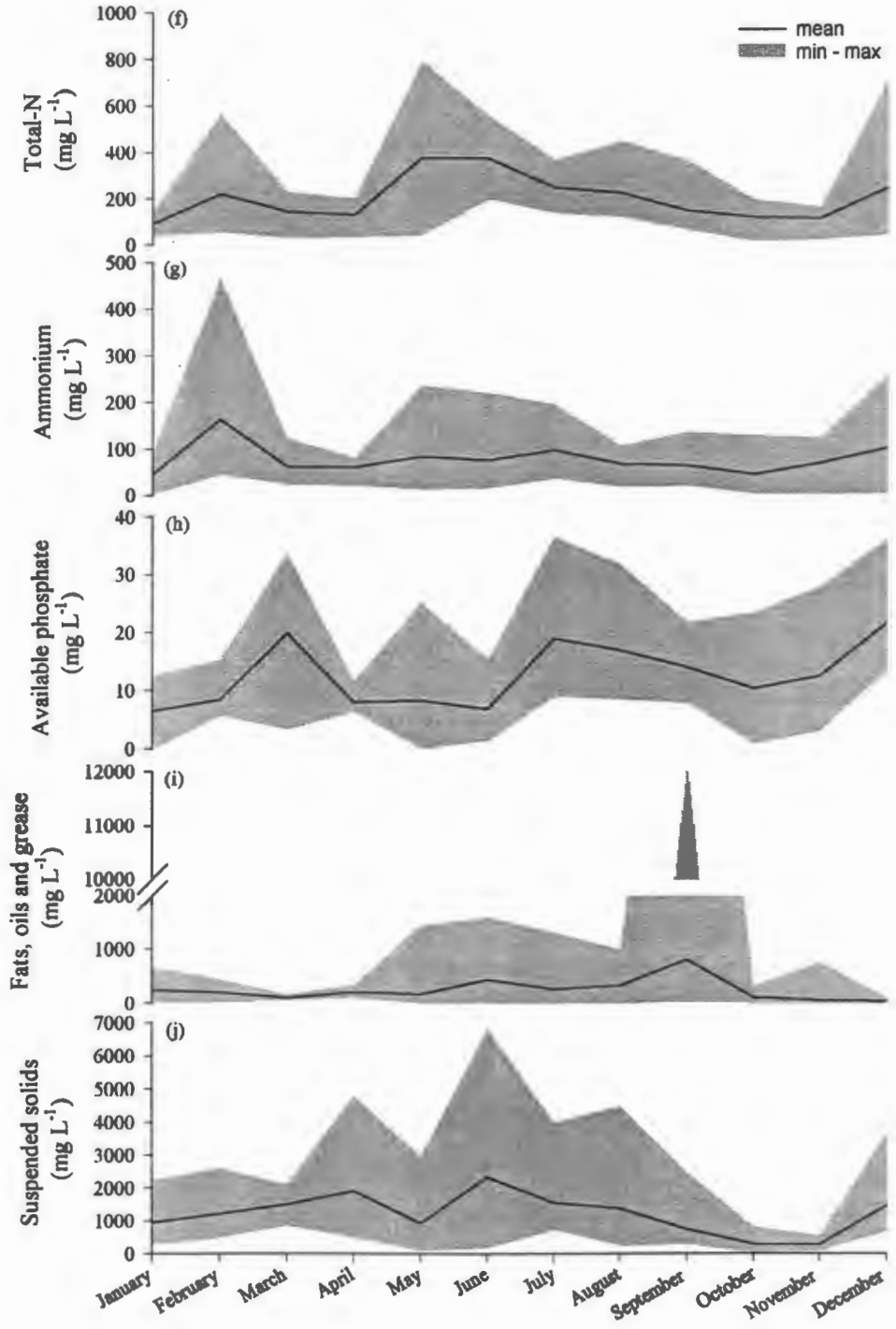

Figure 2 (cont.). Monthly total-N (f), ammonium (g), available phosphate (h), fats, oil and grease (i) and suspended solid (j) concentrations in effluent water from the Sea Harvest fish processing plant released into Small Bay during 1996. Mean, minimum and maximum given. Data: DWAF permit returns.

Figure 3 shows the $^{15}N/^{14}N$ frequency distribution of all sediment samples collected in Small Bay and Langebaan Lagoon. The sample mode is between 7 and 8 ‰ indicating that most samples contain traces of effluent-derived nitrogen. $^{15}N/^{14}N$ ratios of sedimentary nitrogen, total-N (from both marine and effluent origin) and organic matter content are summarised in Table 3. Using a variation of the standard two-source mixing model, the percentage of total sedimentary-N originating from the fish factory outfall and the actual amount of effluent-N and marine-N in the sediment can be calculated (Owens, 1987). The mixing model is based on the distribution of $\delta^{15}N$ in the sediments, and is given by:

$$\delta^{15}N_{obs} = \delta^{15}N_{eff} f \cdot f + \delta^{15}N_{det} \cdot (1-f) \quad \textbf{[Eq. 2]}$$

where $\delta^{15}N_{obs}$ is the $\delta^{15}N$ observed in the sample, $\delta^{15}N_{eff}$ and $\delta^{15}N_{det}$ are the δ15N values for the sedimentary effluent-N and sedimentary marine detrital-N respectively and f is the relative proportion of $\delta^{15}N$ in the two sources. Values of 9.2 and 4.6 ‰ were used as end-members in the equation, with the highest and lowest values going to the effluent and detrital N sources respectively (see Section 3.4). Results from calculations involving the mixing model are presented in Table 3. This model assumes that the organic matter used as a tracer is conservative, i.e. minimal changes due to diagenesis occur after deposition, and that observed isotope ratios are the result of physical mixing of the two sources in the system (Sweeney and Kaplan, 1980b; Cifuentes *et al.*, 1988).

Sedimentary $\delta^{15}N$ values within 10 m of the effluent-release pipe range from 6.3 - 9.2 ‰, with the highest values recorded to the north and west of the outfall. Nitrogen in the sediment near the outfall pipe, especially at PN and PW, is clearly of fish origin since values are similar to $\delta^{15}N$ measured for anchovy and roundherring bone and gut contents sampled in the southern Benguela ecosystem (see Discussion, section 3.4.2). These sediments also contained large amounts of fish scales. Organic matter and sedimentary total-N lie in the range 3.7 - 16.4 % and 0.1 - 1.1 % for organic matter and total-N respectively, with the highest values occurring in the most enriched sediments. Jackson and McGibbon (1991) recorded similar organic matter contents for sediments close to the effluent pipe in 1989 and 1990. The control site at Schapen Island had $\delta^{15}N$ values ranging from 3.6 - 5.3 ‰ which are similar to sedimentary $^{15}N/^{14}N$ ratios recorded in the

southern Angola Basin (4.6 ‰ at 100 m; Holmes *et al.*, 1996). Total-N and organic matter range from 0.03 - 0.04 % and 2.5 - 3.6 % respectively in Langebaan Lagoon sediments. Since this site is situated approximately 7.6 km from the fish factories and water from Saldanha Bay does not move into Langebaan Lagoon to a significant degree (Monteiro *et al.*, in press), nor is it perturbed by anthropogenic activities, the $\delta^{15}N$ values of the organic matter contained in sediments of this region are produced in the surface waters when phytoplankton takes up 'natural' ammonium or nitrate. The remainder of the sites in Small Bay sampled during February had $\delta^{15}N$ values ranging from 6.0 - 8.1 ‰ with a mean of 7.2 ‰, but did not show a gradient of greater depletion ^{15}N with increasing distance from the outfall. Surprisingly, the sedimentary nitrogen from sites D4 to D10 has $\delta^{15}N$ values of between 6.3 - 6.9 ‰ that is isotopically light compared to sediments adjacent to the effluent pipe. $\delta^{15}N$ values measured at these sites increased with increasing distance up to about 790 m away from the pipe.

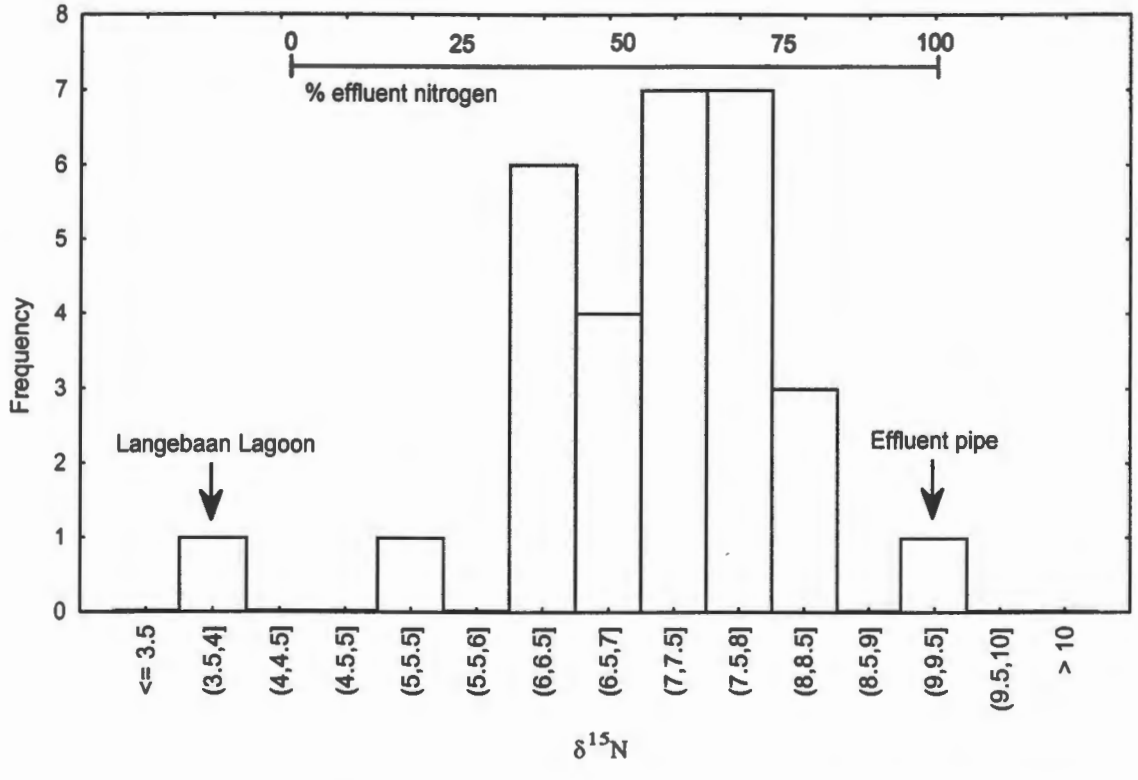

Figure 3. Frequency distribution of Small Bay and Langebaan organic sedimentary $\delta^{15}N$ values. Marine and effluent derived nitrogen are indicated by the arrows while the scale above the histogram indicates the percentage of effluent derived nitrogen represented by the $\delta^{15}N$ values ($\delta^{15}N$ = ‰, vs. atmospheric N_2).

Table 3. Fraction of the total sedimentary-N comprising fish-factory derived N (f_{eff}), total sedimentary-N (sedimentary effluent-N + sedimentary marine-N), absolute amount of effluent- or marine-derived N in sediments, $\delta^{15}N$, organic matter content of the whole (non-decalcified) sediment sample measured at each sampling site between November 1996 and February 1997.

Site	f_{eff} [%]	Sed. total-N [%]	Sed. effluent-N [%]	Sed. marine-N [%]	$\delta^{15}N$ [‰]	Organic matter [%]	Distance from pipe [m]	Month collected
FR	63.3	0.04	0.03	0.01	7.5	-	1040	Nov-96
LA		0.02			5.3	-	7600	Nov-96
DR	47.7	0.03	0.01	0.01	6.8	-	2080	Dec-96
DE	63.6	0.03	0.02	0.01	7.5	-	2680	Dec-96
LA		0.02			4.4	-	7600	Dec-96
PE	63.2	0.12	0.08	0.04	7.5	6.79	20	Feb-97
PN	100.0	1.05	1.05	0.00	9.2	16.40	20	Feb-97
PS	37.9	0.07	0.03	0.04	6.3	3.74	20	Feb-97
PW	83.8	0.48	0.40	0.08	8.4	14.68	20	Feb-97
D9	33.5	0.06	0.02	0.04	6.1	3.95	40	Feb-97
D8	31.4	0.05	0.02	0.04	6.0	3.34	140	Feb-97
D10	38.6	0.06	0.02	0.04	6.4	3.38	140	Feb-97
D6	37.7	0.06	0.02	0.04	6.3	2.35	400	Feb-97
D5	42.1	0.04	0.02	0.02	6.5	1.81	600	Feb-97
D4	50.1	0.04	0.02	0.02	6.9	1.97	800	Feb-97
D3	73.2	0.03	0.02	0.01	7.9	1.36	920	Feb-97
D2	56.7	0.03	0.02	0.01	7.2	3.44	1040	Feb-97
B1	48.5	0.04	0.02	0.02	6.8	1.89	1160	Feb-97
B2	55.8	0.06	0.03	0.03	7.2	3.08	1160	Feb-97
B3	77.4	0.03	0.03	0.01	8.1	1.79	1240	Feb-97
D1	73.7	0.03	0.02	0.01	8.0	1.45	1360	Feb-97
B4	52.9	0.03	0.02	0.01	7.0	2.04	1400	Feb-97
B5	60.7	0.03	0.02	0.01	7.4	1.66	1640	Feb-97
DRD	51.9	0.03	0.01	0.01	7.0	1.36	1920	Feb-97
B6	64.7	0.03	0.02	0.01	7.6	1.51	1940	Feb-97
DR	63.9	0.03	0.02	0.01	7.5	-	2080	Feb-97
B7	71.7	0.03	0.02	0.01	7.9	1.64	2580	Feb-97
DE	31.1	0.03	0.01	0.02	6.0	1.36	2680	Feb-97
WR	59.0	0.03	0.02	0.01	7.3	1.91	2960	Feb-97
B8	74.3	0.03	0.02	0.01	8.0	1.88	3440	Feb-97
B9	75.5	0.03	0.02	0.01	8.1	10.29	4320	Feb-97
SR	55.8	0.03	0.02	0.02	7.1	2.01	4320	Feb-97
B10	68.0	0.03	0.02	0.01	7.7	2.12	5160	Feb-97
LAa		0.04			5.3	2.51	7600	Feb-97
LAb		0.03			3.6	3.58	7600	Feb-97

[site names: FR - Fish Raft; LA - Langebaan Lagoon control site; DR – Dial Rock; DE - Deep Site; PE - Effluent release pipe, east; PN - Effluent release pipe, north; PS - Effluent release pipe, south, PW - Effluent release pipe, west; DRD - Dial Rock, deep; WR - Wreck Site; SR - SFRI Raft; B1-B10 - sites along transect B; D1-D10 - sites along transect D]

Cluster analyses based on organic matter content, total-N and $\delta^{15}N$ indicate that sites PN and PW form a distinct grouping at a linkage distance of approximately 8, based primarily on their high organic matter and total-N content (Figure 4) (see Table 2 for an explanation of site names; Figure 1 gives the position of each site in the bay). The role of $\delta^{15}N$ in influencing the clustering of PN and PW is small compared to the other two variables as can be seen from the cluster analysis based on only $\delta^{15}N$ and a dummy variable (Figure 5). The control sites in Langebaan Lagoon (LAA and LAB), including sites DE and D5 to D10, separate out from the other sites sampled in Small Bay at a linkage distance of about 4.5 (Figure 4), and this grouping is caused mainly by their distinct $\delta^{15}N$ and organic matter contents (Figures 5 and 6 respectively). A three dimensional MDS ordination obtained from analysis of the % sedimentary total-N, $\delta^{15}N$ and % sedimentary organic matter distance matrices is given in Figure 7. The ordination plot of % total sedimentary N, $\delta^{15}N$ and % sediment organic material clearly shows the control sites and the most impacted sites to be isolated from the rest of Small Bay in three-dimensional space. The sites D5 to D10, and DE, appear more similar to the control sites in terms of % total-N, % organic matter and $\delta^{15}N$. Site B9 is an outlier and is distinct from the other sites sampled in Small Bay along Dimension 3 (Figure 7). This is brought about mainly by its very high organic matter content.

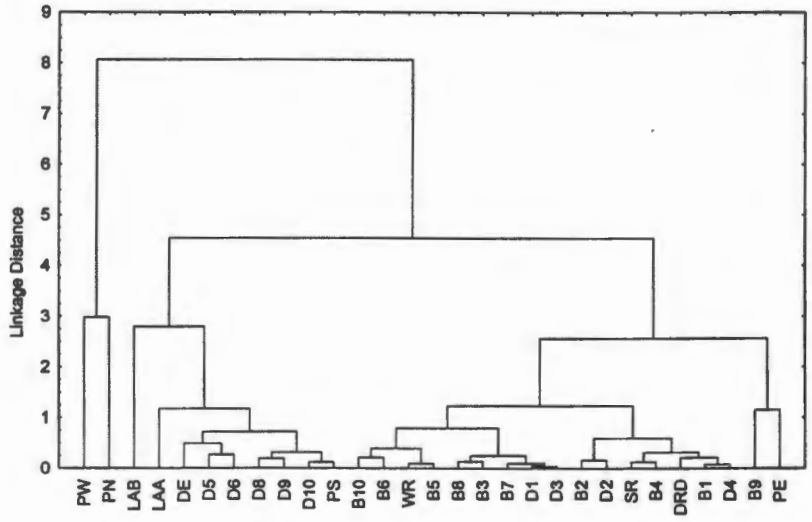

Figure 4. Multivariate cluster analysis based on % sedimentary total-N, $\delta^{15}N$ and % sedimentary organic matter showing the similarity among sites sampled during February 1997. Linkage distance is calculated from Euclidean distances and amalgamation follows the complete linkage rule.

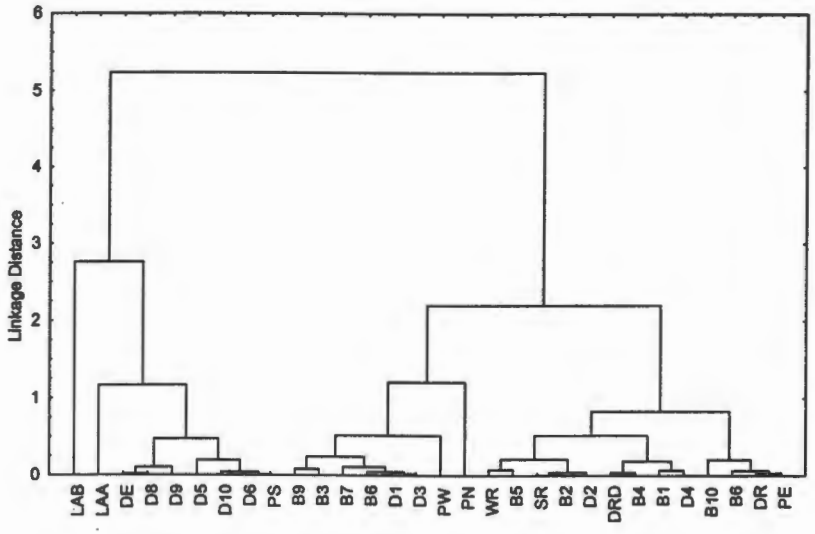

Figure 5. Cluster analysis of sites based on $\delta^{15}N$ and a dummy variable for February 1997 data. Sites with similar $\delta^{15}N$ values are clustered together. Linkage distance is calculated from Euclidean distances and amalgamation follows the complete linkage rule.

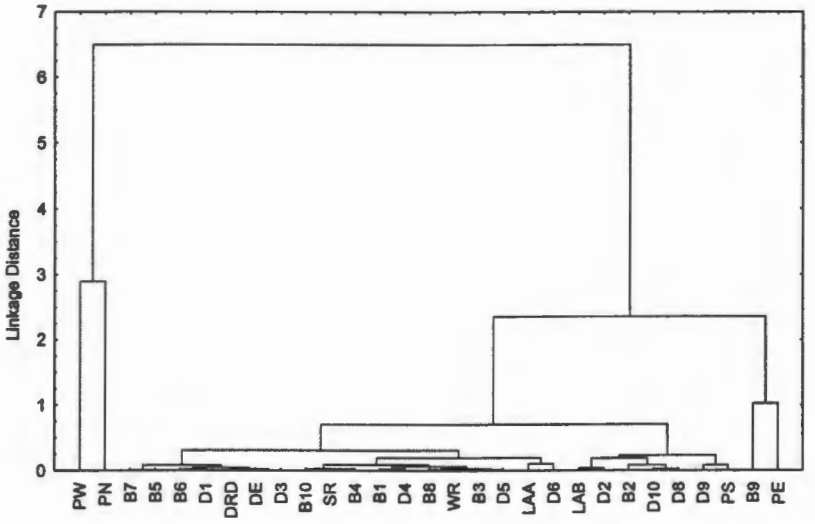

Figure 6. Cluster analysis based on % sediment organic matter and a dummy variable showing the similarity among sites sampled during February 1997. Linkage distance is calculated from Euclidean distances and amalgamation follows the complete linkage rule.

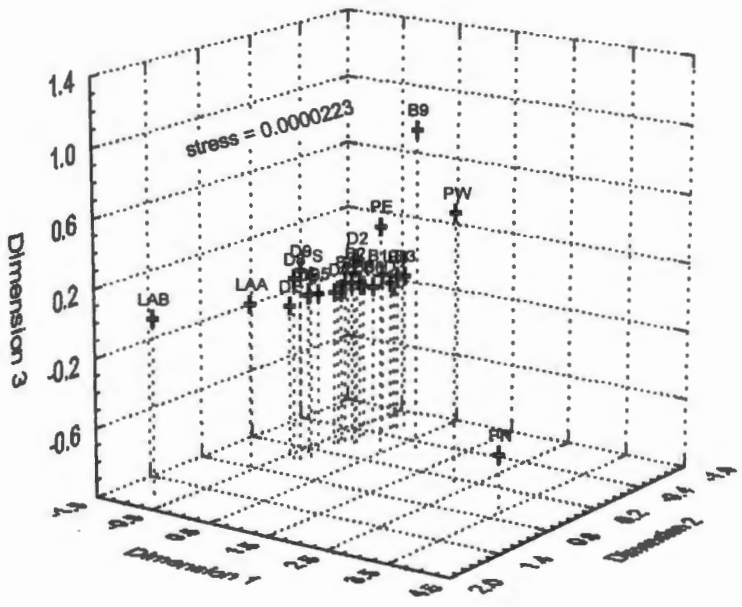

Figure 7. Three-dimensional MDS ordination showing dissimilarity between sites sampled during February 1997 in Small Bay. Ordination is based on % sedimentary N, $\delta^{15}N$ and % sediment organic matter.

3.4 Discussion

3.4.1 Organic pollution

Fish factories have been operating in Saldanha Bay since the establishment of the North Bay Canning Company and the Saldanha Bay Canning Company in 1903 and 1905 (Burman and Levine, 1974). Anthropogenic inputs of waste material into Saldanha Bay from these sources have had major impacts on the distribution, abundance and biomass of benthic macrofauna in the area surrounding the outfall (Jackson and McGibbon, 1991). For example, macrofauna populations in the vicinity of the outfall decreased in biomass and species diversity while the number of small, opportunistic species increased (Jackson and McGibbon, 1991). These changes were attributed to the increased organic loads in the sediments. Similarly, Christie and Moldan (1977b) showed that species diversity, density and biomass increased with increasing distance from the outfall. A change in community structure from one dominated by long-lived, *K*-selected species to one with mainly opportunistic species has been shown to occur in response to hypoxic conditions developing in response to high sedimentary organic loads (Rhoads *et al.*, 1978). Although fish and lobster kills due to organic pollution were reported prior to 1974 (SA Fishing

Industry, 1973; Newman and Pollock, 1973), it is now known that the impact of fish waste disposal into the bay is compounded by the presence of the ore jetty constructed in 1974 (Monteiro *et al.*, 1990). The ore jetty effectively divided Saldanha Bay in two, resulting in an increased residence time for water in Small Bay (Monteiro *et al.*, in press).

Small Bay has an estimated surface area of approximately 10 million m^2 (Anonymous, 1996). With an input of anthropogenic nitrogen into the system of at least 650 tons during 1996 (Table 2), the anthropogenic aerial nitrogen flux equates to approximately 15 mg N m^{-2} yr^{-1}. This flux is placed into perspective by comparing it to the total nitrogen loadings (all sources of nitrogen) for other coastal and estuarine systems (data presented in Table 4). Small Bay has a natural nitrogen input of about 12.3 g N m^{-2} yr^{-1} (from Brundrit, 1996) and it is likely that this value would increase further if sources of nitrogen such as anthropogenic 'new' nitrogen are considered. Primary productivity in Saldanha Bay is controlled by upwelling from the Benguela system and any nutrient input resulting from anthropogenic activities in the area therefore occurs in an ecosystem which is already highly productive.

Nitrogen loadings such as those listed in Table 4 only compare nitrogen inputs into the environment but it is the residence time of the nitrogen in the bay or lagoon that ultimately determines the trophic status (i.e. oligotrophic, mesotrophic, eutrophic) of the water body. The residence time of water, and hence nutrients, is determined by the bathymetry of the system and prevailing barotropic conditions which influence water circulation patterns and flushing rates (Waldron, 1985; Wulff *et al.*, 1990). Therefore, to understand the distribution and fate of pollutants released into the bay it is essential to understand the forcing factors and flushing rates that determine the hydrodynamics (Weeks *et al.*, 1991). As discussed in some detail in Chapter 4, eutrophication in Saldanha Bay requires the presence of an environmental 'window' which is brought about by the coincidence of anthropogenic and natural 'events' (Monteiro *et al.*, 1990).

Table 4. Comparison of annual total nitrogen flux in several coastal and estuarine systems (Small Bay data represents only upwelled nitrogen). All data except the value for Small Bay obtained from Boynton *et al.* 1995, 1996, and papers cited therein). Small Bay data calculated from Brundrit, 1996.

Location	Total-N flux [g N m^{-2} yr^{-1}]
Kaneohe Bay, Hawaii	2.2
Lower Maryland coastal bays	2.4 - 3.1
Baltic Sea, Sweden	3.0
Choptank River, Maryland	4.3
Upper Maryland coastal bays	4.1 - 6.5
Albermarle Sound, North Carolina	7.1
Apalachicola Bay, Florida	7.8
North Sea	9.4
Pamlico River, North Carolina	12.0
Small Bay (Saldanha Bay), South Africa	12.3
Patuxent River, Maryland	12.7
Mobile Bay, Alabama	17.9
Delaware Bay, Delaware	18.2
Mainstream Chesapeake Bay, Maryland	20.5
South San Francisco Bay, California	22.6
Narragansett Bay, Rhode Island	27.6
Maryland coastal bays tributaries	15.7 - 39.7
Potomac River, Maryland	29.3
Patapsco River, Maryland	49.0
Tokyo Bay, Japan	89.1

There are several possible fates of nitrogen after entering Small Bay, both as dissolved inorganic and suspended particulates:

1. suspended particulate organic nitrogen (PON) settles onto the sediment where remineralisation occurs followed by nitrification or denitrification, or is accumulated, depending on sediment chemistry;

2. suspended PON is exported from Small Bay into Big Bay, Outer Bay or the coast, depending on water exchange rates and current velocities;

3. dissolved inorganic nitrogen (DIN) is taken up by phytoplankton or existing macroalgae;

4. DIN results in the bloom of unwanted macroalgal species;

5. DIN is exported from Small Bay.

We know that the anthropogenic input of total-N into Small Bay provides potential for eutrophication (Anderson *et al.*, 1996b). The contribution of fish-derived DIN to the nitrogen budget and the associated effect on *Gracilaria* will be discussed in Chapter 4.

3.4.2 $\delta^{15}N$ values of mixing model end-members

The range in sediment $\delta^{15}N$ values representative of the end members (i.e. organic material purely from marine or fish origin) agrees very well with published data for similar systems. For example, the isotopic signature for the control site at Schapen Island is similar to values recorded for the southern Angola Basin near the Cunene River (17°30'S 11°30'E) (Table 5). Sediments in the southern Angola Basin are rich in marine derived nitrogen and contain very little organic matter from terrestrial origin despite being near a river mouth (Holmes *et al.*, 1996). This area receives water from the Benguela Coastal Current which is split off from the Benguela Current off the coast of southern Angola (Holmes *et al.*, 1996). The Benguela Current is also the source of nutrients to the west coast of South Africa (Bailey and Chapman, 1985; Bailey, 1987; Waldron and Probyn, 1992), supplying *inter alia* new nitrogen to the surface during episodic upwelling events. Since both the Saldanha Bay-Langebaan Lagoon system and the southern Angola Basin are under the influence of the same oceanic current it is very likely that the same source nitrogen is available for phytoplankton production in both areas. This is reflected in the similarity of $\delta^{15}N$ values recorded for Langebaan Lagoon and southern Angola sediments. Small differences in the range of $\delta^{15}N$ values between the two areas can be explained by the degree to which available nitrogen in the euphotic zone is utilised. The light nitrogen isotope is preferentially taken up by phytoplankton when inorganic nitrogen is abundant, but as the source nitrogen is depleted, phytoplankton become progressively more enriched as they have to consume the remaining fraction which is now rich in ^{15}N (Holmes *et al.*, 1996). Localised processes such as denitrification and the mineralisation of sedimentary organic matter can also alter local isotopic signatures. $\delta^{15}N$ measured in Langebaan Lagoon sediments are also similar to suspended POM measured in the north-west Gulf of Mexico, the Sargasso Sea and several warm core rings in the Atlantic Ocean (see Table 5).

To calculate the fraction of sedimentary nitrogen derived from effluent released into the bay, a $\delta^{15}N$ value of 4.6 ‰ was chosen to represent the isotopic signature of pure marine detrital matter. This value was deemed appropriate because it is the mean $\delta^{15}N$ value obtained for the Langebaan Lagoon sediment samples in this study and it is the lowest value reported for the southern Angola Basin (at 110 m) by Holmes *et al.* (1996).

Sediment samples taken from near the Southern Sea Fishing factory effluent outlet are clearly enriched in ^{15}N compared to samples taken from the rest of Saldanha Bay. Of the

four samples taken in a 10 m radius around the pipe, the ones collected to the north (PN) and west (PW) of the outfall had the highest values, while the area just south of the pipe (PS) had the lowest value (Table 2). The difference of 2.8 ‰ between $\delta^{15}N$ at PN and PS is most likely the result of prevailing winds and currents at the time of effluent release. This can be seen by the accumulation of 'fish waste' with high $\delta^{15}N$ values down-current from the pipe. The prevailing winds in the Saldanha Bay region are from the south-west quarter, which in turn induce north- to north-eastward flowing surface currents in Small Bay (Weeks *et al.*, 1991; Bilski, 1996). Because the plume is initially buoyant (AJ Smit, pers. obs.), organic matter is carried along with the current and the heavy particles settle out first in close proximity to the pipe, causing localised organic enrichment.

In the current study, very high sedimentary $\delta^{15}N$ values were measured closest to the effluent pipe. Such high values are not unexpected as figures of 10 - 14 ‰ were recorded for anchovy and roundherring tissue by Sholto-Douglas *et al.* (1991). The large amount of organic material in the effluent presumably contains mainly bone and other fish waste such as the contents of the intestines, and is ultimately reflected in the nitrogen isotope ratios in sediments surrounding the outfall. In 1993, an estimated 31 thousand tons of fish waste was disposed into Saldanha Bay (Steenveld *et al.*, 1993) and it is therefore not surprising to find such high $\delta^{15}N$ values in the vicinity of the outlet. $\delta^{15}N$ in these fish reflects their food source in the Benguela ecosystem which is comprised mainly of zooplankton in the 200 - 500 µm and >500 µm size classes, with $\delta^{15}N$ values of 9.0 ± 0.8 and 10.2 ± 1.1 ‰ respectively (Table 5). The nitrogen isotopic ratio of the zooplankton, in turn, is determined by that of the microzooplankton and phytoplankton they feed upon. So, each successive level in the food chain had a $\delta^{15}N$ value determined by the previous level in the trophic structure, starting at $\delta^{15}N$ of dissolved NO_3^- (5 - 6 ‰; Cline and Kaplan, 1975) which is the ultimate source of nitrogen to the ecosystem. Fractionation of $^{15}N/^{14}N$ associated with the incorporation of nitrogen derived from the lower trophic level results in the increasing $\delta^{15}N$ values observed in higher levels (Fry *et al.*, 1983; Schoeninger and DeNiro, 1984; Monteiro *et al.*, 1991). This change between trophic levels, $\Delta\delta^{15}N$, is typically between 1.2 - 2 ‰ in the Benguela system (Sholto-Douglas *et al.*, 1991). A $\delta^{15}N$ value of 9.2 ‰ was chosen to represent the effluent component in the mixing model (Equation 2) as this was the highest value recorded for sediments in Small Bay and it is

very close to the mean $\delta^{15}N$ value for anchovy and roundherring waste released into the bay (Sholto-Douglas *et al.*, 1991).

This study clearly shows that 140 m beyond the effluent pipe there is no clear correlation between percentage organic matter and distance. Spatially, these sites lie in a curve extending from the effluent pipe towards the northern shores of Hoedjiesbaai (Figure 1). Drogue studies and aerial photographs show water movement induced by south-westerly winds to be in a north to north-easterly direction towards Hoedjiesbaai, thus transporting the buoyant effluent plume containing large amounts of suspended organic material with it (Monteiro *et al.*, in press). In fact, the aerial photograph published by Weeks *et al.* (1991) shows exactly the distribution of such a plume under typical south-easterly wind directions, while this present study shows the sites with the highest sedimentary organic load to be situated directly beneath the plume. Similarly, Jackson and McGibbon (1991) showed that the effect of organic loading on benthic macrofauna was generally limited to sites closer than 100 m from the outfall. It was suggested that site specific differences in water movement, sediment composition and depth made it difficult to assess the impact imposed by pollution on macrofaunal assemblages beyond this distance. Furthermore, organic loading may not have an apparent effect on animal communities at all unless its accumulation in sediments exceeds a certain critical level (Anger, 1975). In the present study, stable isotope analysis allowed us to assess the influence of pollution on the rest of Small Bay, the area outside the 100 to 140 m limit imposed by the evaluation of organic loading and macrofaunal community structure.

Table 5. $\delta^{15}N$ values of DIN and various forms of particulate organic matter (POM).

Type	Locality	Source	^{15}N (‰)	Ref.
copepod faeces	NW Gulf of Mexico	surface	11.0 ± 0.0#	[1]
copepods	NW Gulf of Mexico	surface	8.8 ± 0.3#	[1]
suspended POM	NW Gulf of Mexico	surface	3.0 ± 0.2#	[1]
deep NH_4^+	Pacific Ocean	new nitrogen	3.5	[2]
deep NO_3^-	Pacific Ocean	new nitrogen	5.1 to 7.5	[2]
biologically fixed	Pacific Ocean	euphotic zone	-2 to -1	[3], [4], [5]
deep NO_3^-	Atlantic (Sargasso Sea)	new nitrogen	3.7	[6]
deep NO_3^- (summer)	North Atlantic	new nitrogen	7.8	[7]
suspended POM (summer)	Atlantic (Sargasso Sea)	sinking particles	~3	[8]
deep NO_3^-	eastern tropical North Pacific Ocean	water column	5 to 6	[9]
suspended POM	Atlantic (Sargasso Sea)	euphotic zone	4.1 to 4.4	[10]
suspended POM	Atlantic warm-core rings	euphotic zone	2.6 to 11.0	[10]
sediments	Southern Angola Basin	110 m	4.6 to 6.16	[11]
Zooplankton				
• phytoplankton / microzooplankton (20 - 200 µm)			7.5 ± 0.4@	
• copepods (200 - 500 µm)			9.5 ± 0.8@	
• euphausiids (>500 µm)	Atlantic (Benguela upwelling system)		10.2 ± 1.1@	[12]
Anchovy (*Engraulis capensis* Gilchrist)				
• gut contents			8.6 ± 0.7@	
• bone collagen	Atlantic (Benguela upwelling system)		10.6 ± 0.5@	
• muscle			12.9 ± 0.4@	[12]
Roundherring (*Etrumeus whiteheadi*)				
• gut contents				
• bone collagen			8.8 ± 1.2@	
• muscle	Atlantic (Benguela upwelling system)		11.3 ± 1.1@	
			13.7 ± 0.8@	[12]

#± SE; @± SD; [1] Checkley and Entzeroth, 1985; [2] Miyake and Wada, 1967; [3] Wada and Hattori, 1976; [4] Macko *et al.*, 1987; [5] Miinagawa and Wada, 1986; [6] Altabet, 1988; [7] Voss *et al.*, 1996; [8] Altabet and Deuser, 1985; [9] Cline and Kaplan, 1975; [10] Altabet and McCarthy, 1986; [11] Holmes *et al.*, 1996; [12] Sholto-Douglas *et al.*, 1991.

When data for all sites are pooled, the mode of sedimentary $\delta^{15}N$ in Small Bay is 7 to 8 ‰ (Figure 3). By applying the mixing model it immediately becomes apparent that sediments at most sites sampled in Small Bay contain between 53 and 74 % nitrogen derived from the fish factory with the remainder made up by normal marine detritus. The effect of the

plume extends to about 5 km away from the origin into the eastern corner of Small Bay to the site furthest away from the outfall sampled here, which contains up to 68 % effluent derived nitrogen. Closer sites (i.e. D5, D6, D8, D9, D10 and DE), on the other hand, have $\delta^{15}N$ values of between 6.0 and 6.5 ‰ with only a maximum of 50 % of their total nitrogen derived from the effluent according to the mixing model. These results are surprising as these sites are directly in the path of the effluent plume and relatively close to the pipe so one would therefore expect most of the nitrogen to be of fish origin. Furthermore, sites D8 to D10 have the highest organic matter content apart from the four sites located 10 m away from the pipe. There are two possible explanations for these $\delta^{15}N$ values. Firstly, the nitrogen comprising the bulk of the sedimentary total-N is marine-derived so that 'fish-nitrogen' makes up a smaller proportion relative to the rest of the bay. This would imply some deviation from the expected water circulation pattern in this area but it seems unlikely according to the aerial photograph in Weeks *et al.* (1991). A more likely explanation for this anomaly is the presence of a further source of sedimentary nitrogen, the origin of which is uncertain. These sites are situated within the general harbour area so organic matter or other waste disposed from boats can not be ruled out. If this unknown source of nitrogen can be identified, the mixing model used to determine the amount of nitrogen coming from different sources would have to be modified to include it.

Stable isotope and sedimentary organic matter data show that the effects of fish-waste disposal in Small Bay can be grouped into two categories: (a) eutrophication of benthic habitats, and (b) nitrogen enrichment of sediments. Here, sedimentary eutrophication refers to an accumulation of organic matter resulting in an alteration of the benthic community structure or the development of severely anoxic conditions. Sedimentary eutrophication occurs in Small Bay in a localised area between the outfall and approximately 100 - 140 m away. Sedimentary nitrogen enrichment in the context of this study refers to the deposition of anthropogenic sources of nitrogen onto the sediment. This may or may not result in the alteration of biogeochemical processes taking place between the sediment-water interface or deeper down in the sediment's vertical structure, but the low levels of enrichment are not likely to have any serious effects on the community structure.

Consequences of sedimentary nitrogen enrichment can be significant and the numerous possible results depend on sediment chemistry and bioturbation. The processes of nitrogen

cycling can strongly influence primary production in coastal marine systems (Henriksen and Kemp, 1988) and become increasingly important in progressively shallower systems (Harrison, 1980). For example, in systems less than 50 m deep, benthic regeneration can provide between 30 - 80 % of the nitrogen requirements for phytoplankton growth (Nixon, 1981; Blackburn and Henriksen, 1983; Boynton and Kemp, 1985). This study does not provide any evidence for local regeneration, but the possibility cannot be excluded.

Sedimentary organic nitrogen produces ammonium through ammonification. Part of the ammonium is oxidised to nitrite and subsequently to nitrate by the bacteria *Nitrobacter* spp. and *Nitrosomonas* spp. (Fenchel and Blackburn, 1979; Henriksen and Kemp, 1988). The regenerated nitrate may diffuse to overlying water where it becomes available for primary production, or alternatively, it may undergo nitrate reduction mediated by denitrifying bacteria to release gaseous nitrogen or nitrous oxides (denitrification). Denitrification shunts nitrogen away from assimilatory photoautotrophic pathways and when nitrification and denitrification are coupled, it provides a sink for regenerated nitrogen. A second (alternative) product of nitrate reduction is ammonium. This process is important in organic rich sediments with low nitrate concentrations (Tiedje *et al.*, 1982). Denitrification is limited by low sedimentary nitrate concentrations in most systems (Tiedje *et al.*, 1982; Jenkins and Kemp, 1984) and for this reason the total rate of denitrification in sediments is strongly enhanced by high nitrate concentrations in the water column. For instance in some systems up to about 50 % of the total-N input can be eliminated by the sediments before it becomes available for primary production (Billen *et al.*, 1986). Nitrification is an obligate aerobic process (Billen and Lancelot, 1988) and utilises significant amounts of oxygen in the sediments that can cause a change in the balance between aerobic and anaerobic processes (Henriksen and Kemp, 1988; Kemp *et al.*, 1990). Denitrification on the other hand is an obligate anaerobic process (Focht and Verstraete, 1977; Jørgensen, 1977; Jørgensen and Sørensen, 1985). For the two processes to take place in the same sediment, the oxic and anoxic layers must be separated by a horizontal redox discontinuity profile across which ammonium and nitrate diffuse upward and downward, respectively (Vanderborght and Billen, 1975; Huttel, 1990). Under anaerobic conditions the potential for sulphate reduction develops resulting in the degradation of organic matter. Nitrifying bacteria are inhibited by a range of volatile,

sulphur containing compounds (Yoshida, 1967; Oslund *et al.*, 1989) and many of them, such as hydrogen sulphide are directly toxic to macrofauna and seaweeds.

The consequence of sedimentary nitrogen enrichment in Small Bay will have to be studied in detail before the system's nitrogen cycle can be modelled. This would allow us to assess the effects of nitrogen disposal into Small Bay in order to understand the development of eutrophic events. Such an understanding is necessary not only because of the importance of nitrogen to Saldanha Bay and mariculture in the area, but because mismanagement could result in more disasters such as the *Ulva* bloom during the summer of 1993/1994 (Anderson *et al.*, 1996b).

4 *Fish Factory Pollution in Saldanha Bay: Assessment of Water Column Nitrogen Enrichment Using a Natural Abundance Stable Isotope Tracer Approach*

4.1 Introduction

Large volumes of fish-factory effluent containing high concentrations of suspended solids, ammonium, phosphate and fats, oils and greases are disposed into Small Bay (Department of Water Affairs and Forestry, DWAF). The fate of the suspended solids is discussed in Chapter 3 where it was shown that fish-derived organic matter contributes in some instances up to 75 % of the total sedimentary nitrogen input in Small Bay, even at sites up to 5 km distant from the outfall. Similarly, it can be expected that fish-derived inorganic nitrogen (DIN) is distributed throughout Small Bay. If this is true, we may currently be underestimating the input of nitrogen into the bay.

Currently it is thought that because harbour development in Saldanha Bay during the mid 1970's constrained circulation and rates of water exchange with the adjacent coast, the upper limit of primary production in the system was decreased due to a decrease in the maximal rate at which oligotrophic water in Saldanha Bay could be replaced and mixed with nutrient rich upwelled water. Nutrient limitation affects both micro- and macroalgae, including a seaweed important for mariculture. The macroalga, *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham, has been cultivated experimentally in Saldanha Bay since 1989 (RJ Anderson, pers. comm.), and results of some of the first mariculture experiments are discussed by Anderson *et al.*, (1996a). According to this study low nitrogen levels caused reduced growth rates of *Gracilaria* cultivated on the suspended experimental rafts in the region at certain times. In several other systems similar seasonal changes in seaweed growth rates and production have been ascribed to nutrient limitation (Rosenberg and Ramus, 1982; Lapointe and Duke, 1984; Fujita *et al.*, 1989; Borum and Sand-Jensen, 1996) with nitrogen as the primary limiting nutrient in many marine systems (Dugdale, 1967; Rosenberg and Ramus, 1982).

The dynamics of Saldanha Bay and the factors giving rise to the oligotrophic surface waters are discussed in detail in Chapter 1 and by Anderson *et al.*, (1996a) and Monteiro and Brundrit (in press). It must be stressed that the distribution of nutrients in Saldanha

Bay between the two water layers in summer has been extrapolated from models based on the relationship between seawater temperature and nitrate concentration for the Benguela upwelling system (Bailey and Chapman, 1985; Probyn, 1992; Monteiro and Brundrit, in press), and no empirical evidence is available to suggest that this holds true for all areas in the bay, especially the parts of Small Bay that receive anthropogenic inputs of nitrogen each year. For instance, according to Spolander (1996) the only mechanism for nutrient input into the oligotrophic warm surface layer is by entrainment during active upwelling. Entrainment is the process by which nutrients are made available to the surface layer when this water body moves over a colder, nutrient rich layer deeper down. The flux of nitrate into the surface layer has been calculated by Brundrit (1996) using the Spolander (1996) entrainment model. Similarly, Monteiro and Nelson (1996) used a turbulent diffusion model and high-resolution thermistor chain data to model the flux of nitrate across the thermocline into the oligotrophic surface layer. This turbulent diffusion model made use of the same temperature-nitrate relationship mentioned before.

Monteiro *et al.* (1991) linked the presence of a long-term apparent oxygen utilisation (AUO) level in Small Bay to the combined effect of low flushing rates and effluent discharge. However, if reduced water exchange rates affect the rate at which oxygen is utilised, then a similar argument can be applied to nitrogen entering the system. Anthropogenic activities should result in a significant input of 'new' nitrogen into Small Bay because the loss of nitrogen from the system is reduced. One similarity among all previous eutrophication-related studies in Small Bay is the failure to recognise the importance of the second source of nitrogen to the surface layer: that of fish-derived DIN. During 1996, the Southern Sea Fishing and Sea Harvest fishing companies released approximately 3 A 10^6 m^3 of effluent into Small Bay, containing about 650 tons of total-N (DWAF data; see Table 2 in Chapter 3). To place the volume into perspective, the volume of the effluent released into Small Bay [during 1996] was approximately 54 % that of the annual run-off of the Berg River (Chapman and Shannon, 1985). Furthermore, the nitrogen flux resulting from fish waste disposal into Small Bay is about 530 % that resulting from entrainment under moderate wind conditions. Fish-derived nitrogen could therefore be expected to contribute significantly towards the nitrogen budget of Saldanha Bay, and specifically Small Bay. Photoautotrophic production is generally assumed to increase with increased nutrient loading resulting from pollution (Boynton *et al.*, 1982;

Nixon *et al.*, 1986; Paerl, 1993), however, why then is seaweed production on suspended seaweed rafts in Small Bay limited by nitrogen availability as is suggested by Anderson *et al.* (1996a)? It is the aim of this chapter to examine the distribution of the fish-derived DIN in Small Bay, and its role in the nitrogen nutrition of natural and cultivated *Gracilaria* in the area. Understanding the nutrient dynamics of Saldanha Bay has important implications for the system's nutrient budget, the control of eutrophication, and seaweed mariculture site selection.

As in Chapter 3, this study makes use of a nitrogen stable isotope tracer approach to show the incorporation of effluent nitrogen into *Gracilaria* biomass, and hence the spatial distribution of DIN. The usefulness of the stable isotope technique was extended by combining findings with growth experiments conducted on *Gracilaria* in the region, and also by examining wind and temperature records for Small Bay over the study period. The absolute concentration of DIN (ammonium and nitrate) and phosphate was also measured rather than modelling the nutrient fluxes. Calculated nitrogen fluxes are of very little use when it comes to understanding the physiological response of a seaweed to nutrients in the growth medium, but flux calculations are included here to allow comparisons with similar systems.

4.2 Materials and methods

4.2.1 Study area and effluent chemical analysis

The physical and chemical features of Saldanha Bay are discussed in Chapter 1. A description of site names where seaweed samples were collected, and transects along which water samples were taken from can be found in Section 3.2.1 (Table 1), and the spatial distribution of these sites and transects is shown in Figure 1 of the same chapter. The physico-chemico properties of organic effluent released into Small Bay were summarised in Section 3.3.1 (Table 2; Figure 2a-h).

4.2.2 *Gracilaria* growth comparisons on suspended seaweed rafts

The Fish raft, so named because of its proximity to the fish factories and the source of pollution (Section 3.2.1, Figure 1), was installed in September 1996 about 1 040 m north of the Southern Sea effluent release pipe. During summer months prevailing south-easterly winds cause the visible effluent plume to flow directly over the raft. This study

was initiated before the need for stable the isotope analysis was realised (see below), and initially the main purpose of this raft was to test the hypothesis that *Gracilaria* cultivated on it would receive a large proportion of fish-derived nitrogen which would prevent it from becoming nitrogen limited in waters that would otherwise be oligotrophic in summer. Prolonged nutrient limitation causes reduced growth rates (Smit *et al.*, 1997), therefore to test whether receiving fish-derived nitrogen would result in better growth performance, growth rates were compared to rates measured for *Gracilaria* on the SFRI raft, about 5 160 m away in the north-east corner of Small Bay. The SFRI raft was chosen as control raft since it was expected to be in a more oligotrophic area of the bay compared to the Fish raft.

Construction of the suspended seaweed rafts is described by Dawes (1995) and the exact specifications are given by Anderson *et al.* (1996a). Relative growth rates (RGR, refer to Equation 1, Section 2.2.1) for the SFRI and Fish rafts for the period September 1996 to June 1997 were obtained by using ropes and the cable tie method outlined in Chapter 2 (see Section 2.2.2), with tufts weighing approximately 20 g (fresh) as seeding material. In most cases the seedstock was obtained from the natural population situated near the SFRI raft at a depth of about 6 m. Growth data for *Gracilaria* seeded onto netlons were also obtained for the period January 1995 to June 1996 for *Gracilaria* cultivated on the SFRI raft and another raft previously installed in the north-west corner of Small Bay near the mussel rafts (the old Sea Harvest raft) (RJ Anderson, unpubl. data). For these experiments *Gracilaria* was seeded onto netlons using the wire-hook method of Dawes (1995) (see Section 2.2.1; Anderson *et al.*, 1996a). C:N ratios were obtained using a Carlo-Erba NA 1500NC.

4.2.3 Stable isotope analysis of *Gracilaria* in Small Bay

Stable isotope analysis of *Gracilaria* allowed us to test the hypothesis that the contribution of fish-derived nitrogen to the total-N content of *Gracilaria* would decrease with increasing distance from the outfall, i.e., seaweed samples collected closer to the pipe would utilise a greater proportion effluent nitrogen relative to normal background marine nitrogen in the area. *Gracilaria* samples were collected from various sites in Small Bay between November 1996 and May 1997. A SCUBA diver randomly removed triplicate seaweed samples from natural populations at each of the sampling locations. Although the

ends of *Gracilaria* thalli are normally loosely buried in the sand, they often become dislodged which allows the populations to shift spatially due to the influence of currents or swells. Because the distribution of the seaweed varies both spatially and temporally it was not possible to obtain a continuous record for each site over the sampling period. In addition to natural populations, *Gracilaria* samples were obtained from the Fish and SFRI rafts one month after seeding. *Gracilaria* collected during earlier studies in Small Bay (1995) was also analysed for its isotopic composition. Although the latter samples do not form part of the main sampling plan, they do provide valuable insight into nitrogen distribution in an area of the bay previously occupied by an experimental seaweed raft. For reference material, seaweed that has not been exposed to any form of pollution was obtained from a site near Schapen Island in Langebaan Lagoon (a nature reserve).

After collection the seaweed samples were immediately placed in polythene bags and stored in the dark on ice until they could be frozen for longer-term storage. Processing involved thawing the seaweed and rinsing it in deionised water to remove all traces of salt and most of the microscopic epiphytes such as diatoms. Care was also taken to remove all macroscopic epiphytic seaweeds such as *Ceramium* sp. To obtain a fine powder for isotopic analysis, cleaned samples were oven dried at 60 °C and ground using liquid nitrogen and a mortar and pestle. The powder was stored in a desiccator until required for analysis.

Simultaneous $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were determined from approximately 0.6 mg samples on a Finnigan MAT 252 isotope ratio mass spectrometer as detailed in Chapter 3 and Fry *et al.* (1992). Simultaneous carbon and nitrogen analysis also allowed us to obtain C:N ratios for each of the samples. $\delta^{15}N$ and $\delta^{13}C$ were calculated using Equation 1 in Chapter 3, substituting $^{15}N/^{14}N$ with $^{13}C/^{12}C$ in the case of carbon. The laboratory reference gases were high purity tank nitrogen (99.995 %) and carbon dioxide (99.995 %) calibrated against atmospheric nitrogen and Pee Dee Belemnite (PDB) respectively. Analytical precision was ± 0.3 and ± 0.2 ‰ (± 1 SD) for nitrogen and carbon isotope ratios respectively. Although the resulting $\delta^{13}C$ values are mentioned briefly, only findings from the nitrogen isotope analysis are discussed in detail here.

Some *Gracilaria* samples were divided into young lateral branches and the thick main axis and analysed separately for $\delta^{15}N$, $\delta^{13}C$ and C:N ratios. When $\delta^{15}N$ values were required for the whole thallus (*T*), they were calculated according to:

$$\delta^{15}N_T = \frac{\delta^{15}N_a \cdot x_a + \delta^{15}N_b \cdot x_b}{x_a + x_b} \quad \textbf{[Eq. 1]}$$

where x is the quantity of nitrogen in the tissue, a is the young branches and b the main axis (Shearer and Kohl, 1992).

4.2.4 Seawater chemical analysis

Seawater samples for the analysis of ammonium, nitrate and phosphate were collected along four transects across Small Bay (Section 3.2.1, Figure 1) on the 12/02/97. Water was collected directly into 250 mL acid washed Pyrex glass bottles and covered with Teflon lined caps. Samples were also taken from below the thermocline, if it was present, at stations along Transects B and D. Deep-water samples were taken using a 5 L NIO bottle and subsamples were immediately transferred to glass storage bottles as above. Samples were stored on ice in the dark until they could be analysed, usually within two hours after collection. Nutrients were analysed manually using modifications of the standard methods of Solórzano (1969), Murphy and Riley (1962) and Grasshoff (1983) for ammonium, phosphate and nitrate respectively. Details of each method are provided in full in Appendix A.

To supplement nutrient data, profiles (*vs.* depth) of seawater temperature were measured at each site using a YSI water quality logger. In addition, wind data for February 1997 were obtained from the South African Weather Bureau (SAWB). Detailed temperature records were obtained from temperature recorders (manufactured in-house by SFRI) placed at the SFRI raft and Fish raft for the duration of the project. Two recorders were placed on the SFRI raft, one above the thermocline at a depth of approximately 1.5 m and the other about 0.5 m from the bottom (5 m).

4.3 Results

4.3.1 *Gracilaria* growth rates

Seasonal trends in seaweed growth rates between January 1995 to June 1996, and September 1996 and June 1997 are shown in Figure 1a-b; C:N ratios obtained from seaweed harvested after cultivation on the rafts are shown in Table 1. The 1995 data show that growth rate peaked on the SFRI raft between August and November and was significantly higher than the period leading up to August (one-way ANOVA; $p < 0.01$, d.f. = 11, F = 59.04). The highest growth rates for *G. gracilis* on the old Sea Harvest raft were obtained in October and November (one-way ANOVA; $p < 0.01$, d.f. = 9, F = 14.25). Low growth rates of between 1 and 3 % d^{-1} occurred in the summer months, particularly January of both years. C:N ratios determined during the winter months (1995) varied between approximately 7 - 9, but was significantly higher during mid- to late summer (Student's t-test, data pooled per season; $p < 0.01$). After December 1996, seaweed cultivated on the SFRI raft generally had the lowest C:N ratio, although the difference was not significant from C:N ratios obtained from material cultivated on the Fish raft (two-way ANOVA; $p > 0.05$, d.f. = 1, F = 0.09). There was also a significant interaction between C:N ratio and month (two-way ANOVA; $p < 0.05$, d.f. = 4, F = 8.05). Available data show that C:N ratios obtained at the Sea Harvest raft was significantly higher than both at the SFRI and Fish rafts during December 1996 (MANOVA; $p < 0.05$, d.f. = 2, F = 10.00). Months × site interactions and between month (December 1996 vs. May 1997) differences were also significant at $p < 0.05$. During October and November 1996 all the *Gracilaria* cultivated on the SFRI raft died. Subsequently during November 1996 and January 1997 growth rates obtained on the SFRI raft were higher than those on the Fish raft. Growth rates indicated in Figure 1b were generally higher than to those shown in Figure 1a because of the different cultivation methods employed.

4.3.2 Stable isotope analysis

Although isotope ratios were determined for both the main axis and the lateral branches, only $\delta^{15}N$ values for the lateral branches are reported here in detail. The difference in $\delta^{15}N$ appears to show a seasonal trend with the biggest difference observed in raft cultivated *Gracilaria* ($\delta^{15}N_{young} - \delta^{15}N_{old}$, here called $\Delta\delta^{15}N_{young - old}$) in summer (2 - 3 ‰), decreasing towards winter (approx. 0.2 ‰). During summer $\Delta\delta^{15}N_{young - old}$ for the Langebaan

material is approximately 0.5 ‰, and although there are no data for winter, it is not expected to deviate much from the value obtained in summer (see discussion).

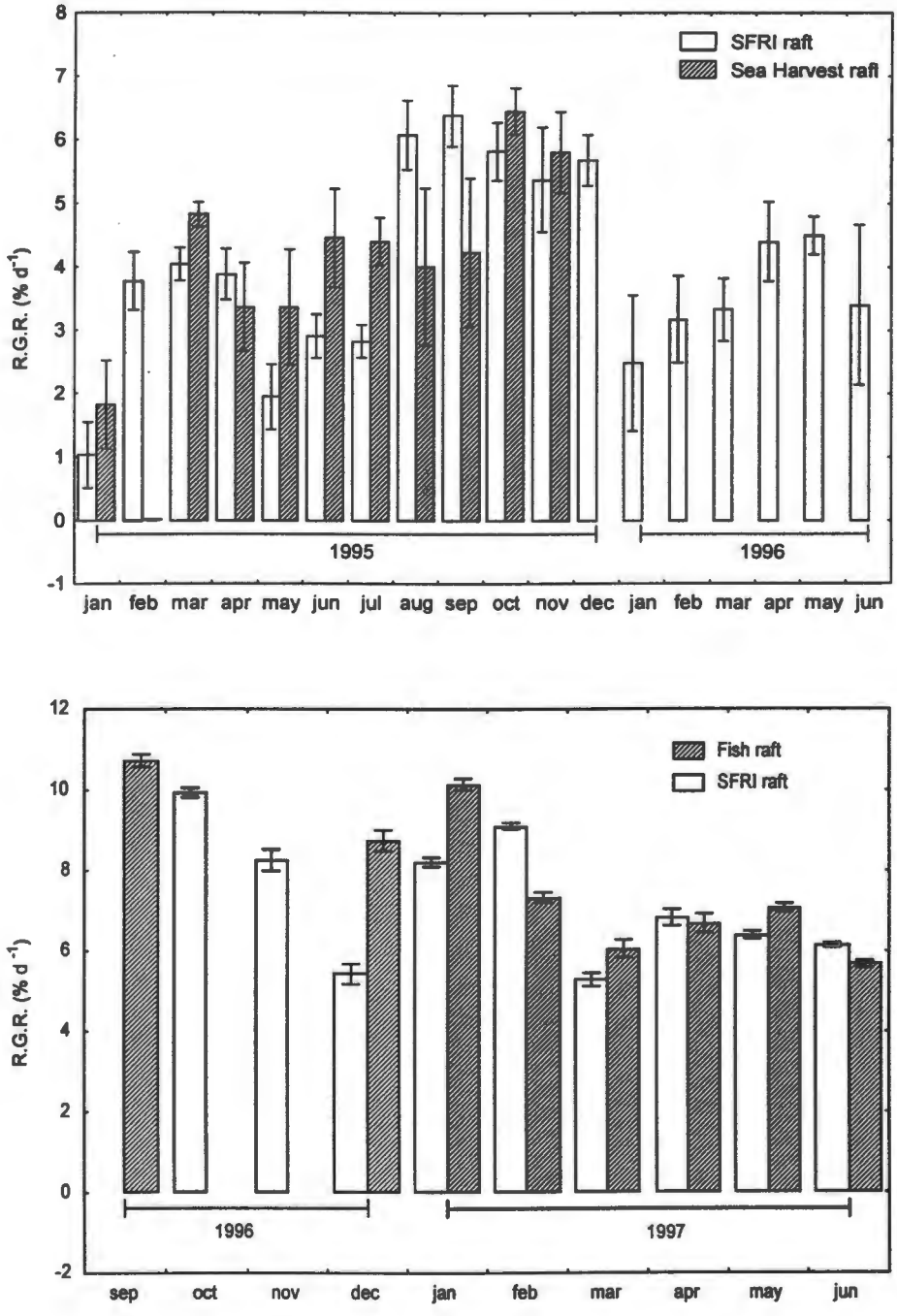

Figure 1. Growth rates of *Gracilaria* obtained in Small Bay from January 1995 to June 1996 for the SFRI and old Sea Harvest rafts using netlons (a), and from September 1996 to June 1997 for the Fish and SFRI rafts using the ropes (b). Whiskers = ± 1 SD.

Table 1. C:N ratios obtained for *Gracilaria* cultivated on three rafts in Small Bay for several months between June 1995 to May 1997 (± 1 SD).

month	C:N	C:N	C:N
	SFRI raft	**Fish raft**	**old Sea Harvest raft**
June 1995	8.47 ± 0.41		7.23 ± 0.01
July 1995	7.58 ± 0.22		8.04 ± 0.07
August 1995	8.49 ± 0.55		
September 1995	10.59 ± 0.22		
October 1995	7.88 ± 0.34		9.30 ± 0.44
November 1995	8.95 ± 1.31		
	SFRI raft	**Fish raft**	**Sea Harvest raft**
December 1996	14.39 ± 3.25	16.16 ± 1.44	21.1 ± 1.1
February 1997	15.82 ± 7.94	18.33 ± 7.54	
March 1997	15.52 ± 2.81	11.79	
April 1997	17.62 ± 3.07	20.81 ± 6.06	
May 1997	8.53 ± 0.15	6.66 ± 1.22	7.9 ± 0.2

For the control site in Langebaan Lagoon, $\delta^{15}N$ values (only young tissue discussed hereafter) ranged from 8.4 ± 0.1 ‰ (± 1 SD) in December 1996 to 9.4 ± 03 ‰ in February 1997. $^{15}N/^{14}N$ ratios for seaweed obtained from the two suspended rafts between December 1996 and May 1997 are shown in Figure 2. *Gracilaria* cultivated on the SFRI raft in the north-eastern corner of Small Bay had mean $\delta^{15}N$ values in the young lateral branches ranging from 11.2 ± 0.0 - 13.0 ± 0.1 ‰ over the sampling period, while the Fish raft had values ranging from 8.6 ± 0.5 to 11.4 ± 0.1 ‰. Similar differences were found when total thallus tissue values were compared, although the $\delta^{15}N$ values were approximately 1 ‰ lower. Overall, *Gracilaria* sampled from the SFRI raft was significantly more enriched in ^{15}N compared to seaweed obtained from the Fish raft closer to the outfall (one-way ANOVA, $p < 0.05$). Nitrogen isotope ratios reached a maximum during February in *Gracilaria* cultivated on both rafts. Differences in $\delta^{15}N$ values between cultivated *Gracilaria* on the SFRI raft (~0.4 m) and the natural population beneath the raft (~7 m) were determined for the months indicated in Figure 3. During December and February the raft populations had more enriched $\delta^{15}N$ values in lateral branches (and total thallus tissue) compared to the natural populations, this difference being significant at $p < 0.001$ (one-way ANOVA). In May however, the $\delta^{15}N$ value of cultivated seaweed decreased so that there was no significant difference between cultivated and natural seaweed populations ($p = 0.0557$, Student's t-test).

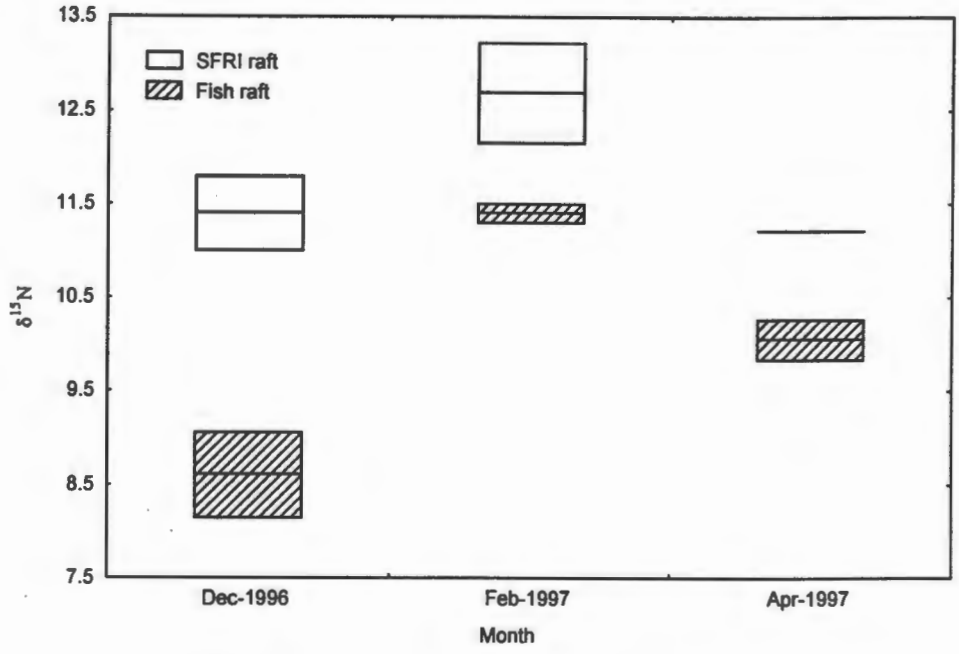

Figure 2. Mean ± 1 SD of $\delta^{15}N$ in lateral branches of *Gracilaria* cultivated on two suspended rafts in Small Bay between December 1996 and April 1997 ($\delta^{15}N$ = ‰, vs. atmospheric N_2).

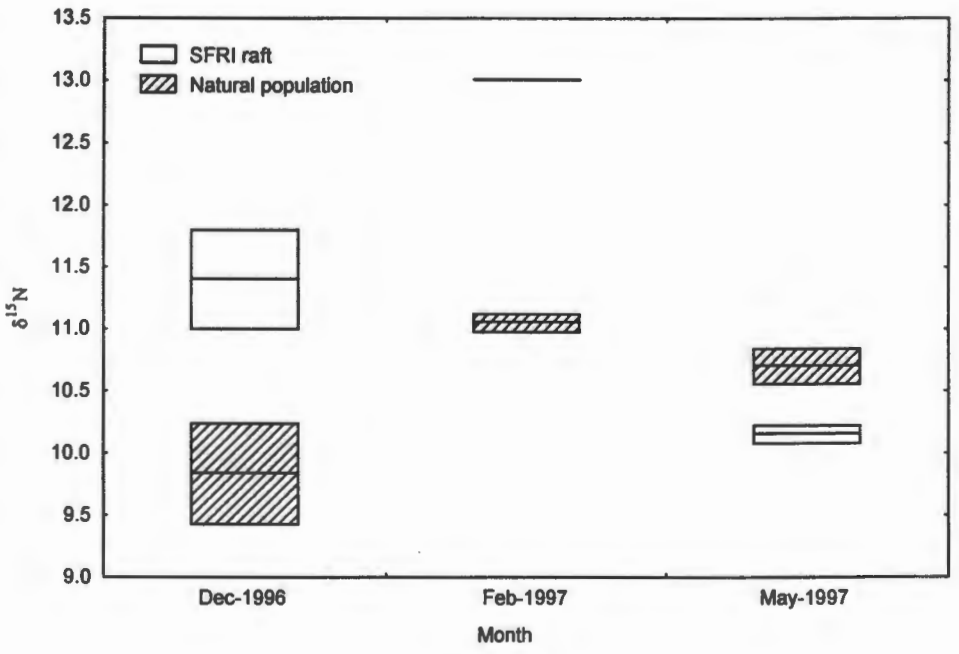

Figure 3. $\delta^{15}N$ in lateral branches of *Gracilaria* sampled from natural populations at a depth of 7 m and from the SFRI raft suspended at approximately 0.4 m beneath the surface. Means and 1 SD shown ($\delta^{15}N$ = ‰, vs atmospheric N_2).

The range in $\delta^{15}N$ for the seaweed (total tissue) cultivated on the SFRI and Sea Harvest experimental rafts during 1995 is shown in Figure 4. *Gracilaria* samples were not collected from the Sea Harvest raft for the months August, September and November because heavy epiphyte fouling and the general poor condition of the seaweed rendered the material unusable for isotopic analysis. $\delta^{15}N$ ranged from 10.4 - 14.3 ‰ for the SFRI *Gracilaria*, while the range in the Sea Harvest raft was between 8.4 - 9.5 ‰. This difference is significant at $p = 0.0117$ (Wilcoxon matched pairs test).

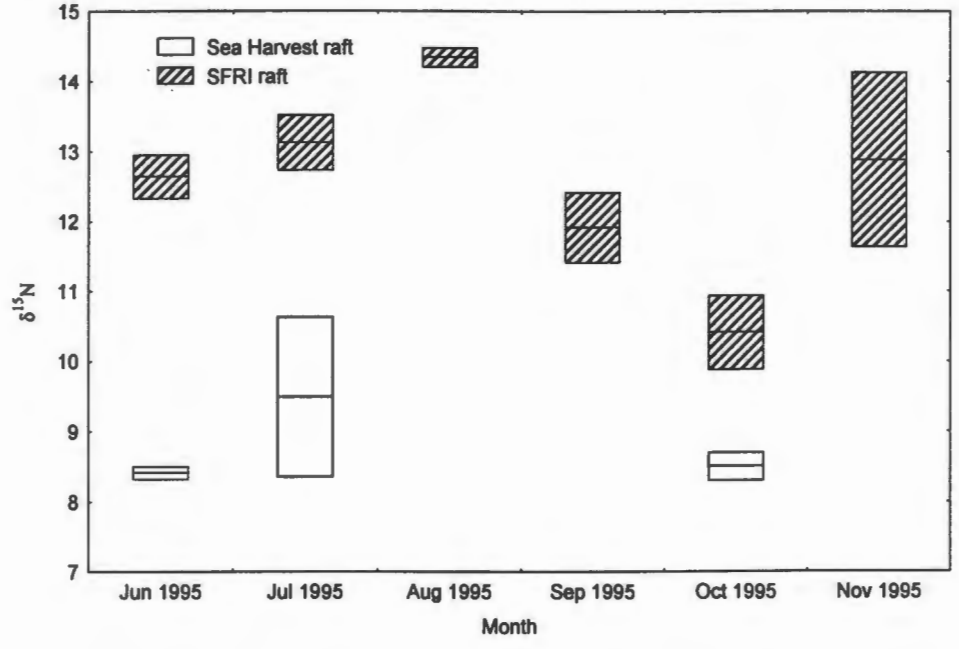

Figure 4. $\delta^{15}N$ values recorded for *Gracilaria* total tissue collected from two seaweed rafts in Small Bay during 1995. Means and SD shown ($\delta^{15}N$ = ‰, vs. atmospheric N_2).

4.3.3 Seawater nutrient analysis

Only nitrate data for the four transects (see Figure 1, Section 3.2.1 for a map) are presented here (Figure 5a-d). Nitrate concentrations measured for Transects B and C generally fall within similar ranges (10.7 ± 3.1 and 12.3 ± 2.9 µM NO_3^--N transect average ± 1 SD for B and C respectively), while levels measured along Transect A are very variable and lower than along B or C (6.2 ± 4.2 µM NO_3^--N). Although the thermocline was not well developed (see below), nutrients were analysed in the colder bottom water as well as the warm surface water at five stations along Transect D. Nitrate concentrations in both water layers were higher than in the other transects due to the transect following the Southern

Sea effluent plume, but there was no significant difference between nitrate concentration in the two layers (dependent t-test, $p > 0.05$). The measurement of nitrate concentrations in effluent water is not required by DWAF, but the very high nitrate values measured during this study near the effluent source are proof that this nitrate originates from the Southern Sea fish factory. The concentration of phosphate varied little among transects, with the highest values (1.4 ± 0.3 µM PO_4^{2+}-P) recorded in the five bottom water samples along Transect D, and lower concentrations in surface water along Transects C and D. Ammonium-N concentration near the outfall was about 4 µM and decreased to 0.8 ± 0.6 µM over the rest of Small Bay.

The above data were obtained under typical summer conditions with winds from the south-west quarter dominant during the sampling period (Figs 6a-b) on 12/02/97. Sampling started around 08:30 and lasted until 15:30 during which period the wind speed increased from about 4.4 to 9.4 m s^{-1}. Thermocline development (Figure 7a-d) was weak with temperature decreasing from 19.7 to 19.3 °C over the first 2.7 m below the surface. Below this depth the temperature gradient increased sharply, but remained constant all the way to the bottom where the temperature fell to 13.9 °C at 7.8 m (site D10; Figure 7a). This vertical temperature gradient was consistent from Sites D10 to D3 which had depths of greater than 5.2 m, but when the water was shallower than this (D2 and D1) the warm layer extended all the way to the bottom without the development of a temperature gradient. The warm surface layer extended all the way into the north-eastern corner of Small Bay (B10; Figure 7c) where the temperature remained constant at 20.5 °C down to a depth of 5.4 m, whereafter it decreased by 0.8 °C over the next 0.9 m to the bottom. Further to the south in Small Bay (e.g. C5; Figure 7d) the vertical water structure still resembled that seen at D10, with an increase in the temperature gradient below 5.1 m. Seawater temperatures for the period November 1996 to July 1997 are shown in Figure 8.

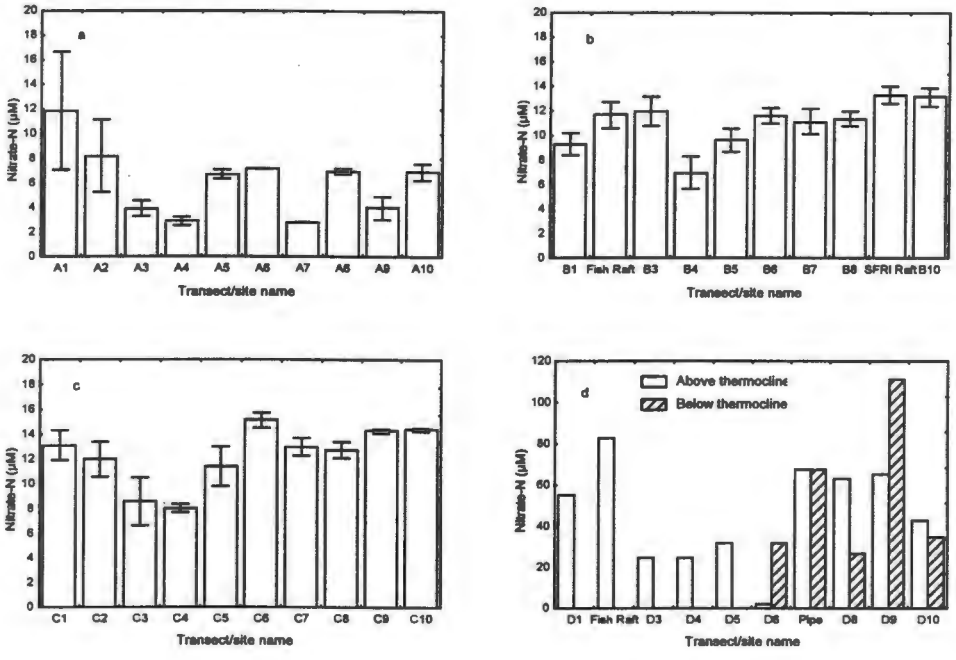

Figure 5. Nitrate concentration measured along transects A (a), B (b), C (c) and D (d) across Small Bay on the 12th of February 1997. Whiskers = ± 1 SD (no standard deviations given for Transect D).

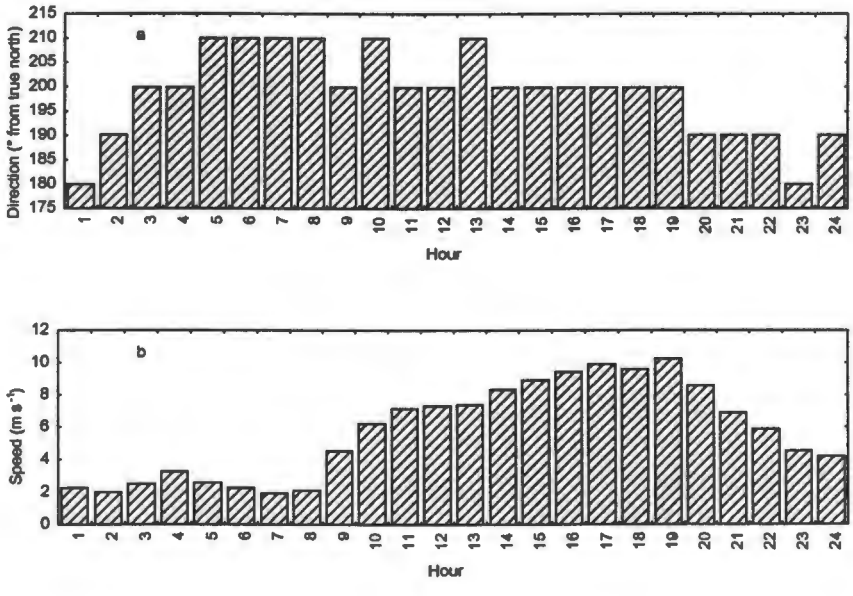

Figure 6. Hourly wind speed (a) and direction (b) on the 12th of February 1997 showing the dominance of south-westerly winds prevailing over Saldanha Bay. Data for Langebaanweg (SAWB).

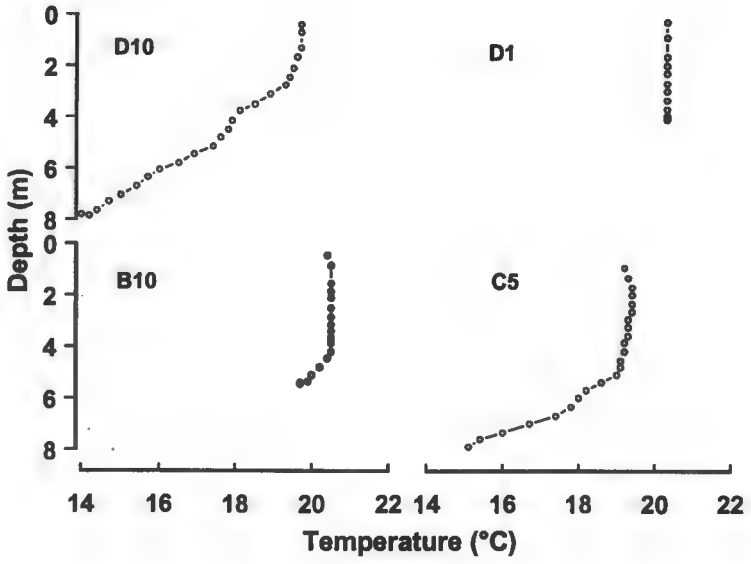

Figure 7. Examples of temperature profiles taken on the 12th of February 1997 at sites D10, D1, B10 and C5 along three transects in Small Bay.

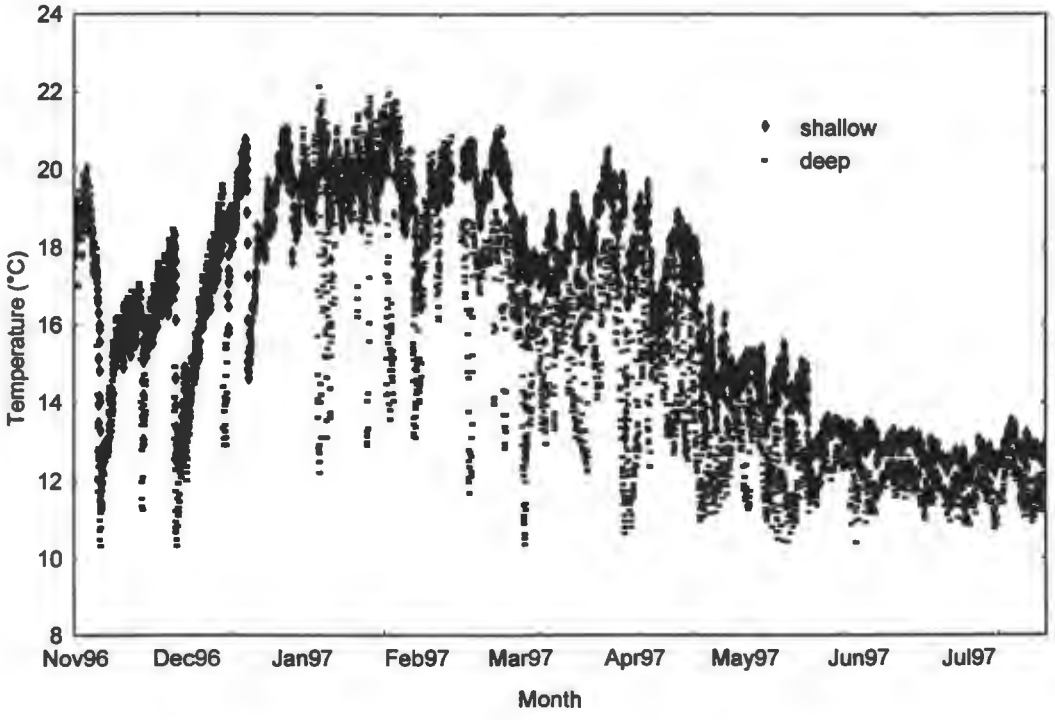

Figure 8. Seawater temperatures at the SFRI raft measured at 1.5 m beneath the surface and 0.5 m from the bottom from the period November 1996 to July 1997. Hourly temperatures are shown.

4.4 Discussion

4.4.1 Distribution of DIN

It is difficult to provide a generalised overview on the interaction between meteorological conditions (and the resulting hydrography) and anthropogenic perturbations in Small Bay because disturbance in the system takes place as transient 'events' rather than a gradual change (see later). To obtain a detailed understanding of the temporal and spatial changes in nutrient dynamics in Small Bay, many intensive 'event-scale' studies need to be conducted. This part of the study focuses only on one event and discusses possible effects it may have on seaweed mariculture in the area, especially site selection for *Gracilaria* or other seaweed cultivation.

The fate of fish-derived particulate organic nitrogen (PON) was addressed in the previous chapter where it was shown that more than 50 % of the particulate nitrogen is retained in Small Bay. The remainder of the DIN released into Small Bay is subject to biological uptake or further transformations through a variety of biogeochemical processes. At this stage it is uncertain which of the biogeochemical cycles are important for nitrogen cycling in the system. However, it is likely that the very high annual inputs of effluent have significantly changed the nitrogen budget of Small Bay and possibly the adjacent Big Bay.

Vertical and horizontal temperature and nutrient profiles for Small Bay on the 12th of February 1997 show the typical hydrography during summer to be dominated by moderate to strong south-westerly winds. Following the terminology of Monteiro and Brundrit (in press) (also see Chapter 1) used to describe the spatial boundaries of three horizontal zones of water in Saldanha Bay during stratification, both Zones B and C were present. Zone C, which extends from where the depth of the thermocline is equal to, or exceeds, the depth of the bathymetry, covered the shallow part of Small Bay no deeper than approximately 5 m. This area is characterised by a layer of isothermal warm water (about 19 °C) extending down to the bottom. Zone B was present in water of more than 5 m deep with a weak thermocline of about 1.1 °C m^{-1} extending down from that depth. The horizontal and vertical structure of the water, and the presence of south-westerly winds, indicates this to be the 'Relaxation Phase' after the switch from active upwelling to 'stratification', when there is an isothermal warm surface component (see Chapter 1 and Monteiro and Brundrit, in press). The wind record for the week prior to sampling shows the dominance of

moderate (~6 m s^{-1}) south-westerly winds, suggesting that the present hydrography was well developed and was in existence for at least five days. This is confirmed by the long-term seawater temperature records measured at the SFRI raft. Since phytoplankton removes all available nutrients from the euphotic zone within 3 to 5 days after upwelling (Brown and Hutchings, 1987) it might have been assumed that the surface layer would be oligotrophic.

Measurement of the nutrient concentration along Transects A to D, however, shows this assumption to be invalid. Ammonium-N and phosphate-P concentrations varied between 1 - 1.5 µM over most of Small Bay with high concentrations near the outfall. Recorded nitrate-N values were above 10 µM in most instances, with very high concentrations (60 - 110 µM) near the effluent pipe. The presence of very high nitrate-N concentrations in the effluent raises the question of why nitrate values are never reported in the fish-factory permit returns. Nevertheless, the presence of a second nitrogen source is clearly indicated, making the validity of the turbulent diffusion (Monteiro and Brundrit, in press) and entrainment (Spolander, 1996; Brundrit, 1996) models questionable when applied to Small Bay. The nitrogen flux resulting from the disposal of fish-derived nitrogen into Small Bay can be calculated from DWAF data. Assuming the waste only affects Small Bay (surface area of 1 A 10^7 m^2), an annual release of approximately 650 tons of total-N is equivalent to a N-flux of 0.53 mmol m^{-2} hr^{-1}. The nitrate-N flux resulting from entrainment on the other hand, is approximately 0.1 mmol m^{-2} hr^{-1} under moderate wind conditions (Brundrit, 1996). Anthropogenic nitrogen input into Small Bay therefore contributes significantly to the nitrogen budget of the bay, with an N-flux of about 530 % that under moderate entrainment. A more conservative estimate of anthropogenic N-flux is obtained by taking the surface area of the entire Saldanha Bay into account (7 A 10^7). When this is done the flux equates to 0.08 mmol total-N m^{-2} hr^{-1} (40 % of the figure obtained by Brundrit, 1996). Since our study (Chapter 3) did not examine the extent of the effluent plume in Big Bay the latter estimate should be used, at least until future work shows the exact area of Saldanha Bay that is influenced by fish-derived nitrogen. Although only about 214 tons of the 650 tons of total-N is available for immediate uptake in the form of ammonium, particulates settle onto the sediment and are remineralised with time to provide new nitrogen to the water column in Small Bay (and Big Bay). After thermocline development during summer when Zone C covers a large area of Small Bay, remineralised nitrogen is

released across the sediment-water interface directly into the 'oligotrophic' surface layer. Furthermore, other biogeochemical processes involving bacteria, phytoplankton and zooplankton cycle various forms of POM, DIN and dissolved organic nitrogen (DON) through the water column and the sediments, thus making regenerated nitrogen available for primary production (Gilbert, 1988). There is also the possibility that nitrate is lost from the system to the atmosphere through nitrate reduction to N_2 or N_20.

An understanding of the nature and variability of the hydrography in Small Bay is essential to our understanding of the distribution of pollutants in the system and to predict possible eutrophic conditions that might result from effluent release. Data from this study show the distribution of high concentrations of nitrogen resulting from fish-waste disposal into Small Bay during a part of February 1997. According to G Pitcher (pers. comm.) these high nitrogen levels are not typical for Small Bay with a stable, well-developed thermocline, and it is possible that our fieldwork coincided with a previously unobserved hydrodynamic state. This stresses the importance of intensive sampling to follow the development of stratification subsequent to an upwelling event, combined with water-parcel tracking in the stratified system under the influence of surface water transport brought about by south-westerly winds. It is unlikely that eutrophication would develop under conditions dominated by south-westerlies, but severe cases of nutrient enrichment resulting in macroalgal blooms have been recorded (e.g. Anderson *et al.*, 1996b) for Saldanha Bay. These eutrophic conditions are suggested to result from the interaction of natural barotropic forcing mechanisms such as wind direction and strength, combined with anthropogenic perturbations (Monteiro *et al.*, 1990). The timing of natural and anthropogenic forcing events is related to the degree of disturbance in the bay, resulting in environmental change taking place as a series of isolated events rather than a continuum of gradual change towards disturbance. Potential for disturbance is greatest during spring and summer under warm water, still/low wind conditions. This is because any effluent released into the bay is trapped in the surface layer in the north-western corner of Small Bay (surface currents brought about by winds from the south-west quarter are needed to distribute the waste over the rest of Small Bay). The window for the eutrophic event in Small Bay during 1993/1994 is thought to have been brought about by strong stratification which developed concurrently with high anthropogenic nutrient inputs (Anderson *et al.*,

1996b) which caused a localised build-up of nitrogen and the development of an *Ulva lactuca* bloom.

Although the interaction of natural populations of *Ulva lactuca* and *Gracilaria gracilis* has been studied under eutrophic conditions (Anderson *et al.*, 1996b), very little is known about other primary producers in Small Bay. Apart from macroalgae, benthic and planktonic microalgae also utilise DIN and would therefore be expected to respond to nitrogen enrichment as has been shown by Ryther and Dunstan (1971) and Nixon and Pilson (1983). Similarly, Borum and Sand-Jensen (1996) compiled data from different temporal coastal ecosystems and found planktonic microalgal primary production to be a hyperbolic function of nitrogen loading. Furthermore, they propose that in certain systems benthic macroalgae show the opposite response to increased nutrient loading compared to phytoplankton because productivity in seaweed populations is limited by light availability rather than nutrients. In this study, C:N ratios obtained for *Gracilaria* living in benthic populations are lower than in cultivated 'populations' in the surface layer on suspended rafts, thus indicating that the same is true for seaweed populations in Saldanha Bay. During summer nitrogen becomes available to benthic populations through periodic intrusions of nutrient-rich upwelling water into the bay (Anderson *et al.*, 1996a) which allows the populations to replenish their internal nitrogen resources (Smit *et al.*, 1997). It is suggested that due to the presence of a fluctuating thermocline during this time, this nitrogen is more often available to natural populations than to the cultures suspended near the water surface, hence the difference in C:N ratios between the two populations. When the primary production of a seaweed population is limited mainly by light availability, the increased light attenuation associated with enhanced phytoplankton productivity under high nutrient loads could result in a reduction of macroalgal distribution and biomass (Borum and Sand-Jensen, 1996). If this occurs in Saldanha Bay and the biomass of natural *Gracilaria* populations decreases because of competition with plankton for light, it could negatively affect the seaweed industry that relies on regular beach casts of *Gracilaria*. There is the possibility that this has already happened. Prior to 1974 the *Gracilaria* industry obtained seaweed wash-ups of up to 7 000 kg (fresh) material per year, but this decreased drastically after the construction of the iron-ore jetty and breakwater that year. The initial collapse has been attributed directly to harbour-development (Rotmann, 1990) but the several subsequent collapses have been shown to be partly due to the grazing

pressure by herbivores (Anderson *et al.*, 1990). High concentrations of anthropogenic nitrogen are released into Small Bay and one would therefore expect to find dense phytoplankton populations. It is claimed not to be the case (G Pitcher, pers. comm.): on the contrary the surface layer in summer is often very transparent (pers. obs.). Therefore, despite the high flux of nitrogen into the system, phytoplankton is surprisingly unresponsive. Once again detailed water-parcel studies to determine the ageing of effluent water are needed.

4.4.2 Seaweed $\delta^{15}N$ as an indicator of effluent distribution

The $\delta^{15}N$ value of a seaweed provides an indication of the usage of nitrogen integrated throughout its lifetime, and because different sources of inorganic nitrogen available for uptake have different $\delta^{15}N$ signatures, it enables the identification of the source of nitrogen used by the seaweed. Inorganic nitrogen contained in the fish factory effluent, and that regenerated from the decomposition of fish-derived organic matter, is enriched in ^{15}N compared to natural oceanic nitrate or ammonium brought into Saldanha Bay through upwelling (Monteiro *et al.*, in press). It should therefore be possible to determine what source of nitrogen was available for uptake during the lifetime of *Gracilaria*'s growth.

Gracilaria samples taken from the control site have higher $\delta^{15}N$ values than sediment samples removed from the same site. The former values are similar to those recorded by Monteiro *et al.*, (in press) for *Ulva lactuca* in Langebaan Lagoon (8.1 to 8.9 ‰) and those by AJ Smit (unpubl.; Table 2) for a variety of seaweed species collected on the Cape Peninsula at Kommetjie. It would be safe to assume that these values are typical of seaweed not exposed to fish-derived nitrogen. In Small Bay $\delta^{15}N$ values measured in *Gracilaria gracilis* vary widely both temporally and spatially, but in most cases significant differences between sites are consistent over time. High $\delta^{15}N$ values of up to 14.3 ± 0.1 are nearly always associated with *Gracilaria* grown on the SFRI seaweed raft near site B9. Such high values have also been recorded by Monteiro *et al.* (in press) in samples of *Ulva* after its bloom in 1993/1994 in Small Bay. These very high $\delta^{15}N$ values occur during the winter months when concentrations of total-N and ammonium in the effluent are highest and the water column well mixed, as well as during summer when under stratified 'oligotrophic' conditions. Although, in some cases there is no overlap and direct comparison is difficult, available data suggest that *Gracilaria* grown on the Fish raft and

the Sea Harvest raft (Figure 1) received less fish-derived nitrogen during their period of cultivation on the rafts. Due to the mainly clockwise circulation pattern in Small Bay, the Sea Harvest raft is upcurrent of the site of effluent release and thus receives mainly natural marine nitrogen from outside the bay: it is thus not surprising that $\delta^{15}N$ values measured there (8.4 ± 0.1 to 9.5 ± 1.1 ‰) are lower than those of *Gracilaria* sampled at either the Fish or SFRI rafts. Furthermore, C:N analysis indicates that *Gracilaria* cultivated on the Sea Harvest raft is more prone to nitrogen limitation than sites to the east of the effluent release pipe. This is also indicated by the very poor growth rates obtained during summer with *Gracilaria* cultivated on the Sea Harvest raft (Anderson *et al.*, 1996a). The low $\delta^{15}N$ values measured in *Gracilaria* cultivated on the Fish raft (relative to the SFRI raft) are surprising because of its proximity to the effluent plume which on occasion passes straight through the raft (pers. obs.).

Table 2. $\delta^{15}N$ of a variety of seaweeds.

Type	Locality	Source	$\delta^{15}N$ (‰)	Ref.
Unspecified seaweed			8.1	[1]
Ulva lactuca	Langebaan Lagoon	subtidal	8.9	[2]
seaweed				
• Chlorophyta [A]	Kommetjie, Cape Town		8.5 ± 0.8[@]	[3]
• Phaeophyta [B]	Kommetjie, Cape Town	intertidal /	6.1 ± 1.5[@]	[3]
• Rhodophyta [C]	Kommetjie, Cape Town	subtidal	7.0 ± 1.3[@]	[3]

[@] ± SD; [1] Hoering, 1955; [2] Monteiro *et al.*, in press [3] AJ Smit, unpublished.
[A] [*Codium duthieae* Silva, *Chaeotomorpha robusta* (Aresch.) Papenfuss; *Ulva lactuca*.]; [B] [*Ecklonia maxima* (Osbeck) Papenfuss, *Laminaria pallida* Greville ex J. Agardh, *Macrocystis angustifolia* Bory, *Chordariopsis capensis* (C. Agardh) Kylin, *Splachnidium rugosum* (L.) Greville]; [C] [*Hymenena venosa* (L.) Kylin, *Nothogenia ovalis* (Suhr) Parkinson, *N. erinacea* (Turner) Parkinson, *Porphyra* sp., *Aeodes orbitosa* (Suhr) Schmitz, *Iridaea capensis* J. Agardh, *Gigartina radula* J. Agardh (gametophyte), *G. bracteata* (S.G. Gmelin) Setchell and Gardner *Carpoblepharis flaccida* (C. Agardh) Kuetzing, *Neuroglossum binderianum* Kuetzing].

Gracilaria cultivated on the SFRI raft suspended at about 0.4 m beneath the water surface has higher $\delta^{15}N$ values than seaweed from the benthic populations at a depth of approximately 6 m. This difference is significant during summer when the water column is stratified and indicates that seaweed suspended above the thermocline has access to fish-derived nitrogen that is not available to the benthic populations deeper down. With the onset of northerly winds during May the stratification is broken down and upwelled water containing new nitrogen with a lower $\delta^{15}N$ value becomes available for uptake. We suggest that during this time fish-derived nitrogen is well mixed throughout the water column, and is moved out of the bay with surface currents flowing in a north to north-westerly direction away from the SFRI raft.

Differences in $\delta^{15}N$ measured in *Gracilaria* reflect site specific differences in the relative distribution of two sources of nitrogen, and one conclusion from the above discussion is that DIN derived from the fish factories is more abundant in the north-eastern corner of the bay due to the clockwise direction of water circulation in Small Bay during summer (Weeks *et al.*, 1991). This argument is further supported by the very low $\delta^{15}N$ values measured in *Gracilaria* cultivated on the Sea Harvest raft to the south-east of the effluent pipe. Furthermore, it appears that the DIN originating from the effluent is kept buoyant in the surface layer above the thermocline as is seen from the comparison of $\delta^{15}N$ values of *Gracilaria* in the natural and raft populations. Apart from the high seaweed $\delta^{15}N$ values measured in the north-east corner of the bay, the conclusion from the isotope data is consistent with our knowledge of the hydrodynamics of Saldanha Bay (refer to Chapter 1 and references therein). Therefore, if effluent-derived nitrogen makes up a significant proportion of the total nitrogen utilised by *Gracilaria* on the SFRI raft, then growth rates obtained there should be higher than at the Fish raft. However, up to this point in the discussion seaweed growth data and C:N ratios obtained during isotopic analysis have not been considered in detail. Paradoxically, these data suggest that the growth of *Gracilaria* on the suspended rafts is limited by the availability of nitrogen, despite the fact that cultivated *Gracilaria* on the SFRI raft is significantly enriched with the heavy isotope of nitrogen which is indicative of fish-derived nitrogen. One possible explanation for this contradiction is that at times when there is little wind, much of the available DIN is taken up by phytoplankton before it reaches the SFRI seaweed raft. Under higher wind strengths, however, surface currents are stronger and the nitrogen reaches the far corner of the bay quicker, leaving insufficient time for complete removal by phytoplankton. It is under these conditions that *Gracilaria* is given a chance to take up fish-derived nitrogen and its $\delta^{15}N$ value is changed depending on the amount of effluent nitrogen taken up.

Young lateral branches show a faster response of tissue $\delta^{15}N$ values to change in environmental DIN $\delta^{15}N$ than main axes. During summer a difference of up to 3 ‰ is observed between the young and old tissue of the same thallus (here called $\Delta\delta^{15}N_{young\text{ - }old}$). This difference is especially pronounced when *Gracilaria* is moved from a benthic population with a history of natural marine-N consumption onto a seaweed raft exposed to effluent-derived nitrogen. The effect seems to disappear during winter when surface currents change direction and the water column becomes well mixed, resulting in the

dilution of effluent-N to sufficiently reduce the overall $\delta^{15}N$ signature of total DIN to close to that of normal marine-N. Also, $\Delta\delta^{15}N_{young\ -\ old}$ observed in populations exposed to only one source of DIN throughout their lifetime such as the Langebaan Lagoon population is very small. A difference in $\delta^{13}C$ is also observed between the two tissue types but there are insufficient data to detect seasonal or spatial patterns. In some instances $\Delta\delta^{13}C_{young\ -\ old}$ can be as much as -5 ‰. Observed carbon isotope ratios are likely to results from changing organic or inorganic carbon sources, however nothing is known about the biogeochemistry of carbon in this area and is it therefore not possible to speculate about how these changes occur.

The reason for a difference in δ-values between lateral branches and main axes lies in turnover rate of nitrogen or carbon, and in the case of a seaweed, it is a function of growth rate and metabolism. In his classical study, Tieszen *et al.* (1983) showed that different animal tissue types can be expected to have different isotope 'memories' which are determined by the $^{13}C/^{12}C$ ratio for source (food) carbon at the time of feeding, the $^{13}C/^{12}C$ of subsequent foodstuff, presumably after it has been taken up into the bloodstream and fractionation has occurred, and the biochemical turnover rate of carbon in the tissue. Metabolically active tissues such as fats and liver have a faster turnover of carbon (the rate at which carbon from one source is replaced by carbon from another source) than hair or the brain. Although the lateral branches are strictly speaking not a different tissue type than the main axis, the difference stems from the growth rates and nutrient uptake rates associated with each, and by analogy, lateral branches in seaweeds behave much like metabolically active tissue in animals. It is well known that growth and nutrient uptake rates are higher in finely branched seaweeds than those with a coarser thallus construction (e.g. Littler, 1981 and Littler and Arnold, 1982), and the same is likely to apply to fine lateral branches and the thick main axis. Because the lateral branches have a faster growth rate their $\delta^{15}N$ (or $\delta^{13}C$) memory should be less than the slower growing main axis and therefore the original nitrogen pool is continually diluted by nitrogen taken up to sustain growth, and for that reason shows a quicker response to the change in ambient DIN source. In other words, if *Gracilaria* is moved from a natural population exposed to only marine-N throughout its lifetime to a raft which receives fish derived nitrogen, the older main axis will still show predominantly marine $\delta^{15}N$ values while the value in young laterals will rapidly approach that of effluent DIN.

Results from this study are valuable for assessing the effect of effluent-N on natural and cultivated seaweed populations in Small Bay. In summary, fish derived nitrogen is assimilated by raft-cultivated seaweed in Small Bay to the north and east of the outfall during summer. Natural *Gracilaria* populations inside Small Bay, as well as those cultivated to the south-west of the outfall show very little evidence of taking up effluent-N. During winter the $\delta^{15}N$ signal in cultivated seaweed on rafts inside Small Bay decreases, showing that the contribution of effluent-N to total-N accumulation decreases, either because of dilution of the effluent-N pool with marine-N, or because of a shift in direction of surface currents. These results are not only important in terms of the distribution of pollutants in the bay, but also from a site selection point of view, because they explain differences observed in earlier studies (Anderson *et al.*, 1996a) between rafts situated inside Small Bay and those near the mussel rafts close to the mouth of Small Bay. The results suggest that sites inside the bay are more suited for *Gracilaria* cultivation because effluent-N could help to alleviate nitrogen limitation, providing better growth rates. Rafts near the mouth of Small Bay do not receive effluent-N in summer when surface water is oligotrophic, and have poor growth rates compared to those inside Small Bay.

Although these results are useful for explaining growth and productivity-related problems faced by seaweed mariculturists, they do not give any indication of the system's trophic status and the paradoxically low phytoplankton density in the area. Answers to this question lie in the dynamics of phytoplankton populations in Small Bay. One method for determining a system's trophic status is to find relationships between nutrient loadings and phytoplankton biomass. Vollenweider (1976) used a simple linear model to relate chlorophyll-*a* concentration in the upper mixed layer of a lake in relation to annual phosphorus loading corrected for surface area, depth and water turnover time. Boyton *et al.* (1996) used a similar model based on aerial nitrogen fluxes and chlorophyll-*a* concentrations. In Saldanha Bay such a model will have to be refined because of the system's physical characteristics, in particular the well mixed nature of the water column in winter and stratification in summer. Further corrections will have to be made for water residence time. At present such a model cannot be developed for Small Bay due to the lack of detailed phytoplankton biomass data for the system. To thoroughly understand Saldanha Bay's nutrient dynamics and the system's response to effluent disposal in the

area it is essential that all sources and sinks of nitrogen and phosphorus be studied in detail.

5 *Nitrogen Uptake Kinetics*

5.1 Introduction

A knowledge of the factors affecting nutrient uptake by seaweeds is critical to our understanding of the effects of nutrient availability on growth and production (Fujita, 1985). The availability of nitrogen has long been implicated as one of the major factors limiting the growth of micro-and macroalgae in the sea (Dugdale, 1967; Chapman and Craigie, 1977; Rosenberg and Ramus, 1982; Lavery and McComb, 1991), and it has been shown that the addition of nitrogen can greatly enhance the growth rate and production of mariculture species (Lapointe and Ryther, 1979; Smit *et al.*, 1997). An increase in nitrogen loading in natural waters due to anthropogenic activities has also been shown to result in the development of blooms of opportunistic species such as *Ulva lactuca* (L.) (Anderson *et al.*, 1996b), *Gracilaria tikvahiae* (McLachlan) and *Cladophora vagabunda* (L.) (Peckol *et al.*, 1994). The development of these blooms has been ascribed to morphological and hence physiological characteristics of the algae and can often be predicted by the functional form model (Littler, 1980; Littler and Arnold, 1982). According to the model, seaweeds with a large surface area to volume ratio such as membranous *Ulva* spp., or finely branched species such as *Cladophora* spp. have faster rates of nutrient uptake than species with a coarser or thicker thallus construction (Wallentinus, 1984). According to Rosenberg and Ramus (1982) the higher rate of nutrient uptake of opportunistic species (with high surface area to volume ratio) imparts a competitive advantage over other groups of macroalgae under eutrophic conditions.

The functional-form model is also important from a mariculture perspective where the requirement for rapid biomass production favours seaweeds with fast growth, and hence fast photosynthetic and nutrient uptake rates. Morphological plasticity and hence variability of surface area : volume ratios is well known among many red algae of which *Gracilaria* is probably the best known (Santelices *et al.*, 1995). This suggests that the functional-form model, initially developed to integrate the adaptive significance of thallus morphology relative to seaweed productivity and survival, can also be applied in selecting strains or clones within one species (Hanisak *et al.*, 1990) which might be best suited for mariculture applications.

Three sources of dissolved inorganic nitrogen (DIN) are available for uptake by macroalgae in the marine environment, *viz.* ammonium, nitrite and nitrate. Many studies have focused on the uptake kinetics of ammonium and nitrate (e.g. Haines and Wheeler, 1978; Wallentinus, 1984; Friedlander and Dawes, 1985; Lewis and Hanisak, 1996) but fewer have looked at the kinetics of nitrite uptake (e.g. Topinka, 1978; Probyn, 1985) because of its low natural concentration. In Saldanha Bay, on the southern African west coast, ammonium, nitrite and nitrate can reach concentrations of up to 5, 0.6 and 20 µM nitrogen respectively (unpublished data). According to Probyn (1985) nitrate is the major nitrogen source utilised by the kelp *Ecklonia maxima* (Osbeck) Papenfuss, while ammonium contributes less than 4 % of the total nitrogen uptake during upwelling. Up to now no study has identified the preferred source of nitrogen for *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham.

The uptake of nitrogen by algae can be measured *in situ* (Probyn and McQuaid, 1985) or under controlled conditions (Pedersen, 1994) in the laboratory. Nutrient uptake is often determined by measuring the disappearance of the nutrient from the culture medium over a time interval after the addition of the alga. The uptake rate (V) of nutrients is most often described as a hyperbolic function of substrate concentration (S), by analogy to the Michaelis-Menten expression used to model enzyme catalysed reactions (Dowd and Riggs, 1965). The Michaelis-Menten equation assumes that uptake is unidirectional so that no losses occur after uptake. Mathematically, it is equivalent to the Monod equation (Droop, 1973; Droop, 1977; Pasciak and Gavis, 1974; Gavis, 1976; Rosenberg *et al.*, 1984), but where the Michaelis-Menten relationship describes nutrient uptake rate as a function of substrate concentration, the Monod model relates growth rate to substrate availability [by assuming that cells are always in equilibrium with their surroundings and that growth is exponential]. The coupling of nutrient uptake to growth rate is discussed by Droop (1973, 1977) who uses a two-compartment model to describe the flow of nutrients into the cell where it forms the internal pool on which growth depends.

Two parameters can be estimated from fitting the Michaelis-Menten model to V vs. S data: V_{max}, which is the [extrapolated or theoretical] maximal rate of uptake of the nutrient of interest under the experimental conditions, and K_s, which the half saturation constant and is numerically equivalent to the value of S where $V = ½\ V_{max}$ (Dowd and Riggs, 1965). The hyperbolic geometry of the V vs. S curve suggests that in most cases uptake is not

simply a passive process relying on diffusion alone, but that it is active or facilitated (Lobban and Harrison, 1994). Alternatively, it may be the case that some other upper limit is imposed on the rate at which nutrients can be incorporated into thallus tissue (Pedersen, 1994). The parameters V_{max} and K_s are ecologically important since they describe the nutrient uptake ability of a species under conditions of nutrient limitation and allows for comparisons of nutrient uptake kinetics among species and studies (Harrison *et al.*, 1989). The past nutritional history of the seaweed can complicate matters since it often has a marked effect on the shape of the V vs. S relationship. For example, nitrogen limitation frequently changes the typical hyperbolic response to a biphasic (D'Elia and DeBoer, 1978) or linear type (Fujita, 1985). Additionally, in some instances the uptake of nutrients (e.g. ammonium) does not appear to be saturated even under high experimental concentrations irrespective of past nutritional history and then other models must be sought to describe the relationship (D'Elia and DeBoer, 1978).

Water motion is another important factor which determines the rate of nutrient uptake by macroalgae and it has been shown that increased water movement enhances the rate of nutrient uptake (Wheeler, 1980a; Neushul *et al.*, 1992). A better understanding of the effect of water motion on seaweed cultivation has led to significant advancements in suspended seaweed raft design (Neushul *et al.*, 1992). Although water motion is not a factor likely to be controllable in the open sea, knowledge of relative water motion at different localities can help with site selection and significantly enhance the productivity of seaweed cultivation systems.

The aim of this paper is to compare the ammonium and nitrate uptake kinetics of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham from Saldanha Bay, South Africa. No attempts were made to relate nutrient uptake rate and cellular nitrogen status to growth rate. For comparative purposes, different treatments are compared using the Michaelis-Menten 'constants'. Furthermore, the influence of thallus morphology and water motion on the nutrient uptake parameters are also investigated as a knowledge of these factors could be useful for future strain- and site-selection purposes important for *Gracilaria* cultivation. This knowledge of ammonium and nitrate uptake will enable us to model the *in situ* utilisation of the two forms of dissolved inorganic nitrogen in Saldanha Bay. This can be used to assess the possible importance of the seaweed as a biofilter if grown on commercial scale suspended rafts in the Bay (see Chapter 6). The nitrogen

uptake kinetics of several other species of *Gracilaria* have been looked at in detail (D'Elia and DeBoer, 1978; Fujita, 1985; Lapointe, 1987; Peckol and Rivers, 1995), but this is the first attempt to understand these processes in a South African species.

5.2 Materials and methods

5.2.1 *Gracilaria* preparation

Gracilaria specimens were collected from Saldanha Bay during winter and brought to the laboratory where they were placed in tanks of recirculating seawater. To obtain N-replete thalli, healthy specimens were transferred to Erlenmeyer flasks containing 500 mL enriched (16 µM NO_3^--N and 20 µM NH_4^+-N), filtered seawater (0.45 µm) and kept at temperatures of 15 and 20 °C at a stocking density of approximately 4 g fresh seaweed per 500 mL. The culture medium was replaced daily for a period of two weeks to prevent nitrogen limitation, and water movement was achieved by supplying compressed air to the flasks via disposable pipette tips attached to plastic airlines. Illumination was provided by cool white fluorescent tubes at 90 µmol photons m^{-2} s^{-1} and was sufficient to saturate growth (Engledow and Bolton, 1992; own data, unpublished). A second batch of *Gracilaria* was maintained under the same conditions described above, but the nutrient medium was replaced only once a week for two weeks to bring about nitrogen limitation (termed N-limited *Gracilaria* hereafter). Nitrogen uptake kinetics were determined for N-replete and N-limited *Gracilaria* acclimatised to the two temperatures in a series of perturbation experiments.

5.2.2 Perturbation experiments

NH_4^+-N and NO_3^--N uptake kinetics were determined using the perturbation method (Harrison *et al.*, 1989; Pedersen, 1994) which follows the depletion of a nutrient from the incubation medium over short time intervals after initial spiking to a high concentration. The experiment was initiated by adding 150 mL, 0.45 µm filtered seawater enriched to 50 µM NH_4^+-N or NO_3^- -N to three replicate 250 mL Schott bottles, each containing approximately 1.5 g fresh seaweed. The bottles were capped and attached to a Stuart Scientific Model SF1 flask shaker set to 150 oscillations per minute to provide turbulent water motion, were illuminated with 90 µmol photons m^{-2} s^{-1} and held at 15 or 20 °C. Water samples were drawn at 0, 15 and 30 minutes after the start of the experiment and

every 30 minutes thereafter until all nitrogen had been taken up. Controls showed that volatilisation of NH_4^+-N and uptake of NH_4^+-N and NO_3^--N were undetectable over the experimental period. NH_4^+-N was determined manually using the phenol-hypochlorite method of Solórzano (1969) and NO_3^--N was analysed on a Technicon AutoAnalyser II using the standard method of Grasshoff (1983). The analysis of water samples for NH_4^+-N concentration was done in duplicate but this was not necessary for NO_3^--N analysis due to the high analytical precision of the instrument (1 SD < 0.09). At the end of the experiment the seaweed samples were dried in an oven at 60 °C for determination of dry mass and analysed for the relative content of C and N on a Carlo-Erba CN analyser.

5.2.3 Data reduction

The effect of inorganic nitrogen concentration on the uptake rate of *Gracilaria* was modeled using the Michaelis-Menten equation when uptake rate was saturated at high substrate concentrations. To obtain the V (μg N-at g^{-1} (dry) hr^{-1}) vs. S (μM NH_4^+-N or NO_3^--N) plot, the slope between consecutive sample pairs on the S vs. time (t) depletion curve was determined and plotted against the average substrate concentration of that interval. Corrections were made for seaweed dry mass and change in incubation volume due to interval sampling. After the data were transformed to obtain V at a given S, the Michaelis-Menten model was fitted and V_{max} (μmol N g^{-1} (dry) hr^{-1}) and K_s (μM N-at) estimated using an iterative procedure in the non-linear regression module of Statistica for Windows Release 5.1. A third parameter, alpha (α), is introduced here, and is calculated as the initial slope of the Michaelis-Menten curve at substrate concentrations of less than K_s. This value is similar to α used to describe the initial slope of photosynthesis-irradiance curves (quantum efficiency) and gives an indication of the sensitivity of uptake rate to changes in substrate concentration. The greater the value for α, the greater the increase in uptake rate associated with an increase in substrate concentration and the more effective the species, strain or morphotype at taking up nutrients at low substrate concentrations. α used here is similar in concept to V_{max}/K_s (Harrison *et al.*, 1989), also denoted as α.

Since the Michaelis-Menten equation is not always applicable the data were subjected to the Hanes-Woolf linear transformation (S/V against S) and then to a linear regression (Dowd and Riggs, 1965). The Hanes-Woolf transformation was chosen above the Lineweaver-Burk or double-reciprocal ($1/V$ vs. $1/S$) and Eadie-Hofstee (V vs. V/S)

transformations for reasons given by Dowd and Riggs (1965) and because it maintains an independent variable (S) on the horizontal axis. The kinetic parameters can be obtained from the Hanes-Woolf plot with the intercept on the horizontal axis as $-K_s$ and the slope as $1/V_{max}$. Normal probability plots were used to examine the distribution of residuals and p- and correlation coefficients (r) were calculated using Pearson's product-moment correlation analysis. When residuals deviated from the expected distribution or when correlation analysis resulted in $p > 0.05$, a model with an added diffusion component was used to describe the NH_4^+-N V vs. S data. The latter model is applicable to data showing unsaturated uptake kinetics and has successfully been applied by Muscatine and D'Elia (1978) and D'Elia and DeBoer (1978) and is given by:

$$V = V_{\max} \cdot \frac{S}{K_m + S} + (K_d \cdot S) \quad \textbf{[Eq. 1]}$$

where V_{max} and K_s are the same as in the Michaelis-Menten model and K_d is the diffusion constant. The diffusion constant was obtained from the slope of the linear portion of the V vs. S plot and the product of K_d and S (rate of diffusion at that substrate concentration) was used to correct the V vs. S data (V minus rate of diffusion) to obtain a hyperbola approximated by the Michaelis-Menten equation. All statistical analyses were done using Statistica for Windows Release 5.1.

5.2.4 The effect of water movement on nutrient uptake rate

Gracilaria collected from Saldanha Bay was maintained for one week in an aquarium of recirculating seawater (0 µM N-at DIN and 2 µM inorganic-P) under saturating light conditions (90 µmol photons m^{-2} s^{-1}) at 15 °C. To determine the uptake rate of NH_4^+-N and PO_4^{2+}-P under different rates of water movement, thalli each weighing approximately 1.5 g (fresh), were placed in Schott bottles containing 150 mL enriched filtered seawater (4 µM NH_4^+-N or 2 µM PO_4^{2+}-P) with separate bottles for each nutrient. Three replicate incubation vessels were attached to flask shakers set to 0 (control), 250 or 500 oscillations min^{-1} (later referred to as still, low or high respectively). The experimental temperature and light conditions were 15 °C and 80 µmol photons m^{-2} s^{-1}. The rate of nutrient uptake was determined by measuring the disappearance of the nutrient from the medium over one 30-minute incubation period. PO_4^{2+}-P was analysed using the method of Murphy and Riley (1967) and NH_4^+-N as above. Rates of disappearance were corrected for incubation

time, volume and seaweed dry mass and uptake expressed as μmol ammonium-N or phosphate-P g^{-}1 (dry) h^{-1}.

In a subsequent experiment, the effect of water motion on the Michaelis-Menten parameters was examined by using the perturbation method as described previously. Only nitrate (~60 μM) was included in the incubation medium. The seaweed was acclimatised for a week in Erlenmeyer flasks (4 g per 500 mL) containing low nutrient (<4 μM NO_3^--N; 0 μM NH_4^+-N) water under 90 μmol photons m^{-2} s^{-1} at 15°C. This experiment allowed the assessment of the interaction between nutrient concentration and the rate of water movement (0, 250 and 500 osc. min^{-1}).

5.2.5 The effect of thallus morphology on the uptake rate of NH_4^+-N

Normal and branched morphotypes of *Gracilaria* were used in this experiment to evaluate the effect of thallus morphology on the rate of NH_4^+-N uptake. Branching was induced by maintaining seaweed fragments ranging in mass between 1 - 1.5 g (fresh) in unialgal culture in 500 mL Erlenmeyer flasks. One-third strength Provasoli Enriched Seawater (PES; McLachlan, 1973) was used as culture medium and was replaced twice a week to ensure nutrients did not become limiting. Light intensity, photoperiod and temperature were maintained at 90 μmol m^{-2} s^{-1}, 16:8 (L:D) and 20 °C. After the seaweed had been growing under these conditions for two to three months without removal of biomass, it developed a high frequency of branching and hollow interior (Plates 1 and 2, Section 2.3.1) of 14.9 ± 8.3 branches per cm.

To acclimatise the seaweeds (normal and branched) to the experimental conditions they were placed in culture at a light intensity of 90 μmol photons m^{-2} s^{-1} at 15 °C for three weeks prior to the start of the experiment. Nutrients were supplied once a week in the form of 20 μM NH_4^+-N and 2 μM PO_4^{2+}-P. The experiment was initiated by placing four replicates of each branching form into individual 250 mL Schott bottles containing 150 mL 0.45 μm filtered seawater enriched to 20 μM NH_4^+-N, and attached to a flask shaker set at 500 oscillations per min. The disappearance of NH_4^+-N was measured over the following three hours by using the perturbation method discussed previously. NH_4^+-N determination was done according to the method of Solórzano (1969). Rates of NH_4^+-N uptake were determined after correction for seaweed dry mass and incubation volume and expressed as μmol N g^{-1} (dry) hr^{-1}.

5.3 Results

5.3.1 Nutrient uptake kinetics

NH_4^+-N depletion curves of N-replete *Gracilaria* incubated at 15 and 20 °C are shown in Figure 1. The maximal rate of NH_4^+-N uptake (the slope over consecutive time intervals) decreases with increasing incubation time and is non-linear with time. The shapes of the curves are virtually identical for the two temperature treatments. The two parts of the curve suggest a biphasic uptake response representing the diffusive (60 – 140 minutes) and saturable (0 – 60 minutes) components.

Both linear and Michaelis-Menten models fit the uptake rate (V) vs. substrate concentration (S) data for N-replete and N-limited *Gracilaria* extremely well with Pearson's product-moment correlation coefficients (r) of greater than 0.97 in all cases (Table 1). However, because linear Hanes-Woolf transformations did not yield significant correlation coefficients and resulted in deviations from the expected distribution of residuals, it was felt that linear regressions are most appropriate. Linear models fitted to V vs. S data are represented in Figure 2a-b. The linear response shows that uptake is not saturated within the range of NH_4^+-N concentrations used in these experiments for either N-replete or N-limited material at the two temperatures. Slopes of the regression lines were 1.81 and 2.01 for N-replete *Gracilaria* at 15 and 20 °C respectively, and increased to 2.45 and 3.16 in the N-limited material (Table 1).

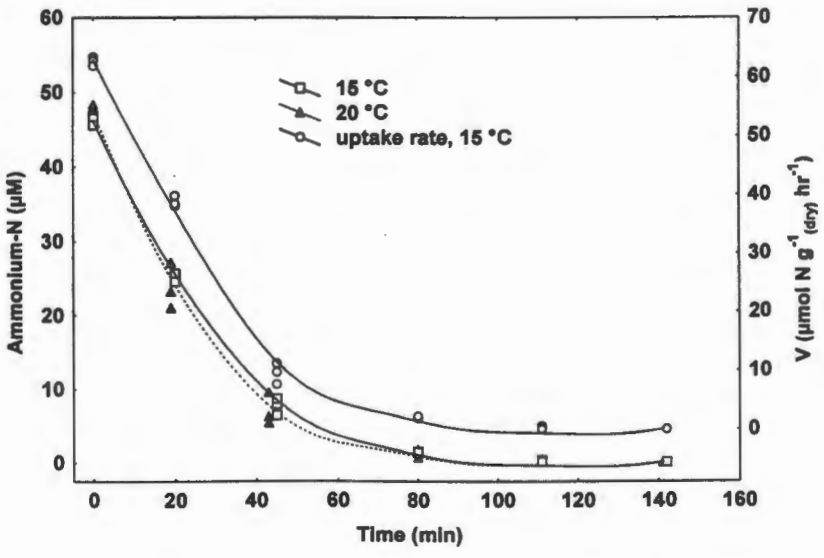

Figure 1. Time-course of NH_4^+-N depletion for N-replete *Gracilaria*. Curves fitted using least-squares estimation.

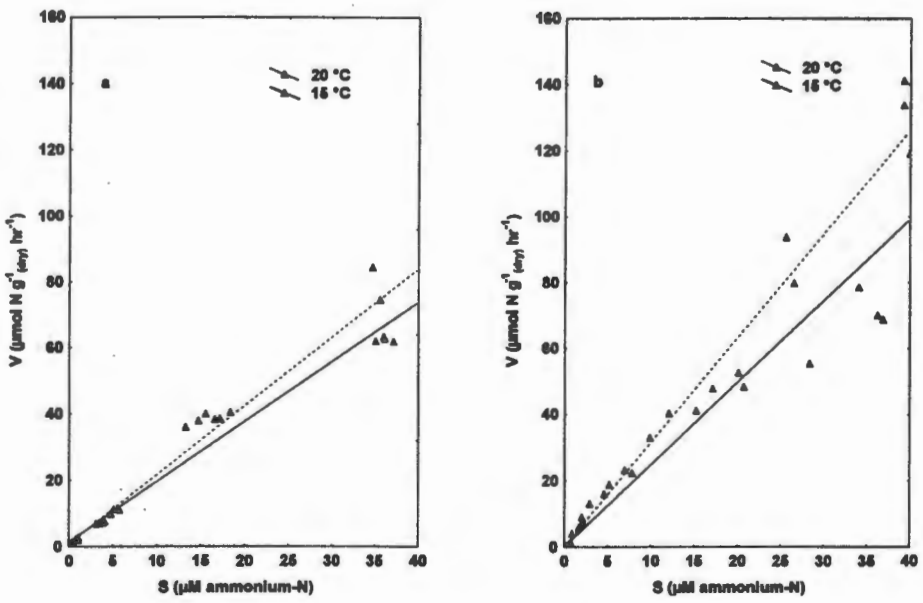

Figure 2. Linear regressions of NH_4^+-N uptake results for N-replete (a) and N-limited (b) *Gracilaria*. Regression statistics are given in Table 1.

Corrections were made to compensate for diffusion and the resulting Michaelis-Menten curves are shown in Figure 3a-b. Estimates of V_{max}, K_s and K_d are given in Table 1, together with the associated correlation coefficients and percentage variance explained by the function. The curves in Figure 3 indicate the high NH_4^+-N affinity component of uptake. A linear transformation of the diffusion corrected data shows that the NH_4^+-N uptake kinetics of only the N-replete *Gracilaria* acclimatised to 15 °C can be described by the diffusion-corrected Michaelis-Menten expression.

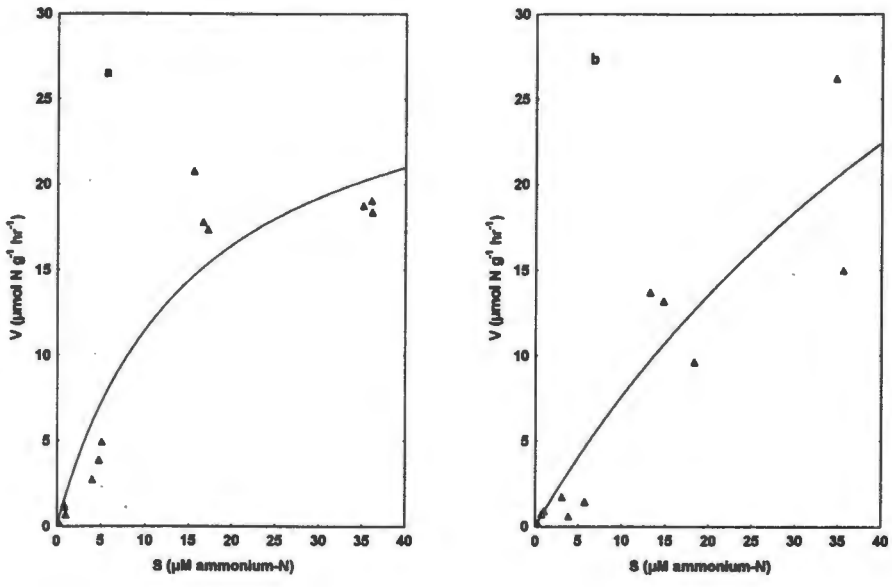

Figure 3. Michaelis-Menten curves applied to diffusion-corrected NH_4^+-N uptake data for N-replete *Gracilaria* at 15 °C (a) and 20 °C (b). Curve-fit statistics are shown in Table 1.

The uptake kinetics of NO_3^--N by *Gracilaria* are distinctly different from those of NH_4^+-N. Examples of NO_3^--N depletion over time typical of Michaelis-Menten kinetics are shown in Figure 4. The linear part of the curve between the start of the experiment and 80 minutes shows concentration-independent or rate-saturated transport. The second part of the curve indicates the concentration-dependent or rate-unsaturated uptake. As is the case with NH_4^+-N, the depletion curves at 15 and 20 °C are very similar.

Table 1. NH_4^+-N uptake kinetic parameters and statistics associated with linear and Michaelis-Menten models fitted to V vs. S data (K_s = µM; V_{max} = µmol N-at dry g^{-1} hr^{-1}; K_d = litre g^{-1} (dry) hr^{-1}). Values in {} are the slopes of the regression equations. I - Parameter estimates yielded unrealistically high V_{max} and K_s values that were subsequently omitted from the table. The α-parameter is the slope of the Michaelis-Menten curve estimated below the K_s concentration.

Linear regression:

	Models	p	r
N-repl., 15 °C	$V = 1.4097 + \{1.8089\} \cdot S$	0.000	0.988
N-repl., 20 °C	$V = 0.77001 + \{2.0779\} \cdot S$	0.000	0.981
N-lim., 15 °C	$V = 1.0019 + \{2.4515\} \cdot S$	0.000	0.970
N-lim., 20 °C	$V = -0.2742 + \{3.1636\} \cdot S$	0.000	0.973

Michaelis-Menten:

	K_s	V_{max}	V_{max}/K_s	α	r	% var. expl.
N-repl., 15 °C	55.6	161.4	2.9	2.0780	0.998	99.5
N-repl., 20 °C	96.9	271.0	2.8	1.8089	0.985	96.9
N-lim., 15 °C	I	I			0.970	94.1
N-lim., 20 °C	I	I			0.973	94.6

Michaelis-Menten – diffusion corrected:

	K_s	V_{max}	V_{max}/K_s	K_d	α	r	% var. expl.
N-repl., 15 °C	15.3	29.0	1.9	1.2	0.8472	0.966	93.3
N-repl., 20 °C	75.5	64.7	0.9	1.7	0.4022	0.938	87.9

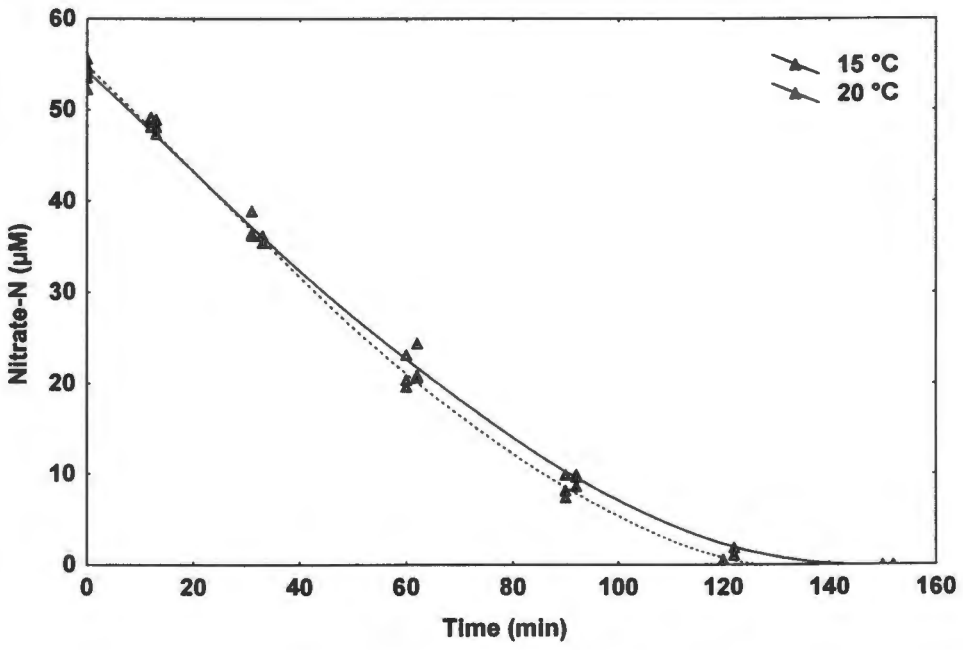

Figure 4. Time-course of NO_3^--N depletion measured for N-replete *Gracilaria*. Curves fitted using least squares estimation.

Michaelis-Menten curves fitted to data obtained for N-replete *Gracilaria* at 15 and 20 °C are virtually identical (Figure 5a), with similar V_{max} values obtained at the two temperatures (34.6 – 35.0 µmol N-at g^{-1} (dry) hr^{-1}). K_s at 15 °C however, is higher than at

20 °C (6.9 and 5.6 μM NO_3^--N at 15 and 20 °C respectively). Table 2 gives the K_s and V_{max} values estimated from the Michaelis-Menten expression, as well as the same parameters obtained from the Hanes-Woolf transformation for comparison. The Michaelis-Menten model explains 90 and 95 % of the variability of the data at 15 and 20 °C, with correlation coefficients of greater than 0.94 in both cases. Linear Hanes-Woolf transformations of the same data are given in Figure 5b-c, showing highly significant linear correlations of S/V and S for each plot ($r > 0.9$). The Michaelis-Menten model is clearly well suited to describing the data.

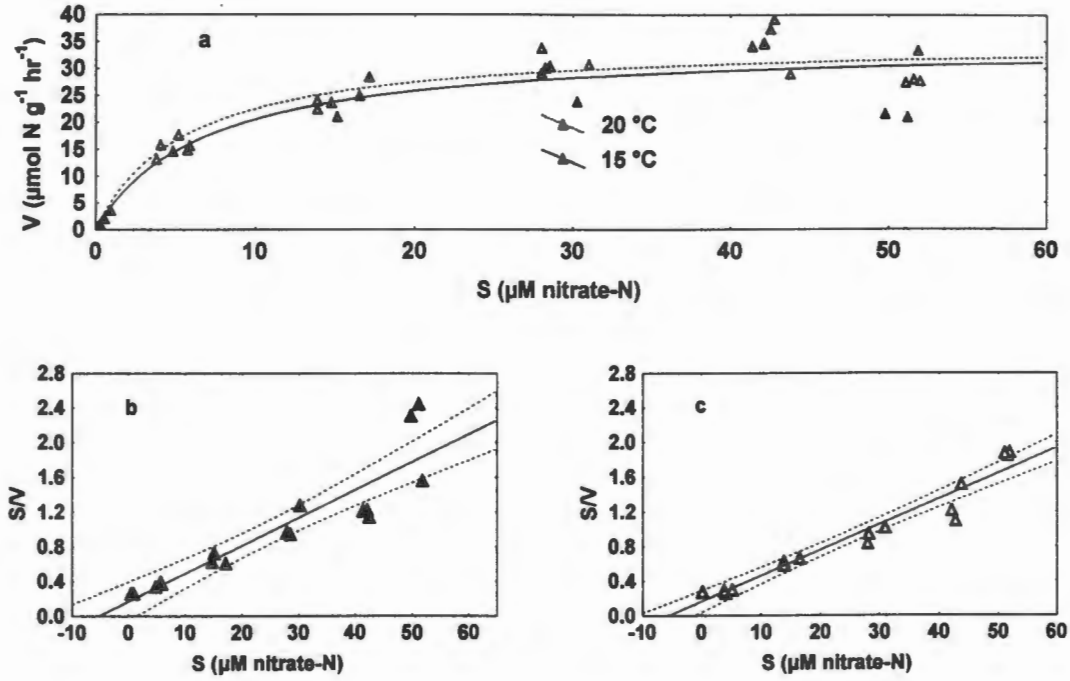

Figure 5. Michaelis-Menten curves of N-replete *Gracilaria* NO_3^--N uptake data (a), and Hanes-Woolf transformed plots of the same data at 15 °C (b) and 20 °C (c). 95 % confidence intervals are given for the linear regressions. Estimates of kinetic parameters are given in Table 2.

Table 2 gives the kinetic parameters and curve-fit statistics of Michaelis-Menten and Hanes-Woolf plots for NO_3^--N uptake by N-limited *Gracilaria* at 15 and 20 °C. Linear regressions of Hanes-Woolf transformed data are not significant and suggest that an alternative to the Michaelis-Menten model be sought in order to adequately explain the V vs. S response determined. The reason for the deviation from the expected linear S/V vs. S

relationship is that uptake rates ceases at NO_3^--N concentrations of less than 2 µM, resulting in disproportionately high *S/V*-values. In the absence of an appropriate kinetic model to explain the *V* vs. *S* relationship observed for N-limited *Gracilaria*, the data were analysed after omitting the *S/V* outliers at low NO_3^--N concentrations. Michaelis-Menten and Hanes-Woolf plots of these data are given in Figure 6a-c, and new estimates of V_{max} and K_s are located in Table 2. The removal of outliers resulted in highly significant ($p < 0.001$) linear regressions when *S/V* was plotted with respect to *S*. Kinetic parameters obtained from corrected data are similar to those obtained prior to removal of outliers, and show the same trends. K_s and V_{max} are both lower than for N-replete *Gracilaria*, with the lowest values estimated for the material acclimatised to 15 °C. A feature of Figure 6a is the low uptake rate at high NO_3^--N concentrations [at the start of the experiment, especially evident for material acclimatised at 20 °C] followed by an increase approximately 30 minutes later. The possibility of inhibition of uptake at high NO_3^--N concentrations and the inhibition of NO_3^--N uptake due to the presence of NH_4^+-N were therefore evaluated in subsequent perturbation experiments below.

Table 2. Kinetic parameters estimated for four NO_3^--N perturbation experiments from Michaelis-Menten* curves and Hanes-Woolf# plots. Correlation coefficients and *p*-values, as well as % variance explained are also present to indicate how well the respective models fit the data. K_s = µM NO_3^--N; V_{max} = µmol N dry g^{-1} hr^{-1}. I - No reliable estimates for K_s or V_{max} could be obtained.

	K_s*	V_{max}*	V_{max}/K_s*	*r*	α	% var. expl.*	K_s#	V_{max}#	*r*#	*p*#
Analysed using complete data:										
N-repl., 15 °C	6.9	34.6	5.0	0.949	2.644	90.0	5.2	31.1	0.918	0.000
N-repl., 20 °C	5.6	35.0	6.3	0.977	3.529	95.4	5.5	33.8	0.972	0.000
N-lim., 15 °C	6.7	14.3	2.1	0.798	1.310	63.6	I	31.0	0.140	0.437
N-lim., 20 °C	5.4	18.4	3.4	0.840	1.066	70.6	I	I	-0.308	0.082
Analysed after removal of S/V outliers:										
N-lim., 15 °C	5.0	13.9	2.8	0.654	1.107	42.7	4.7	12.9	0.900	0.000
N-lim., 20 °C	3.4	17.7	5.2	0.594	1.442	35.3	1.3	14.3	0.892	0.000

5.3.2 Inhibition of NO_3^--N uptake at high nitrate concentrations

The effect of NO_3^--N concentration in inhibiting uptake at high concentrations was examined by conducting two perturbation experiments, one with an initial NO_3^--N concentration of 55 µM and another at 30 µM (Figure 7). Results show that uptake rate is low during the first 35 minutes of incubation whereafter it increases to about 10 – 11 µmol N g^{-1} (dry) hr^{-1} at both 30 and 55 µM NO_3^--N concentrations.

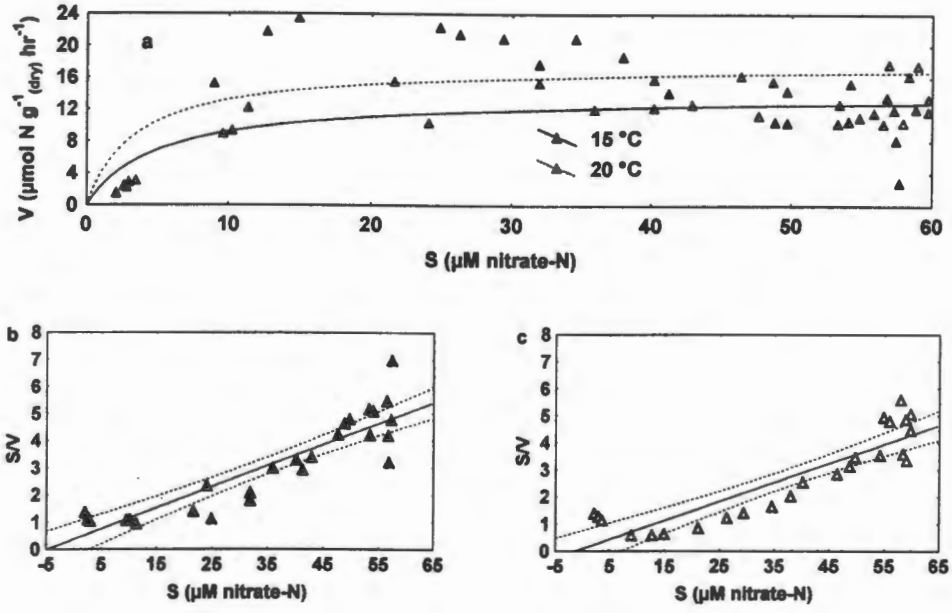

Figure 6. Michaelis-Menten curves of N-limited *Gracilaria* NO_3^--N uptake data (a), and Hanes-Woolf transformed plots of the same data at 15 °C (b) and 20 °C (c). 95 % confidence intervals are given for the linear regressions. Estimates of kinetic parameters are given in Table 2.

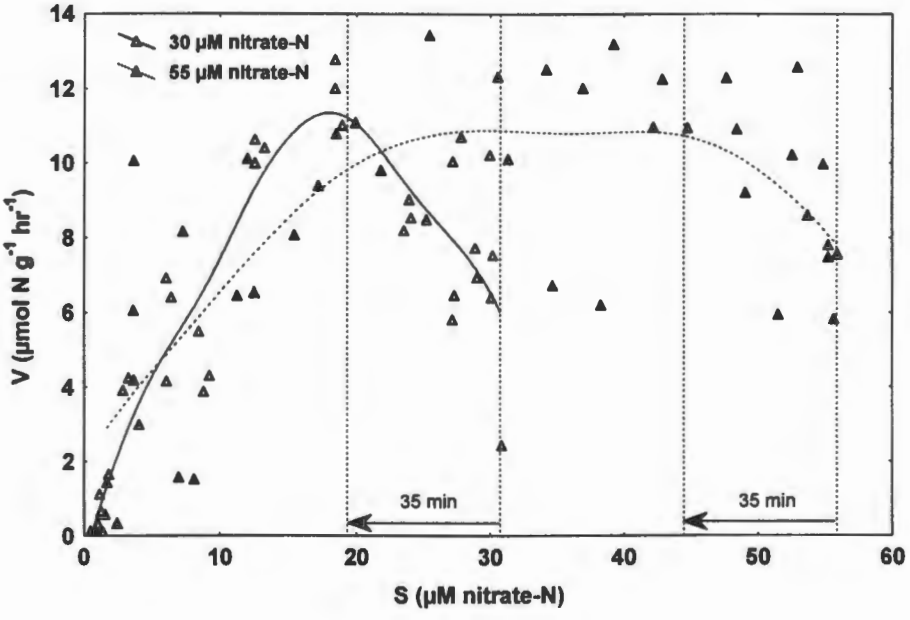

Figure 7. NO_3^--N uptake rates during incubation in two media containing different initial NO_3^--N concentrations. Arrows indicate the first 35 minutes of each experiment (arrows are not meant to be used as a second independent variable); experiments terminated when substrate concentrations reached about 0 µM NO_3^--N. Fitted lines are least-squares regressions.

5.3.3 NH_4^+-N – NO_3^--N interaction

Since the low rate of NO_3^--N uptake at the start of the experiment does not depend on NO_3^--N concentration within the range of concentrations used in the experiments, the role of NH_4^+-N in the inhibition of nitrate uptake was determined in a subsequent experiment. A perturbation experiment was conducted in which *Gracilaria* was placed in an incubation medium containing 50 µM NO_3^--N and 25 µM NH_4^+-N and the depletion of both species was followed over time. The control treatment used NH_4^+-free seawater spiked to an initial NO_3^--N concentration of about 60 µM. Depletion curves of NH_4^+-N and NO_3^--N are given in Figure 8a, showing the low rate of NO_3^--N uptake in the presence of NH_4^+-N at concentration above 5 µM. After the concentration of NH_4^+-N was reduced from about 30 µM to less than 5 µM within the first 50 minutes (Figure 8a), the rate of NO_3^--N uptake increased substantially (Figure 8b). Uptake rates as high as 16 µmol N g^{-1} (dry) hr^{-1} in the absence of NH_4^+-N were maintained until the NO_3^--N concentration reached 20 µM. In comparison, the control had high rates of NO_3^--N uptake from the onset of the experiment at 60 µM NO_3^--N.

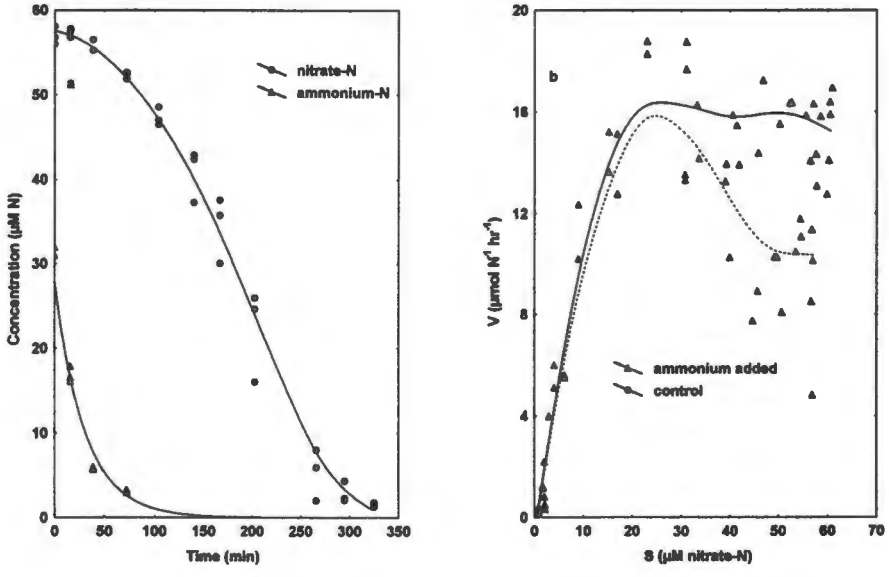

Figure 8. a) Time-course of NH_4^+-N and NO_3^--N depletion after spiking one medium with both NH_4^+-N and NO_3^--N. b) Least-squares regressions fitted to NO_3^--N uptake rate vs. substrate concentration in the presence and absence of NH_4^+-N. Data for NO_3^--N uptake in the presence of NH_4^+-N was obtained from curve shown in (a).

5.3.4 The effect of water movement on NO_3^--N uptake rate

A one-way MANOVA indicates a significant effect of water motion on the rate of NH_4^+-N and PO_4^{2+}-P uptake in *Gracilaria* (Figure 9, $p < 0.001$, d.f. = 1, 4). NH_4^+-N uptake rates at shaker settings of 0 and 250 oscillations min^{-1} are 1.09 ± 0.24 and 1.23 ± 0.22 µmol NH_4^+-N g^{-1} (dry) hr^{-1} respectively and are not significantly different as shown by the Tukey Honest Significant Difference (HSD) test ($p > 0.05$). At 500 oscillations min^{-1} the uptake rate of 1.98 ± 0.36 µmol NH_4^+-N g^{-1} (dry) hr^{-1} is significantly higher (Tukey HSD, $p < 0.005$) than at the still and low water movement treatments. A similar response was found for the rate of PO_4^{2+}-P uptake for the three treatments. At a shaker speed setting of 500 oscillations per minute the uptake rate of 2.09 ± 0.17 µmol PO_4^{2+}-P g^{-1} (dry) hr^{-1} was significantly higher than at 0 and 250 oscillations per minute with rates of 1.25 ± 0.05 and 1.13 ± 0.16 µmol PO_4^{2+}-P g^{-1} (dry) hr^{-1} respectively (Tukey HSD, $p < 0.001$ in both cases).

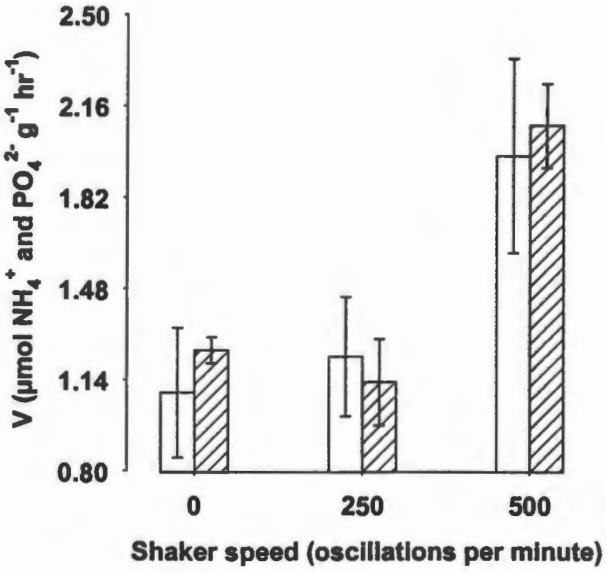

Figure 9. The effect of water movement on the rate of NH_4^+-N and PO_4^{2+}-P uptake by *Gracilaria* (± 1 SD indicated). Rates were obtained by taking the slope of the linear portion of the time-course of nutrient depletion after the start of the experiment.

Michaelis-Menten curves for NO_3^--N uptake at three rates of water motion are shown in Figure 10. An increase in water motion from still conditions to a shaker speed setting of 500 oscillations min^{-1} affects mainly the Michaelis 'constant', K_s (Table 3), so that the

affinity of *Gracilaria* for NO_3^--N increases at high rates of water motion. The effect on V_{max} is less clear, but the highest maximal rate of uptake occurs at 500 oscillations min^{-1}.

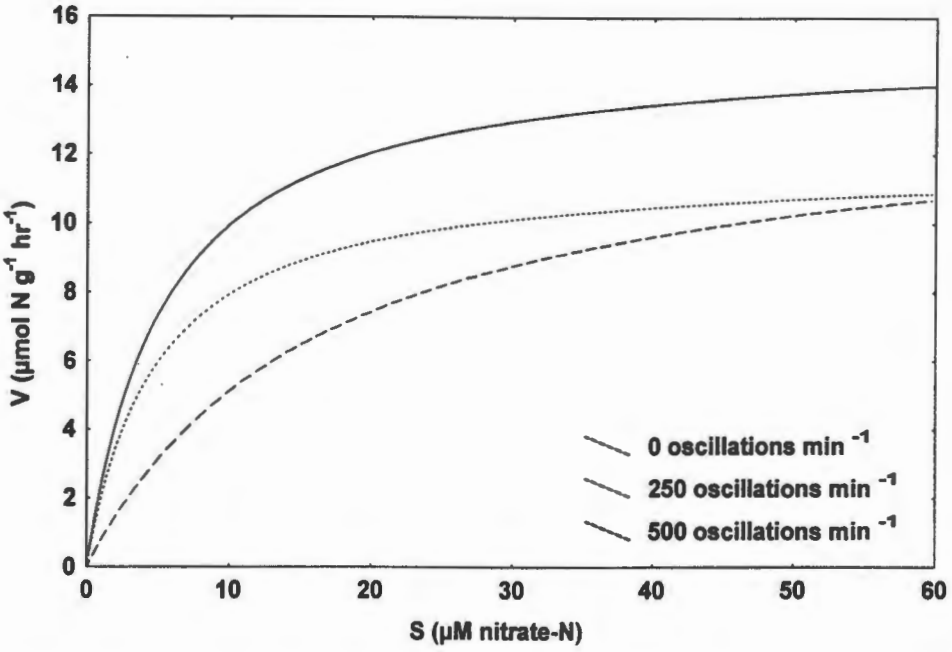

Figure 10. Michaelis-Menten curves for NO_3^--N uptake by *Gracilaria* under three rates of water movement. Data-points are not shown to prevent clutter. Curve-fit statistics and kinetic parameters are given in Table 3.

Table 3. Kinetic parameters estimated for NO_3^--N uptake under three rates of water movement, and statistics associated with the fit of the model to the data. The Hanes-Woolf transformation was significant in all cases ($p < 0.05$).

	K_s	V_{max}	V_{max}/K_s	α	r	% var. expl.
0 osc. min^{-1}	16.8	13.7	0.8	0.447	0.844	71.2
250 osc. min^{-1}	4.8	11.8	2.5	1.269	0.861	74.2
500 osc. min^{-1}	5.4	15.3	2.8	1.991	0.876	76.7

5.3.5 The effect of thallus morphology

Thallus morphology does not have an effect on the NH_4^+-N uptake response at different substrate concentrations (Figure 11a-b). Uptake kinetics of both morphotypes could be described by Michaelis-Menten as well as linear equations, but the complete lack of correlation in the Hanes-Woolf transformed data suggests that a linear model describing rate-unsaturated uptake best suited the data (Table 4). There is virtually no difference between the linear models for the two thallus forms.

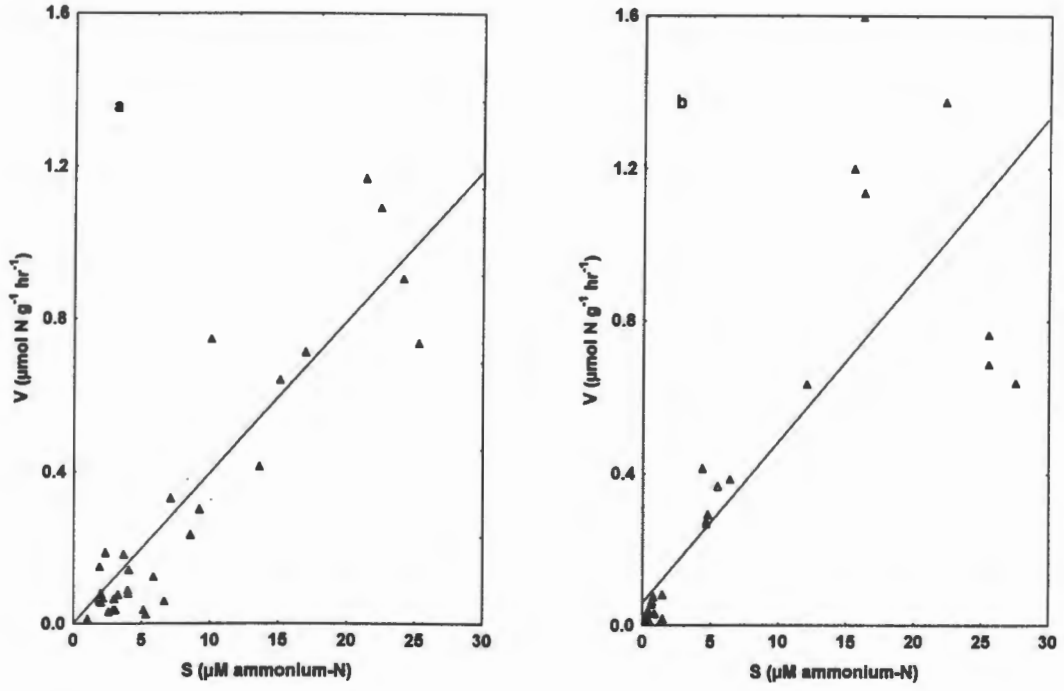

Figure 11. Rate of NH_4^+-N uptake by normal (a) and branched (b) morphotypes of *Gracilaria* as a linear function of substrate concentration. See Table 4 for curve fit statistics.

Table 4. NH_4^+-N uptake kinetics of two *Gracilaria* morphotypes.

	Model	*p*	*r*
Normal	$V = 0.0475 + 0.04296 \cdot S$	0.000	0.924
Branched	$V = 0.05923 + 0.04250 \cdot S$	0.000	0.820

5.4 Discussion

By utilising the perturbation method it is possible to obtain the uptake rates at many substrate concentrations in one experiment, and it gives information on the internally and externally controlled phases of nutrient uptake (McGlathery *et al.*, 1996). This method is also used to describe uptake during transient phases of high nitrogen availability (Probyn and Chapman, 1982). The drawback of the method, however, is that the nutritional status changes during the course of the experiment which has an effect on nutrient uptake rates by the seaweed (Fujita, 1985). An alternative method is the multiple flask method (Harrison *et al.*, 1989; Pedersen, 1994) which measures the disappearance of the nutrient over a very short time interval from several flasks, each enriched to a different initial nutrient concentration. The latter method has the advantage that if applied correctly, the surge uptake of the nutrient can be determined. For this study the perturbation method was preferred because an estimation of the actual uptake kinetics of *Gracilaria* in Saldanha

Bay was sought, in other words, the internally and externally controlled phases of uptake were of greatest interest. Surge uptake was not measured in this study because it was not considered to be ecologically meaningful.

The Michaelis-Menten model has been used in many studies on the nutrient uptake mechanism of algae (e.g. Dugdale, 1967; Chrisholm and Stross, 1976; Topinka, 1978; Probyn and Chapman, 1982; Williams and Fisher, 1985; Parker, 1993; Peckol *et al.*, 1994; Portielje and Lijklema, 1994; Vergara *et al.*, 1995). This equation describes the uptake rate of a nutrient as a function of its concentration in the external culture medium. The rectangular hyperbolic geometry of the uptake rate – substrate concentration relationship is characteristic of a facilitated diffusion or an active uptake mechanism, which shows a saturation of the carriers responsible for transporting the nutrient across the membrane (analogous with enzyme kinetics) (Lobban and Harrison, 1994). Alternatively, the saturation response has been linked to the rate at which amino acids can be incorporated into biomass (Pedersen, 1994). Results from this study show that NO_3^--N uptake by *Gracilaria* is always associated with a rate-saturating mechanism characteristic of the Michaelis-Menten relationship and is influenced by the temperature at which the seaweed has been acclimatised as well as its nutrient history. The two methods of estimating the Michaelis-Menten parameters yielded similar V_{max} and K_s values. An exception was for N-limited *Gracilaria* before correction had been applied by removing outlying S/V values in which case V_{max} was exaggerated. In N-replete *Gracilaria* V_{max}, or its capacity for uptake (Vergara *et al.*, 1995), was not affected by temperature, but K_s was lower at 20 °C indicating a higher affinity for NO_3^--N at that temperature. α was also affected by temperature and the increase in the value of this parameter at 20 °C (for N-replete material) indicates that the seaweed became more efficient at removing NO_3^--N from the incubation medium at low ambient concentrations. Harrison *et al.* (1989) and Pedersen (1994) suggest the ratio V_{max}/K_s (also called α in their studies) as a measure of the initial slope of the Michaelis-Menten curve. However, when α is calculated as V_{max}/K_s, a very different value to the true slope of the line is obtained (from the $V - S$ pairs below the value of K_s), as is shown in Tables 1 – 3. The uptake rate of NO_3^--N is reduced by about 38 % in the presence of NH_4^+-N. The reduction in uptake rate lasts for as long as NH_4^+-N is present in the culture medium at concentrations above 5 µM, typically 30 – 35 minutes in these experiments. The inhibition of NO_3^--N uptake in the presence of NH_4^+-N has also

been shown for *Enteromorpha intestinalis* (L.) Grev., *Gracilaria pacifica* Abbott (Thomas and Harrison, 1987), *Enteromorpha prolifera* (Müller) J. Agardh and *Ulva* sp. (O'Brien and Wheeler, 1987) and phytoplankton (Parker, 1993).

In contrast to the uptake of NO_3^--N, NH_4^+-N uptake was not saturated within the experimental concentrations indicating a multi-component uptake response to substrate concentration. A similar switch from Michaelis-Menten kinetics to non-saturable uptake has also been demonstrated for *Neoagardhiella baileyi* (Harvey ex Kützing) Wynne and Taylor (D'Elia and DeBoer, 1978), *Gracilaria tikvahiae* (Fujita, 1985) and *Ulva rigida* C. Agardh (Lavery and McComb, 1991). Non-saturable kinetics have been shown for corals with symbiotic algae (Muscatine and D'Elia, 1978), *N. baileyi* and G. *foliifera* (Forsskål) Børgesen (D'Elia and DeBoer, 1978), *G. tikvahiae* (Friedlander and Dawes, 1985) and *Chaetomorpha linum* Kützing and *U. rigida* (MacFarlane and Smith, 1982; McGlathery *et al.*, 1996). The two parts of the curve seen in the time-course of NH_4^+-N depletion (Figure 1) correspond to the low- and high-affinity systems. The high-affinity mechanism operates at low substrate concentrations and has a characteristic low K_s-value. The low-affinity system is seen at high NH_4^+-N concentrations and is dominated by a diffusive component, which is unsaturated and proportional to the substrate concentration (MacFarlane and Smith, 1982; Friedlander and Dawes, 1985; Lobban and Harrison, 1994). The physiological basis of biphasic kinetics has been discussed in detail by MacFarlane and Smith (1982) and to some extent by Lobban and Harrison (1994). Basically, the high rate of NH_4^+-N uptake at high external substrate concentrations is due to a pH gradient that exists between the seawater (~8.2) and the cell vacuole and/or cytoplasm (5.0 – 6.0). In seawater, reduced nitrogen occurs as neutral (NH_3 – ammonia) and ionic (NH_4^+ – ammonium) molecules or ions, and the pH determines the proportion of the two species. At a pH of 8.2, about 5 –10 % of the total NH_4^+ is present as NH_3. Ammonia is polar and rapidly diffuses across cell membranes. Once NH_3 is present in the vacuole it becomes protonated to NH_4^+ and cannot diffuse back across the membrane. This process is called acid trapping and in marine diatoms it can theoretically result in the accumulation of NH_4^+ by a factor of 10^3 (Wheeler and Hellebust, 1981). At low substrate concentrations, NH_4^+ is taken up via carriers (or porters) embedded in the membrane and this process is limited by the rate at which diffusion can supply NH_4^+ to the reaction sites across the boundary layer (MacFarlane and Smith, 1982). In the case of biphasic kinetics, K_s and V_{max} can only be

estimated after correction for diffusion has been applied (D'Elia and DeBoer, 1978) in order for uptake to be explained by the Michaelis-Menten relationship.

According to Probyn and Chapman (1982), an ecological advantage of biphasic uptake would be seen in a non-homogeneous environment where NH_4^+-N concentration fluctuates temporally. In such a system, the seaweed would be able to function over a wide range of NH_4^+-N concentrations because it can adjust its uptake kinetics up or down in order to acclimatise to transient patches of varying NH_4^+-N availability (Fujita, 1985; Williams and Fisher, 1985). However, *Gracilaria gracilis* has a much higher affinity for NO_3^--N than for NH_4^+-N as can be seen in Figure 12 and is therefore well adapted to remove NO_3^--N from waters with low ambient concentrations. NO_3^--N would therefore contribute a greater proportion towards the total nitrogen consumption of this species. The significance of this with respect to the removal of nitrogen from Saldanha Bay will be discussed in Chapter 6. The high affinity for NO_3^--N, together with the ability of *Gracilaria* to store nitrogen internally in excess of immediate requirement (Rosenberg and Ramus, 1982; Lapointe and Duke, 1984; Peckol *et al.*, 1994; Vergara *et al.*, 1995; Smit *et al.*, 1997), make it well suited to be productive in an environment dominated by transient pulses of nutrients. Compared to NH_4^+-N uptake, the maximal potential for NO_3^--N uptake is higher at 15 °C, than at 20 °C.

The Michaelis-Menten equation can be applied to uncorrected NH_4^+-N uptake data and fits the data extremely well. However, kinetic parameters estimated consequently are likely to be exaggerated and unreliable due to the uncertainty associated with extrapolation to very high substrate concentrations and uptake rates (Table 1). Furthermore, high V_{max} and K_s values obtained from uncorrected data are not ecologically significant because levels of NH_4^+-N and NO_3^--N are unlikely to reach concentrations higher than 4 and 25 µM *in situ* [in Saldanha Bay]. Essentially, NH_4^+-N uptake is a linear function of the range of substrate concentrations found under natural conditions if diffusion correction is not applied.

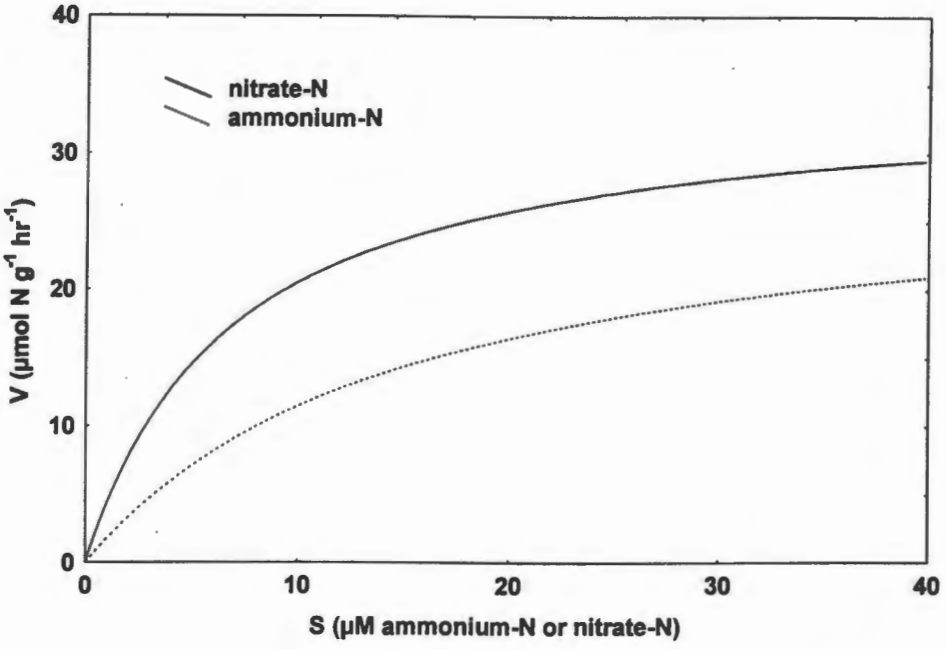

Figure 12. Hypothetical Michaelis-Menten curves for NH_4^+-N and NO_3^--N uptake by N-replete *Gracilaria gracilis* of Saldanha Bay.

Highly significant linear regressions can also be fitted to V vs. S data (Table 1) without prior correction for diffusion but estimation of kinetic parameters is not possible. Two pieces of information can be obtained from linear regressions: the slope (α) and y-intercept. A steep slope (dV/dS) indicates a faster uptake rate at a given substrate concentration, and a positive y-intercept points to the possible presence of a diffusive component (Lavery and McComb, 1991). For instance, the slopes of the linear regressions in this study clearly show that uptake is a function of temperature and tissue nitrogen status, with the lowest slope (lowest response of uptake rate to substrate concentration) in N-replete *Gracilaria* at 15 °C, and the highest at 20 °C for N-limited material. Fujita (1985) has shown that NH_4^+-N uptake is similarly affected by N-limitation in *Ulva lactuca* and *Gracilaria tikvahiae*. The presence of a positive y-intercept for N-replete *G. gracilis* (this study) suggests the presence of biphasic kinetics, which could be confirmed by diffusion correction (below). When linear $V - S$ responses are obtained, it is suggested that a diffusion term be incorporated in the model and kinetic parameters estimated if correction results in significant Hanes-Woolf plots.

Only NH_4^+-N uptake rates for N-replete *Gracilaria gracilis* could reliably be explained by multi-component uptake kinetics with a diffusive component, in which case correction

resulted in more realistic estimates of K_s and V_{max}. Nutrient history seemed to affect the shape of the relationship between uptake rate and substrate concentration as diffusion correction for N-limited *G. gracilis* was not possible, and uptake rate therefore remained a linear function of NH_4^+-N concentration. Results also show that the temperature at which *G. gracilis* had been acclimatised influenced the uptake rate of NH_4^+-N. According to Lobban and Harrison (1994) an increase in temperature has a small effect on nutrient uptake controlled solely by physical processes such as diffusion. This is supported by resultes obtained in this study, which show that an increase in temperature from 15 to 20 °C results in a 29.4 % increase in the value of K_d, the diffusion coefficient, whereas K_s increased by 79.3 %. Temperature also greatly affected the maximal potential for short-term uptake, with the highest V_{max} value estimated for *G. gracilis* acclimatised to 20 °C.

NO_3^--N uptake kinetics for N-limited *Gracilaria gracilis* differs from that of N-replete material in that N-limitation leads to decreases in V_{max}, K_s and α values. The lower K_s values [compared to those measured in N-replete *Gracilaria*] suggest an enhanced affinity for NO_3^--N, while V_{max} indicates a decreased capacity for uptake. Fujita (1985) examined the NH_4^+-N uptake and kinetic parameters of *Ulva lactuca*, *Enteromorpha* spp. and *Gracilaria tikvahiae* grown under high and low N-flux, both before and after starvation. He found that N-limitation increased the affinity for NH_4^+-N uptake while V_{max} remained unaffected. Similarly, Peckol *et al.* (1994) found that NH_4^+-N uptake of *G. tikvahiae* is faster under N-limiting conditions. D'Elia and DeBoer (1978) could not find any correlation between C:N ratio and the kinetic parameters for NO_3^--N uptake, but it was evident that NH_4^+-N uptake was directly related to the C:N ratio. Results of O'Brien and Wheeler (1987) show that NH_4^+-N uptake of *Enteromorpha prolifera* was not correlated with tissue nitrogen content while both kinetic parameters were higher for NO_3^--N uptake under reduced tissue N-content. Various other studies give variable evidence for and against enhanced NH_4^+-N or NO_3^--N uptake under different degrees of N-limitation (e.g. Probyn and Chapman, 1982; Rosenberg *et al.*, 1984; Friedlander and Dawes, 1985; Probyn and McQuaid, 1985; McGlathery, 1996).

Perhaps the most detailed study on the effect of N-limitation on uptake is by Pedersen (1994), who showed changes in the three stages of NH_4^+-N uptake (surge, internally controlled and externally controlled uptake) as a function of internal nitrogen concentration. In the first of these stages, surge uptake for *Ulva lactuca* increased up to a

point with increasing N-limitation, suggesting that this phase is more likely to be related to the decreasing size of small, short-term storage pools during incipient nutrient limitation rather than total-N content. Surge uptake results from a concentration gradient between the seaweed and the external medium, therefore a reduction of surge uptake in N-replete seaweed occurs as a result of feedback inhibition from pools of inorganic nitrogen and amino acids. In the second stage, internally controlled uptake was not affected by N-limitation and remained constant with increasing N-limitation. The rate-limiting step for this phase of uptake is the rate at which amino acids are incorporated into macromolecules and biomass. The last or externally controlled phase (alternatively referred to as the physically controlled phase by Munk and Riley (1952)) was also shown not to vary with increasing N-limitation since a limit is placed on uptake by the rate of mass transport of nitrogen across the boundary layer. The limitation of uptake by externally controlled physical factors is called 'diffusion transport limitation' (Pasciak and Gavis, 1974) and can be alleviated through an increased concentration of nutrients in the external medium, or the rate of water movement across the boundary layer. The rate of transport of a nutrient from moving water to the algal surface takes place through the boundary layer surrounding the seaweed. The rate of diffusion is directly related to the concentration gradient across the boundary layer, but inversely proportional to the thickness of the layer. Diffusion transport limitation can therefore be alleviated by increasing the concentration of the nutrient in the external medium (DeBoer *et al.*, 1978), or by decreasing the thickness of the boundary layer through increasing the water movement past the thallus (Gavis, 1976; Parker, 1982).

Several workers have shown that increased rates of water movement enhance algal growth and nutrient uptake rates above rates obtained under perfectly still conditions. Uptake rates are affected by the velocity of currents or waves past the 'stationary' fronds of attached seaweeds (e.g. Munk and Riley, 1952; Parker, 1981, 1982; Neushul *et al.*, 1992) or the sinking rate or swimming speed of planktonic algae (e.g. Pasciak and Gavis, 1974; Canelli and Fuhs, 1976; Gavis, 1976). Results from this study are consistent with these findings. Increased turbulent water motion was shown to specifically affect the values of α and K_s. These two parameters relate to the initial part of the Michaelis-Menten curve describing the externally controlled phase and are determined by the rate at which nitrogen can diffuse across the boundary layer. In this study an increase in shaker speed from completely still conditions to 250 oscillations per minute is accompanied by a 284 %

increase in α. Doubling the turbulent water motion resulted in a 445 % increase of α above still conditions. K_s is about three times lower at higher rates of water movement so that the efficiency of NO_3^--N uptake is greatly enhanced. It is clear that water motion enhances diffusion transport and reduces transport limitation. Water motion is especially important in facilitating nutrient uptake under low ambient levels of NO_3^--N often experienced in nature. For example, at 4 μM NO_3^--N, uptake rate is increased by a factor of 2.5 when water motion is increased from completely still conditions to 500 oscillations per minute. In environments with low DIN concentrations, either the rate of diffusion across the boundary layer or the affinity for nitrogen may limit growth or determine the alga's nutrient status (Fujita and Goldman, 1985). If the affinity for nitrogen is low, an increased rate of diffusion to the algal surface might exceed uptake capacity. On the other hand, if the affinity for DIN is high, nitrogen uptake might be limited by the rate at which nitrogen can be supplied to the surface.

It is not possible to relate experimental findings to conditions experienced in nature unless the exact hydrodynamic nature of the water body surrounding the suspended seaweed raft is known (Neushul *et al.*, 1992). The turbulent water motion generated in the oscillating flasks used in these experiments is likely to generate conditions similar to those experienced by *Gracilaria gracilis* attached to a suspended raft subjected to only wind-chop. However, water motion past or over a *Gracilaria* thallus attached to a raft is more complicated since it involves a unidirectional flow of water past the thallus due to the combination of surface currents, wave action and wind-chop. These results support the suggestion of Anderson *et al.* (1996a) that site-related differences in growth of *G. gracilis* at Saldanha Bay were caused by seasonal differences in water movement. Results stress the importance of proper raft design (in terms of tensioning of the seaweed lines) and positioning in areas to allow sufficient water motion to overcome nutrient transport limitation. It also shows that a comparison of nutrient uptake studies from various authors is seldom valid because the effect of water motion on the kinetic parameters is often overlooked.

Thallus morphology has been implicated as an important factor affecting seaweed growth and production (Littler, 1980, Littler and Littler, 1980; Littler and Arnold, 1982; Rosenberg and Ramus, 1984; Hanisak *et al.*, 1988) with forms having high surface area : volume ratios showing fast growth rates and high photosynthetic performances. As

discussed in Section 5.1.1, according to the functional – form model, seaweeds with a higher surface area : volume ratio should also have faster rates of nutrient uptake (Rosenberg and Ramus, 1984; Wallentinus, 1984; Hanisak *et al.*, 1990). Findings from this study do not provide supporting evidence for this in *Gracilaria gracilis* as uptake kinetics are virtually indistinguishable between the highly branched morphotype and the normal form. It is therefore possible that the unexpectedly low uptake rate measured for the highly branched form is the result of thallus age and is not a true reflection of its uptake response. These highly branched forms are considered senescent (M Steentoft, pers. comm., Chapter 2). The method used to induce branching in this study involved placing the thallus under optimal growth conditions and ageing it to a state presumably never attained in the wild. Initially the optimal conditions resulted in rapid growth but this decreased steadily as branching developed (Chapter 2). Branched morphotypes are also often found on suspended seaweed rafts when material from previous *G. gracilis* harvests is used to seed subsequent seaweed lines. Whether morphological differentiation takes place on a suspended seaweed raft or in the laboratory under controlled conditions it is probably a function of initial rapid growth rate and extreme age. Apart from low nutrient uptake rates (relative to a hypothetical young branched specimen that does not occur naturally), these branched forms also show a reduced photosynthetic capacity (unpublished data). In support of these findings, Littler and Arnold (1980), Wheeler (1980), Parker (1982) and Gómez *et al.* (1996) have also shown thallus age to greatly affect seaweed productivity.

This paper discusses the first study on the nitrogen uptake ecophysiology of a southern African species of *Gracilaria*. Our results show that *G. gracilis* is well adapted to survive in an environment dominated by the transient availability of DIN through the use of a high affinity system for NO_3^--N and biphasic kinetics with a strong diffusive component for NH_4^+-N uptake. Failing to recognise the presence of biphasic kinetics through diffusion correction could be a possible pitfall that might cause one to incorrectly apply the Michaelis-Menten model. This stresses the importance of rigorously validating the appropriateness of a kinetic model prior to final application to avoid such an error, and techniques to do so are discussed in detail here. It is not certain how uptake rates of *G. gracilis* measured here compare to rates measured *in situ* or in specimens that were not maintained in cultivation for any length of time. Despite this, it is likely that rates

measured in the laboratory are underestimations. For example, Fujita (1985) found that NH_4^+-N uptake rates determined for *Ulva lactuca* and *Gracilaria tikvahiae* were higher when measured in freshly collected material compared to those grown in the laboratory at high or low N-flux for a period of time prior to the experiments. Furthermore, from a mariculture perspective nutrient uptake rates are of limited value in allowing one to judge a species' growth performance. It is therefore suggested that future studies attempt to apply the Droop (1974, 1977) model which allows one to examine the de-coupling between growth rates and external substrate concentrations. By applying this model it should be possible to show to what extent growth rate is controlled by the availability of internally stored nitrogen, while also showing the dependence of thallus nitrogen on environmental nutrient availability through an understanding of uptake kinetics. Doing so would provide not only the desired physiological knowledge, but also information useable by seaweed mariculturists who attempt to link growth rates to environmental conditions.

However, these results are useful for providing conservative estimates of *in situ* rates of nutrient removal from the natural environment, and allow us to evaluate the effectiveness of *Gracilaria gracilis* as a biofilter in Saldanha Bay (see Chapter 6).

6 *Estimation of* In Situ *Nitrogen Uptake*

6.1 Introduction

Seaweeds are important contributors of primary production in nearshore areas (Chapman and Craigie, 1977; Bolton and Levitt, 1987; O'Brien and Wheeler, 1987; Vidondo and Duarte, 1995) and in some instances they may attain production rates greater than that of phytoplankton (Solidoro *et al.*, 1995). This is especially true in the case of shallow coastal areas where benthic macroalgae are not limited by light availability (Lapointe and Duke, 1984). Under these conditions, macroalgae may be very important in affecting nutrient cycling (Viaroli *et al.*, 1993; Pihl *et al.*, 1996; Viaroli *et al.*, 1996). Since biomass production often responds strongly to nutrient additions (Menesguen and Pireou, 1995; Vidondo and Duarte, 1995) they may also act as a sink for excess nutrients released into the sea as result of anthropogenic activities. This opens up the possibility of using seaweeds as natural filters (or biofilters) to remove dissolved inorganic nitrogen (DIN) from water (Ryther *et al.*, 1972; Goldman *et al.*, 1974; Ryther *et al.*, 1975, Smit *et al.*, 1997). Seaweed biofilters have been proposed for use in intensive fish-ponds or cages where more than 70 % of the nitrogen released from food not assimilated into biomass is released back into the environment (Shpigel *et al.*, 1993; Neori, 1996; Neori *et al.*, 1996) and is subsequently responsible for pollution. Benefits to waste-water utilisation as fertilising medium include increased seaweed yields, increased oxygen levels, improvement of water quality and increased ecomonic value. Further discussions on the use of seaweeds as biofilters can be found in Petrell *et al.* (1993), Subandar *et al.* (1993), Friedlander and Levy (1995), Bodvin *et al.* (1996), Buschmann (1996), Buschmann *et al.* (1996), Jiménez del Río *et al.* (1996) and Petrell and Alie (1996).

A similar situation exists in Saldanha Bay where large quantities of nitrogen-rich waste resulting from fish processing are disposed of into the environment. The environmental consequences of this pollution have been discussed by Christie and Moldan (1977b), Jackson and McGibbon (1991) and Anderson *et al.* (1996b) and in Chapters 2 and 3. Large populations of *Ulva lactuca* L. have developed at least once in response to nitrogen pollution in Saldanha Bay (Anderson *et al.*, 1996b). Since opportunistic algae also act as biofilters and increase in biomass as a result of removing nutrients from the environment

(Kautsky, 1982), their presence [at very high densities] is undesirable since they affect natural *Gracilaria* populations through competition for space and light. Furthermore, since they develop as a natural response to eutrophication they are uncontrollable. The aim of this Chapter is to assess the theoretical role of commercial-scale *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham stocked rafts in Small Bay, a sub-section of Saldanha Bay to the north-east of the ore jetty, in removing nitrogen from the water column. Since fish-derived DIN can contribute substantially to the total DIN input it is readily available for uptake by cultivated *Gracilaria* (Chapter 3). Therefore, cultivating *Gracilaria* on suspended rafts in Small Bay that are fertilised by fish factory pollution should result in increased production rates and biofiltration.

6.2 Methods, results and discussion

In situ uptake of nitrogen by *Gracilaria* was estimated by assuming the seaweed maintains an internal nitrogen content of 3.1 % N g^{-1} (dry) and a relative growth rate (RGR) of 4.0 % per day throughout the year. The total-N content of *Gracilaria* used here is the average annual thallus nitrogen content of *Gracilaria* cultivated on the SFRI raft (Chapter 2, Figure 1) obtained by C:N analysis described in Section 3.2.3. The RGR of 4.0 % per day is the annual average growth rate of *Gracilaria* cultivated on the SFRI raft (SFRI data, unpublished) using netlons stocked at an initial density of about 400 g (fresh) seaweed per metre (see Anderson *et al.*, 1996a).

If a netlon is stocked at an initial density of 400 g (fresh) m^{-1}, a RGR of 4.0 % per day results in a net harvest of 928 g (fresh) *Gracilaria gracilis* per metre length of netlon (equivalent to 95 g dry *Gracilaria*) after 30 days. In order to maintain this growth rate, about 2.9 g of nitrogen has to be taken up to keep the thallus nitrogen content at 3.1 %. This equates to an *in situ* uptake rate of approximately 43 µg N g^{-1} (dry) hr^{-1}. Such results contradict those of Probyn and Chapman (1982) and Rosenberg *et al.* (1984). The authors showed that uptake rates measured in batch-mode experiments (e.g. perturbation experiments) over-estimate the 'real' uptake rates measured *in situ* or in continuous-flow experiments by an order of magnitude. The very high *in situ* rates estimated for *G. gracilis* in this study is probably due to the indirect [theoretical] method used to estimate uptake and may fail to take into account the uncoupling of uptake and growth such as is described in the Droop model (Droop, 1968; 1973; 1977). Attempts to apply Droop's equation in

order to model growth rates from the measured size of the internal nitrogen pool failed (r = 0.075) due to limited data and therefore results are not presented here. Apart from uncoupling growth and nutrient uptake, the Droop model is also useful for estimating the length of time a seaweed would maintain positive growth in the absence of external sources of nitrogen. However, Smit *et al.* (1997) estimated this time to be about 7 days for *G. gracilis* from Saldanha Bay. It is suggested here that future growth kinetic studies be conducted on *G. gracilis* in order to find the relationship of growth rate to internally stored nitrogen. Once growth of the seaweed has been described in terms of Droop's two-compartment model, it would be possible to relate the *in situ* growth of *G. gracilis* to seasonal variations in light and temperature using ecological numerical models similar to those of Menesguen (1992), Portielje and Lijklema (1994) and Solidoro *et al.* (1995). Such models are useful for determining how the growth of seaweeds is influenced by the environment in which they occur. Furthermore, they are also useful in determining the response of a system to eutrophication and to changes in hydrodynamic conditions and are therefore an essential tool for the management of coastal water bodies subjected to anthropogenic activities.

Scaling the raft up to a 40 ha commercial system while maintaining the same average annual N-content and RGR used above results in a net yield of 494 tonnes of fresh *Gracilaria* [over 30 days] containing about 1.6 tonnes of nitrogen. Within a year, a 40 ha system has the potential to yield about 5 925 tonnes of fresh seaweed and the new biomass produced would have taken up approximately 19 tonnes of nitrogen. This value is very small considering a total-N input into Small Bay in the order of 650 tonnes per year and based on this it is unlikely that *Gracilaria* would make an effective biofilter or noticeably improve water quality in the bay. Similar studies on kelps (*Laminaria*, *Macrocystis* and *Nereocystis* spp.) and *Ulva rigida* C. Ag. have shown that these seaweeds have good biofiltering capabilities, but that the efficiency of nutrient removal is limited by farm size, rather than the intrinsic capability of the species for DIN uptake (Petrell *et al.*, 1993; Jiménez *et al.*, 1996). Nevertheless, the additional nutrients in the wastewater do allow for enhanced growth rates in the cultivated species (Petrell *et al.*, 1993; Jiménez *et al.*, 1996; Chapter 4), leading to increased economic returns.

In situ uptake rates of NH_4^+-N and NO_3^--N can also be determined from estimated monthly water column DIN values (DIN as a function of seawater temperature, Monteiro and

Brundrit, in press) and Michaelis-Menten parameters for N-replete seaweed determined in Chapter 5. It was shown that the uptake of NH_4^+-N and NO_3^--N follow biphasic and Michaelis-Menten uptake kinetics respectively. For the purpose of this study, the diffusion corrected [Michaelis-Menten] model is used to describe the uptake of NH_4^+-N (i.e. a rate-saturated response to substrate concentration (*S*)). It is likely that the linear *V* vs. *S* response is only seen under natural conditions when the seaweed is exposed to a transient pulse of NH_4^+-N after it had been growing in 0 µM DIN for some time. A rate-saturated response represents steady-state conditions and it is suggested to be the normal condition for *Gracilaria gracilis* found in Saldanha Bay.

In situ uptake rates as well as the proportion of the total nitrogen taken up by *Gracilaria gracilis* provided by each of the two species of DIN are presented in Table 1. Figure 1 shows the relative preference index (RPI) for the two forms of nitrogen (McCarthy *et al.*, 1977). Results clearly show that uptake rates are highest during winter months due to the increased availability of NH_4^+-N and NO_3^--N and that NO_3^--N makes up the greatest proportion (up to about 66 %) of the total DIN taken up. In summer, the seaweed becomes more efficient at taking up NH_4^+-N relative to NO_3^--N, showing that it is well adapted to living in environments with low seasonal ambient nitrogen concentrations. Table 1 also gives measured growth rates for *Gracilaria gracilis* cultivated on netlons on the SFRI raft during 1995 (using method outlined by Anderson *et al.*, 1996a). There is some evidence for nitrogen-limited growth during mid- to late-summer. Highest growth rates occurred during late-winter to early summer (August – December) with rates of up to 6.4 % per day. The low growth rates measured during mid-winter despite high DIN concentrations probably reflect the low water temperatures or reduced light during that time.

Table 1. Estimated *in situ* uptake rates for *Gracilaria gracilis* in Saldanha Bay, also showing the proportion of the total nitrogen taken up supplied by NH_4^+-N or NO_3^--N.

Month	RGR (% per day)	Estimated *in situ* uptake rate (µg N g^{-1} (dry) hr^{-1})		Proportion (%) of total nitrogen taken up provided by	
		NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
Jan	1.0	2.9	3.0	49.2	50.8
Feb	3.8	2.9	2.1	58.0	42.0
Mar	4.0	2.9	3.4	46.0	54.0
Apr	3.9	3.9	7.5	34.2	65.8
May	1.9	6.6	11.1	37.3	62.7
Jun	2.9	9.2	11.4	44.7	55.3
Jul	2.8	6.6	11.0	37.5	62.5
Aug	6.1	7.0	11.0	38.9	61.1
Sep	6.4	7.1	11.1	39.0	61.0
Oct	5.8	3.3	5.8	36.3	63.7
Nov	5.4	3.1	4.7	39.7	60.3
Dec	5.7	3.9	2.1	65.0	35.0

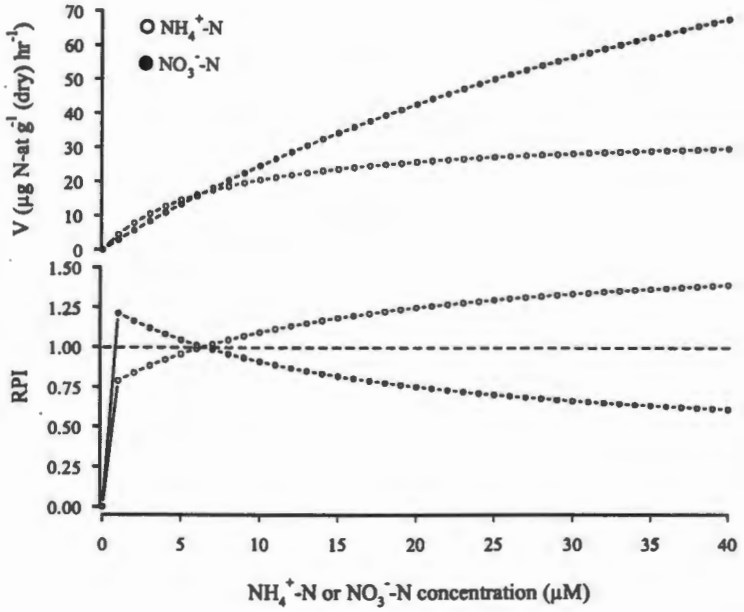

Figure 1. Michaelis-Menten curves and RPIs plotted from Michaelis-Menten parameters and estimated substrate concentrations used to estimate *in situ* uptake. RPI > 1 indicates preference.

A complete seasonal thallus-N record is not available but results from a few analyses (AJ Smit, unpublished data) indicate that tissue-N reaches levels of up to 3.8 % during late-winter when nutrients are abundant. During summer, tissue-N levels decrease to around 1.4 % as nitrogen is mobilised to support faster growth with the onset of favourable conditions. *Gracilaria gracilis* is therefore well adapted to seasonal as well as short-term fluctuations in ambient-N concentrations. The presence of biphasic uptake kinetics for NH_4^+-N enables the seaweed to be effective in assimilating reduced nitrogen during summer months when low fluxes (a function of the frequency of the pulsating thermocline and DIN concentration) of DIN prevail. The rate-saturated uptake mechanism for NO_3^--N is responsible for accumulating nitrogen in the internal pools during winter in excess of immediate requirements (luxury consumption). These pools provide most of the nitrogen to support growth during early summer (Rosenberg *et al.*, 1984). The presence of multi-component uptake kinetics coupled with large internal nitrogen stores makes *G. gracilis* well suited to buffer the effect of transient nitrogen availability on growth over periods of days to weeks (Rosenberg *et al.*, 1984).

Chapter 4 showed that *Gracilaria* does utilise anthropogenic sources of nitrogen when it becomes available, and that this nitrogen is often sufficient to sustain growth when natural background nitrogen levels becomes undetectable. Furthermore, this Chapter showed that *Gracilaria* is well suited to efficiently remove nutrients from the water column under a wide range of DIN concentrations. As was mentioned previously (Chapter 4), seaweed populations in Small Bay, be it natural or cultivated, seldom experience continuous high levels of nitrogen sources unless the required 'environmental window' persist for an extended period of time. Under conditions of long periods of high nitrogen availability, however, one might expect to find a significant increase in

This Chapter demonstrates the efficiency of *Gracilaria gracilis* from Saldanha Bay as a nutrient sink using theoretical calculations. However, despite the efficiency of DIN uptake which makes it a good biofilter, it is unlikely that *Gracilaria* would effectively improve water quality when cultivated in large scale in Saldanha Bay, simply due to the large volume of nitrogenous effluent released into Small Bay each year.

7 *Synopsis*

This thesis provides an insight into the functioning of the environment in which *Gracilaria gracilis* thrives and the inter-relationships of the seaweed within it. Most of the work presented here deals with the nitrogen ecophysiology of *G. gracilis* as it was realised early on in the study that this is the factor most likely to limit growth and production on suspended rafts in Small Bay. However, before a seaweed can be cultivated successfully, irrespective of nutrient availability, a knowledge of seeding practices and the effect on growth and regeneration is required. Other technical requirements such as raft design, stocking densities, seeding substrata, etc. are discussed by Dawes (1995) and Anderson *et al.* (1996a).

Chapter 2 discussed the role of organismic determinants in the growth and regeneration of *Gracilaria gracilis* in cultivation. It was evident that length and quality of initial seedstock greatly affects the potential growth rate and therefore the yield that is obtainable from *Gracilaria* rafts. Furthermore, it was suggested that damage incurred during harvesting and the subsequent reseeding of *Gracilaria* lines using harvested material could lead to morphological differentiation of the thalli resulting in a moribund form with a hollow main axis and a high branching frequency. The initiation of branching is most likely the result of an apical dominance effect whereby control over lateral branch inhibition is lost after damage to the apical region. Therefore, high growth rates can be obtained by seeding netlons with newly collected long, unbroken thalli.

Saldanha Bay has received organic rich pollution resulting from waste disposal by fish-processing factories since the early 1900's. This nitrogenous waste has been linked to at least one *Ulva lactuca* bloom in the system (Anderson *et al.*, 1996b) and has been implicated as responsible for changes in the macrobenthic community structure close to the waste outfall (Jackson and McGibbon, 1991). Chapters 3 and 4 examined the distribution of fish-derived particulate organic matter (POM) and dissolved inorganic nitrogen (DIN) in Small Bay by using a natural abundance stable isotope study. $\delta^{15}N$ analysis of sedimentary organic matter indicated that up to 75 % of the total sedimentary nitrogen pool in Small Bay is comprised of fish-derived nitrogen. $\delta^{15}N$ of *Gracilaria* indicated a similar distribution of DIN in Saldanha Bay. It also provided evidence that

effluent-nitrogen makes up a significant proportion of the total nitrogen taken up by seaweed cultivated during summer months when the water column is normally oligotrophic. This is not the case in winter months when the water column is well mixed. The success of this study is two-fold showing that stable isotope analysis is a valuable tool for tracing the distribution of pollutants in the environment and for studying the nitrogen nutrition of seaweeds in nature.

Chapter 5 described the first attempt to understand the nitrogen nutrition of *Gracilaria gracilis* from Saldanha Bay. Results clearly showed that this species is well adapted to long- and short-term fluctuations in ambient-N concentrations. The presence of biphasic uptake kinetics with a strong diffusive component for NH_4^+-N indicated that *G. gracilis* is effective in taking up ammonium during summer months when DIN availability is limited. Furthermore, the Michaelis-Menten, or rate-saturated uptake mechanism for NO_3^--N is responsible for the accumulation of nitrogen in internal pools during winter in excess of immediate requirements for growth (luxury consumption). However, despite the efficiency of nitrogen uptake, Chapter 6 suggests that the seaweed would be largely ineffective as a biofilter due to the large amount of nitrogen released into Saldanha Bay annually.

8 Appendix

8.1 Ammonium determination

(Solórzano, 1969)

This method relies on the treatment of seawater in an alkaline citrate medium with sodium hypochlorite and phenol, using sodium nitroprusside as catalyst. The resulting deep blue indophenol dye formed with ammonium is measured spectrophotometrically. The method is accurate within the range 0.1 - 10 µM N.

Reagents:

Phenol reagent:

Dissolve 20 g crystalline phenol in 200 mL 95 % ethanol. The solution is stable indefinitely.

Sodium nitroprusside solution:

Dissolve 1 g sodium nitroprusside in 200 mL deionised water. The solution is stable for one month only when stored in an amber bottle.

Alkaline reagent:

100 g sodium-citrate and 5 g sodium hydroxide is dissolved in 500 mL deionised water. The solution is stable indefinitely.

Sodium hypochlorite solution:

Use a 10 - 14 % commercially available sodium hypochlorite solution. The solution decomposes slowly, but can be checked according to Parsons *et al.* (1984).

Working solution:

Mix 10 mL alkaline reagent and 2.5 mL sodium hypochlorite solution. The working solution is stable for one day only. Keep stoppered.

Synthetic seawater:

Dissolve 155 g ammonium chloride, 50 g magnesium sulphate and 0.25 g sodium bicarbonate in 5 L distilled water.

Ammonium sulphate stock standard (1 mL ≡ 1.5 μg-at N):

Dissolve 0.100 g ammonium sulphate in 1000 mL distilled water and add 1 mL of chloroform as preservative. The solution is stable for many months when stored in a tightly stoppered bottle in a refrigerator.

Procedure:

0.1 mL phenol reagent, 0.1 mL sodium nitroprusside and 0.25 mL oxidising solutions are added to 2.5 mL sample, and mixed using a vortex mixer after each reagent addition. The test tubes are covered to prevent contamination and allowed to stand for at least one 3 hours in the dark. The blue colour is stable for 24 hours. Absorbance at 640 nm is measured spectrophotometrically after zeroing with distilled water. Three reagent blanks are made using distilled water in stead of sample. Correction can also be made for turbidity if required, i.e. sample without reagent.

Calibration:

A series of working standards is made by making up 0.05, 0.15, 0.25, 0.50 mL stock standard to 50 mL in volumetric flasks using synthetic seawater. The resulting concentrations are 1.00, 3.00, 5.00 and 10.00 μM N. Working standards should be made up fresh before use. Use 2.5 mL the working standard and add reagents as described above. Sample concentrations are determined from the resulting linear standard curve and estimated from the regression equation.

8.2 Nitrite determination

(Grasshoff *et al.*, 1983)

Nitrite present in seawater reacts with sulphanilamide in an acid solution forming a diazo compound. The resulting compound reacts with N-(1-naphthyl)-ethylenediamine (NEDDI) and forms a coloured azo dye. The method has a range of about 0.01 - 2.5 μM N.

Reagents:

Sulphanilamide solution:

Dissolve 5 g sulphanilamide in 50 mL concentrated hydrochloric acid and about 300 mL distilled water, and make up to a final volume of 500 mL. The solution is stable for many months.

NEDDI solution:

Dissolve 0.5 g NEDDI in 500 mL distilled water and store in an amber bottle. The solution is stable for one month.

Nitrite stock standard solution (1 mL ≡ 5 μg-at N):

Anhydrous sodium nitrite is dried at 100 °C for 1 hour and 0.345 g is dissolved in 1000 mL distilled water. The solution is stored in an amber glass bottle with a few drops of chloroform added as preservative, and is stable for several months.

Synthetic seawater:

Dissolve 155 g ammonium chloride, 50 g magnesium sulphate and 0.25 g sodium bicarbonate in 5 L distilled water.

Procedure:

1 mL sulphanilamide is added to 1 mL sample and allowed to stand for at least 2 minutes (no more than 10 minutes). Add 1 mL NEDDI and mix immediately. Allow to stand for between 10 minutes and 2 hours while standing in the dark and measure the absorbance at

543 nm after zeroing the spectrophotometer with distilled water. If samples are turbid it may be necessary to determine a turbidity blank for each sample. This is done by adding only the sulphanilamide reagent to the sample and reading the absorbance at 543 nm. Reagent blanks are determined using distilled water (nitrite-free) in stead of sample, as described above.

Calibration:

Make up a working standard by adding 10 mL stock standard to 1000 mL with distilled water and use the same day. Prepare a series of standards by making up 1.0, 2.0 and 3.0 mL of working standard to a final volume 50 mL using synthetic seawater. The standards correspond to 1.0, 2.0 and 3.0 μM N. Reagents are added as above, and the absorbance determined at 543 nm. Calibration is performed from the resulting standard curve and estimated from the linear regression equation.

8.3 Nitrate determination
(Grasshoff, 1983)

Nitrate in seawater is quantitatively reduced to nitrite using copperised-cadmium. The nitrite produced is determined as outlined under nitrite determination. The method has a range of about 0.05 - 45 µM N.

Reagents:

Preparation of copperised-cadmium:

Approx. 200 g cadmium filings (range 0.5 - 2.0 mm) is washed in 2 M hydrochloric acid to remove oxides, and rinsed in distilled water. 2 % (w:v) copper sulphate is added and the cadmium washed until it turns from light to dark grey. The copperised-cadmium is washed in 0.007 N hydrochloric acid (0.7 mL L^{-1} distilled water) containing 0.005 M EDTA (1.861 g L^{-1}) by shaking vigorously. The acid/EDTA is decanted and the copperised-cadmium washed in running deionised water until the supernatant is clear. The copperised-cadmium is stored under an acid/EDTA solution in such a way as to exclude air. Granules are washed with acid/EDTA after use, and returned to the original solution.

Ammonium chloride buffer:

10 g ammonium chloride is dissolved in 1000 mL distilled water and the pH adjusted to 8.5 with ammonia or ammonium hydroxide.

Synthetic seawater:

Dissolve 155 g ammonium chloride, 50 g magnesium sulphate and 0.25 g sodium bicarbonate in 5 L distilled water.

Nitrate stock standard solution:

Dissolve 0.772 g potassium nitrate in 1000 mL distilled water and store in a sealed bottle. The solution is stable for many months.

Nitrate reduction procedure:

Add 2 mL ammonium chloride buffer to 3 mL sample, followed by approx. 2 g (roughly half a rounded spoon-spatula tip) of copperised-cadmium granules and agitate for 10 minutes. 1 mL of the reduced sample is used for nitrite determination (see procedure under nitrite determination). Blanks are determined using synthetic seawater in stead of sample. To determine the turbidity blank, follow the above procedure, but omit the addition of reagents (not copperised-cadmium).

Calibration:

Prepare a series of working standards by diluting 0.5, 1.0, 1.5 and 2.0 mL nitrate stock standard in 100 L synthetic seawater. The resulting standards are equivalent to 0.5, 1.0, 1.5 and 2.0 ppm NO_3^--N. The solution is prepared fresh on day of use. The concentration of samples is determined from the resulting linear regression equation.

8.4 Phosphate determination

(Murphy and Riley, 1962)

Seawater is reacted with a reagent containing molybdic acid, ascorbic acid and trivalent antimony. A blue complex is given and the absorbance measured at 885 nm. The range of the method is 0.03 - 5 μM P.

Reagents:

Ammonium molybdate solution:

Dissolve 15 g ammonium paramolybdate in 500 mL distilled water. Store away from sunlight in a plastic bottle. Solution is stable, but see note in Parsons *et al.* (1984).

Sulphuric acid solution:

Add 140 mL concentrated sulphuric acid to 900 mL distilled water. Allow solution to cool and store in a glass bottle.

Ascorbic acid solution:

27 g ascorbic acid is dissolved in 500 mL distilled water. The solution should be stored frozen solid. Stable for many months, but should not be kept for longer than a week if stored at room temperature.

Potassium antimonyl-tartrate solution:

0.34 g potassium antimonyl-tartrate (tartar emetic) is dissolved in 250 mL distilled water, warming if needed. The solution is stable for many months.

Working reagent:

100 mL ammonium molybdate, 250 mL sulphuric acid, 100 mL ascorbic acid and 50 mL potassium antimonyl-tartrate solutions are mixed together. The solution is unstable and should not be stored.

Phosphate stock standard:

Dissolve 0.816 g anhydrous potassium dihydrogen phosphate in 1000 mL distilled water. Store in a dark bottle with 1 mL of chloroform as preservative. Solution is stable for many months.

Synthetic seawater:

Dissolve 155 g ammonium chloride, 50 g magnesium sulphate and 0.25 g sodium bicarbonate in 5 L distilled water.

Procedure:

5 mL samples are warmed to room temperature and 0.5 mL working reagent added. Samples are allowed to stand for between 5 minutes to 3 hours, and the absorbance measured at 885 nm. The spectrophotometer is zeroed using distilled water. Blanks are constructed using distilled water in stead of sample. Use a turbidity blank if needed.

Calibration:

Dilute 10 mL phosphate stock standard in 1000 mL distilled water. Make up 2.5, 5.0 and 7.5 mL of diluted standard to a final volume of 50 mL in volumetric flasks using synthetic seawater to obtain the working standards. Concentrations of the working standards are 3.0, 6.0 and 9.0 μM P. To 5 mL working standard, add reagents as discussed under the procedure above. Calibration is done from the resulting linear regression equation.

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