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Upwelling and fish-factory waste as nitrogen sources for suspended cultivation of *Gracilaria gracilis* in Saldanha Bay, South Africa

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Abstract

In Small Bay, Saldanha, the water becomes highly stratified in summer. The cold bottom layer (of upwelling origin) is rich in nitrogen, some of which enters the surface layer by advection. However, the surface water often becomes warm and oligotrophic leading to poor growth or death of *Gracilaria gracilis* grown in experimental suspended systems. At the same time, large quantities of nitrogen-rich fish waste are released at a particular site in the bay. We tested the hypothesis that *Gracilaria* grown close to the site of waste release (1.5 km away and in the waste plume) would grow faster than at the control site 3.5 km away. In October and November (early summer) 1996, all the *Gracilaria* at the control site died, while growth at the fish waste site was good (between 8 and 10% day^{-1}). In November–December control plants grew slightly faster than those from the waste site, in February the reverse occurred, and subsequently (March–June) growth was similar at both sites as winter winds caused mixing of the water column. These results, and analyses of the C/N ratios of the *Gracilaria* tissues provide some support for our hypothesis. Also, analyses of the stable N isotope ratios in the *Gracilaria* tissues indicate that there is considerable uptake of the fish-waste N even at the control site. We conclude that while proximity to the waste site may sometimes benefit the *Gracilaria*, the fish waste would in fact provide a significant source of N for seaweed cultivated throughout the northern area of Small Bay, particularly when the water is highly stratified in summer.

Introduction

Eutrophication of marine waters, particularly bays, is a well-known phenomenon which leads to various environmental problems (Vollenweider, 1992). The Saldanha–Langebaan bay system (Figure 1) is unique in being the only deep, large embayment in the otherwise very exposed west coast of South Africa. It is also the site of numerous and sometimes conflicting human activities. While the shallow southern portion (Langebaan Lagoon) is a nature reserve, the deeper Saldanha Bay is divided into Small and Big Bays by a quay which is used for ore and oil-loading. Small Bay is used as a fishing harbour, for mariculture (it is the centre of mussel cultivation in SA) and various recreational activities. Small Bay, where most of the these activities occur, also receives about 650 tons of nitrogen annually over and above natural fluxes, in the form of liquid fish-processing waste that is discharged from two factories on its west shore (Figure 1), and which has measurable effects on benthic macrofauna (Christie & Moldan, 1977). Fish waste from the larger (pelagic processing) operation was also implicated in causing a problem bloom of the opportunistic green alga *Ulva lactuca* which reduced the benthic *Gracilaria* population and fouled commercial beach-casts of this economic agarophyte in 1993/94 (Anderson et al., 1996b). The waste is discharged at about 8 m depth, and most of it rises to the surface, where it is distributed in the surface water by the wind, often leaving a visible oily slick. In summer prevailing southerly winds drive the plume northwards to the beach, and then eastwards with the circulating current (Anderson et al., 1996b; Weeks et al., 1991). Subsequently, the use of stable isotope ratios of nitrogen confirmed that this pelagic fish-waste was the source of the nitrogen that had fed the bloom (Monteiro et al., 1997). Stable isotope ratios of carbon (^{13}C/^{12}C) and nitrogen

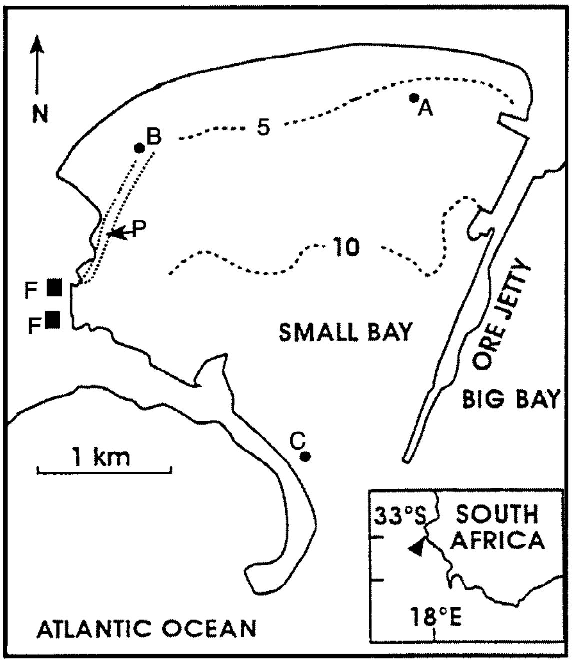

Figure 1. Map of Small Bay, Saldanha, showing positions of Sites A, B and C and fish-factories (F). The usual direction of the pelagic fish-waste plume in summer is shown (P). Depth contours (5 and 10 m) shown.

($^{15}N/^{14}N$) are an accepted method of following trophic pathways in marine systems (Monteiro et al., 1991; Owens, 1987).

Features of the biogeochemistry of Small Bay that are relevant to the growth of seaweeds are summarized by Anderson at al. (1996b). In winter the water column is well-mixed, uniformly cold (12–14 °C) and nutrient-rich, However, in summer the system becomes strongly stratified, with warm, oligotrophic surface water overlying a cold, nutrient-rich bottom layer which originates from upwelling on the adjacent coast. Under natural conditions, nitrogen in the surface layer becomes depleted as it is taken up by phytoplankton, and it can only be replaced by flux across the thermocline. This is estimated at a low rate of mmol m^{-2} h^{-1} of N (P.M.S. Monteiro, pers com.). Nitrogen levels in the surface water may become too low to measure: under these conditions *Gracilaria* cultivated near the surface becomes bleached, and if the conditions persist for more than a week or two, dies (Anderson et al., 1996a). The thermocline usually varies in depth between about 5 and 10 m, on an approximately 6–7-day cycle, effectively pulsing in a manner that appears to supply sufficient nutrients to most of the benthic *Gracilaria* population (which is concentrated at depths from 3–8 m in the gently sloping north of the bay). However, sometimes in summer the southerly winds are sustained for periods of several weeks, warm oligotrophic water persists at 5–6 m depths, particularly in the NE corner of Small Bay, and even benthic *Gracilaria* dies.

The cultivation of the local species, *Gracilaria gracilis,* has been shown to be technically feasible in Small Bay, using suspended ‘rafts’ of rope and netting lines, and on an experimental scale yielded growth rates of 4–7% day^{-1}, suggesting a commercial yield of about 36 t dry wt ha^{-1} (Anderson et al., 1996a), similar to that obtained commercially at Luderitz in Namibia (Dawes, 1995). However, Anderson et al. (1996a) showed that over the 5 years of experimental cultivation in the NE corner of Small Bay, growth rates tended to fall very low at some stage each summer, and in 1974, all cultivated material died; these effects were a result of prolonged southerly winds and the presence of oligotrophic water.

There is thus the paradox that, while in one part of Small Bay the surface nutrient levels can in summer sometimes remain low enough to severely reduce *Gracilaria* growth or even kill the seaweed, several kilometres away there is enough nitrogen waste being released to have caused a massive bloom of *Ulva* over an area of 20 ha (Anderson et al., 1996b). Also, the maritime authority at Saldanha has received several requests for water space for the suspended cultivation of *Gracilaria,* in competition with other potential water uses such as harbour expansion, recreational sailing and fishing, and information is needed to assist in prioritizing water uses and deciding where (if at all) *Gracilaria* farming should be allowed in Small Bay. This study therefore aimed to test whether it would be preferable to cultivate *Gracilaria* in the path of the fish-waste plume, both from the point of view of improved growth and the removal of excess nitrogen from the water. Specifically, we tested the hypothesis that in summer, growth in the waste plume would be better than at the NE (control) site 3.5 km from the outfall.

Materials and methods

Growth experiments were conducted during 1996/1997 at Sites A and B in Small Bay, and information was used from part of a previous study at Site C (Figure 1). Site A was used previously for experiments between 1991 and 1995 (Site 1 of Anderson et al., 1996a) and lay in the NE corner of Small Bay in about 5.5 m deep water, about 3 km from the fish-waste outfall. Site B was adjacent to several granite reefs about 1 km N of the pelagic waste outfall, at 5.5 m depth and directly in the pelagic fish waste plume during southerly, summer winds. Data and *Gracilaria* samples from previous experiments (1995/1996) at Site C were also used: this site (Site 2 of Anderson et al., 1996a) lay adjacent to the breakwater, just inside the mouth of Small Bay, about 2 km SE of the outfall.

At each site, *Gracilaria gracilis* was grown on a rope 'raft' comprising a rectangular frame of 15 mm polypropylene rope 20 × 5 m in size, suspended horizontally about 0.4 m below the water surface, between a series of floats and anchors (see Dawes, 1995, or Anderson et al., 1996a, for details of raft construction). The seaweed lines were tied across the 5 m width of the frame, so that they were suspended horizontally below the water surface, 0.75 m apart to avoid abrasion between thalli on adjacent lines. For the experiments at Sites A and B, small tufts of *Gracilaria* were weighed out (to within 2 g of 20 g, on a spring balance) and attached to a 5.5-m length of 8-mm polypropylene rope at 0.15-m intervals using plastic cable ties (n = 30). After the growth period (about 1 month) the ropes were removed, the cable ties cut and each numbered tuft re-weighed. In most cases re-stocking was done with material that had just been removed from the raft, but when that was missing or in poor condition new thalli from benthic populations were used. Results are expressed as relative growth rates (RGR) here calculated from the compound interest formula

$$\mathrm{RGR} = (n\sqrt{W_2/W_1} - 1) \times 100,$$

where n = no. of days, W_1 = initial wet wt, W_2 = final wet wt. Although epiphytes were sometimes present on thalli, they were estimated never to make up more than a few percent of the total weight, and were thus ignored.

Data from 1994 and 1995 at both sites A and C were obtained using commercial-style netting lines rather than ropes. In this method the *Gracilaria* was threaded sideways through the mesh of a plastic tube of netting ('netlon'). Between four and five replicate lines were used each month, each stocked at about 400 g f wt $m^{-1}$and buoyed in the middle with a plastic bottle (see Anderson et al., 1996a, for details).

Stable isotope analysis was used to assess the relative amounts of fish-derived N in *Gracilaria* grown at Site B, near the fish-waste outfall, and at Site A, as well as in thalli grown the previous year at Site C. *Gracilaria* samples (three replicates) were collected off the rafts each month when plants were harvested and weighed. For reference purposes, *Gracilaria* was also collected from a relatively unpolluted site in Langebaan lagoon.

The seaweed samples were immediately placed in plastic bags and stored on ice in the dark until transfer to a freezer. For analysis the seaweed was thawed and rinsed to remove microscopic and macroscopic epiphytes and salt, then oven dried at 60 °C and ground to a fine powder using liquid N and a pestle and mortar. The powder was stored in a desiccator until analysis.

Simultaneous $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were determined using 0.6 mg samples (three replicates) on a Finnigan MAT 252 isotope ratio mass spectrometer according to the method of Fry et al. (1992). This also provided C/N ratios for the samples. Ratios of $^{15}N/^{14}N$ are expressed as ‰, and were calculated as follows:

$$\delta^{15}\mathrm{N} = \left[\frac{^{15}\mathrm{N}/^{14}\mathrm{N}_{\mathrm{smp}}}{^{15}\mathrm{N}/^{14}\mathrm{N}_{\mathrm{std}}} - 1\right] \cdot 10^3,$$

where $^{15}N/^{14}N_{\ smp}$ is the isotope ratio of the sample and $^{15}N/^{14}N_{std}$ is the isotope ratio of the standard.

δ^{15} N is reported relative to atmospheric nitrogen (Mariotti, 1984). The reference gas was high purity nitrogen (99.995%) calibrated against atmospheric nitrogen. Analytical precision was to within 0.3‰ (1SD). $\delta^{13}C$ was calculated using an equation of similar form to that above, but substituting $^{13}C/^{12}C$ for $^{15}N/^{14}N$. The reference gas was high purity carbon dioxide calibrated against Pee Dee Belemnite. Analytical precision was 0.2‰.

Water temperatures were measured continuously on submersible electronic recorders accurate to within 0.1 °C but calibrated to within 0.5 °C. They were installed at each site on the raft at 0.5 m depth. Underwater irradiance was measured several times at each site on the same days, using a Li-Cor Li-193SA Spherical Quantum Sensor and LI-100 datalogger. Because light is generally not limiting and values were similar at all sites, the results are not presented, but only referred to where necessary.

Results

The *Gracilaria* at Site A grew well in September–October, but died in November–December, when growth at Site B was good (Figure 2). It is notable that at Site A most of the benthic *Gracilaria* (at 5.5 m depth) also died during October–November. Between

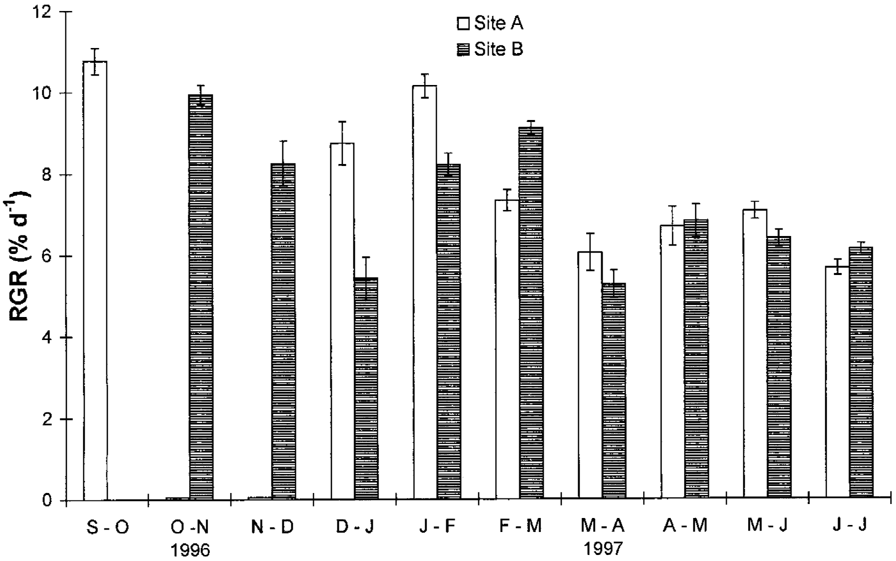

Figure 2. Mean relative growth rates (± 95% confidence limits) of *Gracilaria* tufts grown attached to ropes, at Sites A and B in Small Bay, Saldanha, from mid-September 1996 to mid-July 1997 (n = 30).

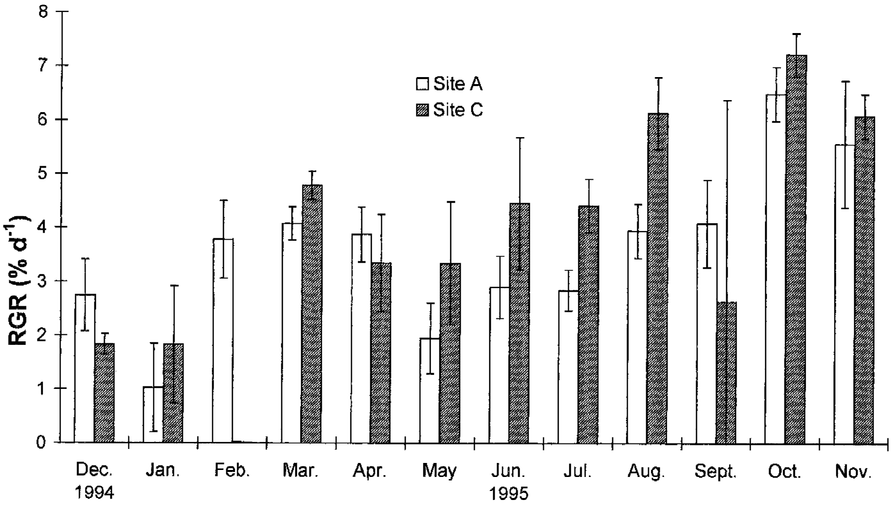

Figure 3. Mean relative growth rates (± 95% confidence limits) of *Gracilaria* grown on commercial-type netting line at Sites A and C in Small Bay, Saldanha, from December 1994 to November 1995 (n = 4–5).

mid-December and February the *Gracilaria* grew better (RGR => 8% day^{-1}) at Site A than Site B and in February–March it grew better at Site B. Subsequent growth rates were similar at the two sites, in autumn and winter.

In December 1994 and January 1995 growth was poor at both sites A and C (Figure 3). In February it improved at A, while the *Gracilaria* at C died. In March, growth at C was better. Subsequently, in winter (May–August), growth was similar at the two sites, except in July and August, when it was higher at Site C, near the breakwater. In September, October and November, RGR values were similar at the two sites. Relative growth rates of *Gracilaria* on the commercial-style netlon lines during 1994/1995 (Figure 3) cannot be compared directly with RGR on ropes (Figure 2), because in the former the commercial stocking weight of 400 g m^{-1} results in high net yields but relatively low RGR, while on the ropes the stocking weight is very low (less than 100 g m^{-1}),

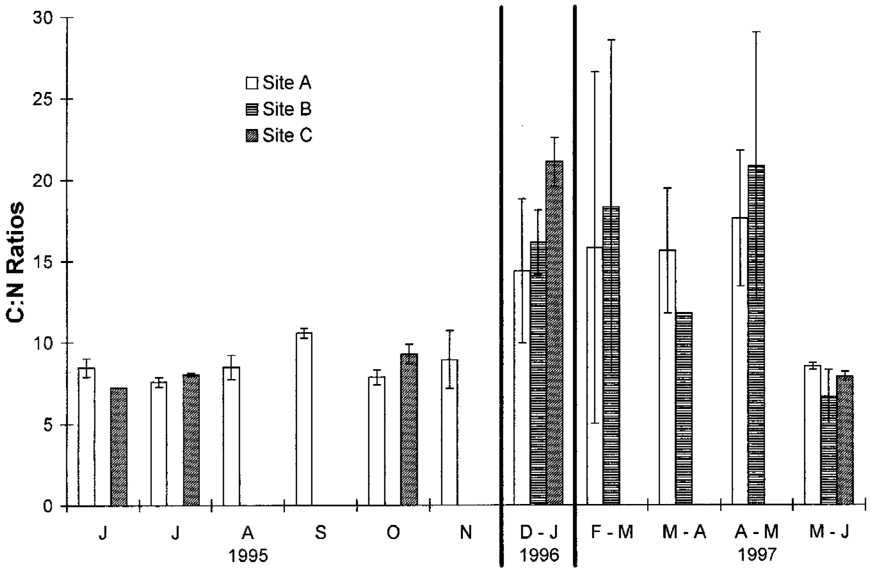

Figure 4. C/N ratios (± 95% confidence limits) of *Gracilaria* tissue cultivated at three sites in Small Bay, Saldanha, at various times ($n = 3$).

Table 1. $\delta\,^{15}N$ values in *Gracilaria* cultivated at Sites A, B and C at various times (95% confidence limits of means shown, $n = 3$ in all cases)

Site	Delta ^{15}N values (‰)			
	Oct '95	Dec '96–Jan '97	Feb–Mar '97	Apr–May '97
A	10.4 ± 0.7	11.4 ± 0.5	13.0 ± 0.2	11.2 ± 0.1
B		8.6 ± 0.7	11.4 ± 0.2	10.0 ± 0.3
C	8.5 ± 0.3	8.7 ± 0.4		

resulting in high RGR but low net yields (Anderson et al., 1996a).

Ratios of C/N at sites A and C (Figure 4) remained low from the start of measurements in June 1995 until November (Site B was not yet in use). In October and November 1996 all the *Gracilaria* at Site A had died, and only bleached fragments remained. The results for midsummer (December) 1996 showed raised C/N ratios at all sites, but particularly Site C. In 1997 the C/N ratios at sites A and B were variable but high between February and April–May (no data for Site C). There was no correlation between RGR values and C/N ratios for the whole data-set.

In all the samples measured (Table 1), the $\delta^{15}N$ values of *Gracilaria* tissue remained consistently high at Site A, reaching a maximum of 13.0‰ in February 1997. In tissue from Site B, this value was low (8.6) in December 1996, but higher thereafter. The two values for Site C (8.5‰ in October 1995 and 8.7‰ in a sample from a pilot commercial raft in December 1996–January 1997) were among the lowest of all the samples.

Surface (0.5 m) water temperature at Sites A and B showed a typical seasonal pattern for Saldanha Bay, with rising averages in summer, and frequent sudden drops, but more stable low temperatures in winter (May–July). Temperatures at the two sites were essentially similar (Figure 5).

Discussion

In September–October 1996, the RGR of *Gracilaria* at Site A was high because daylength was increasing, water temperature increased from 14 to 16 °C, and nutrient levels were likely to still be high after the winter mixing and before the thermocline had been present for long (as discussed by Anderson et al., 1996a). The subsequent death of all plants at Site A (and most of the benthic population) in October–November and November–December is difficult to explain. The material planted at Sites A and B came from the same benthic population. Temperatures at both sites averaged only about 16 °C, and only briefly exceeded 19 °C, and natural nutrients should have been adequate to sustain growth, especially this early in summer. However, at Site A, the completely bleached appearance of the remaining fragments of plants, and of the

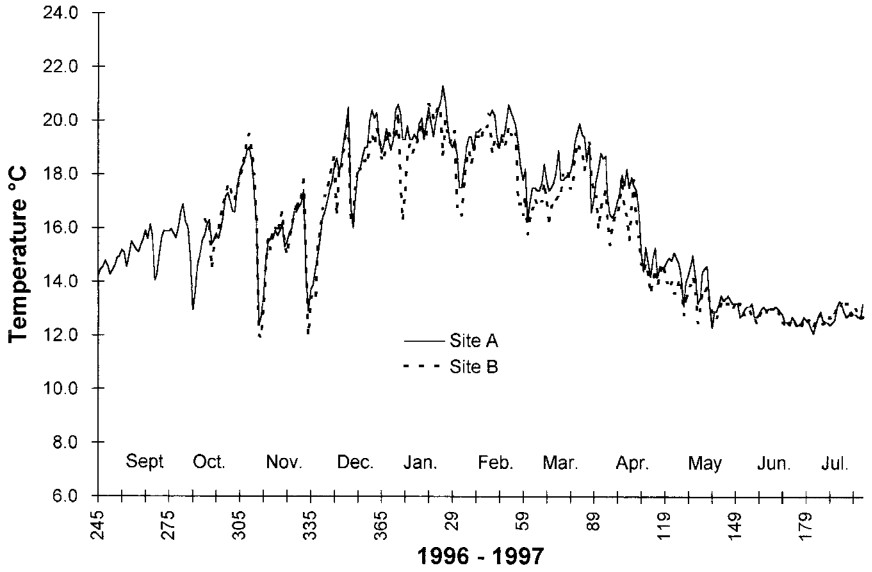

Figure 5. Daily mean water temperatures at Sites A, B and C, Small Bay, from September 1996 to July 1997, at 0.5 m depth.

benthic population, strongly indicated nutrient starvation, although the possibility of a localized disease cannot be ruled out. The important result is that despite almost identical water temperatures (and by inference natural nutrient levels) at Sites A and B, the plants at B in the fish waste plume grew well, providing strong evidence that catastrophic summer die-offs may be avoided by siting close to the fish-waste source. Unfortunately samples were not retained for C/N or δ^{15}N analysis.

In December 1996–January 1997 RGR of *Gracilaria* at Site A was fairly high (>8% day^{-1}), perhaps as a consequence of an improvement in nutrient levels, while at Site B the RGR fell to less than 6% day^{-1}, although temperatures at the two sites remained similar. The C/N values from tissue at both sites were moderately high, indicating low ambient N. However, the explanation for improved growth rates at Site A can be found in the δ^{15}N value of tissue from the two sites. Monteiro et al. (1997) showed that in *Ulva* grown in this waste plume in Small Bay, δ^{15}N values ranged from 8.5 to 13.9, with an average around 10.7‰, while control plants from relatively pollution-free Langebaan Lagoon had values ranging from about 8.0–9.6, with an average of 8.9‰. Values of anchovy muscle protein (the bulk of the fish waste) range from about 12 to 13.4 (a mean of 12.9‰ – Sholto-Douglas et al., 1991), and those of natural oceanic nitrate-N from 6 to 8‰ (Monteiro et al., 1997; Sealy et al., 1987). In December–January the δ^{15}N value of tissue of the *Gracilaria* from Site A was 11.4‰, indicating substantial fish-N input, while that from Site B was only 8.6‰, indicating minimal uptake of fish nitrogen. While it was impossible to follow daily patterns of wind and water flow, this provides evidence that even close proximity to the fish-waste is no guarantee that N from this source will reach the plants. It is possible that either waste was carried to Site A, or that considerable re-mineralization of sediment-N was occurring there. In a separate study, Smit (unpubl.) discusses the potential for re-mineralization of fish-waste N, which he shows to be distributed throughout bottom sediments in the northern area of Small Bay.

The RGRs at the two sites increased and the difference between them narrowed during January–February 1997, despite water temperatures being the highest of that summer, and natural nutrient levels by inference the lowest. In February–March the RGRs remained fairly high although Site B then performed better than Site A, despite similar water temperatures. Over the whole January–March period we would have expected low RGRs on account of low ambient nutrients (as indicated by high water temperatures), in keeping with the known biogeochemistry of Small Bay. By the end of summer nutrient levels should have been very low, since there is little mixing across the thermocline (Anderson et al., 1996a). However, tissue δ^{15}N values were high from both sites, indicating significant uptake of fish-waste N, possibly explaining the relatively good growth rates. The difference between

December–January and February–March was particularly marked at Site B (from 8.6 to 11.4‰, indicating a marked change in N-source over that period.

In March–April growth rates were relatively low at both sites, and C/N ratios moderately high, indicating some nutrient stress. Low growth was typical of this time of year (autumn) in the experiments carried out by Anderson et al. (1996a) and was ascribed to variable and weak winds, little water movement, and low ambient nitrogen levels.

In April–May, RGRs increased somewhat at both sites, to levels that were however still low relative to summer (about 6% day^{-1} compared to $>$8% day^{-1}). Water temperatures began dropping, indicating mixing of the water column and a possible increase in nutrient levels. However, C/N ratios were high, suggesting N-limitation, and δ^{15}N values dropped at both sites, indicating uptake of less fish-waste derived N. From May–July RGRs were steady at just over 6% day^{-1} at both sites, and water temperatures were about 13°C, typical of winter mixed conditions, when high nutrient levels are present, for example typically 5–10 μM of NO_3–N (Anderson et al., 1996a). The low C/N ratios in May–June 1997 are similar to those obtained in previous samples from winter 1995 (Figure 4), and indicate high tissue N levels and a plentiful supply of N relative to growth rates.

Because we used a different cultivation method in 1994–1995, RGR values in Figure 4 cannot be compared directly to those in Figure 3. The former (commercial) method gives lower RGR values because a much higher seeding weight of *Gracilaria* was used. The relatively poor growth of *Gracilaria* at Sites A and C in December 1994 and January 1995 (compared to later in the year) was the result of the prolonged presence of warm oligotrophic surface water (see Anderson et al., 1996a). Weak southerly winds prevailed at the time, and there would have been little movement of fish-waste N to Site A and none to Site C. In February 1995 the *Gracilaria* at Site C died of apparent nutrient starvation, leaving only bleached thalli. This site is upwind of the waste source at this time of year, and would therefore have received no additional nitrogen. Site A was shown to be capable of receiving fish-waste nitrogen by the δ^{15}N ratios of the *Gracilaria* (see above). Anderson et al. (1996a) attributed better growth of *Gracilaria* at Site C than Site A in winter to the greater wind fetch (with winter northerlies), causing more wind chop and thallus movement and increasing nutrient uptake rates at the former site. However, that the northerly winds may also drive fish-waste to site C in winter is a possibility, although we have no δ^{15}N evidence to support this.

The sustained low C/N ratios in *Gracilaria* cultivated at Sites A and C from June–November 1995 indicate high N levels in the tissue (and hence in the water) relative to the growth rates of the plants. This was expected, because the water column is well-mixed and nutrient-rich in winter (see above). Although thermal stratification sets in between September and November, nutrient concentrations in the surface layer generally decline slowly over this period as they are taken up by phytoplankton (Anderson et al., 1996a; Monteiro & Brundrit, 1990). The high C/N ratios for December 1996–January 1997 indicate low tissue N levels, suggesting low N in the water at all sites, but especially Site C (the sample comes from a commercial experiment which was subsequently abandoned due to low growth rates and mussel spat settlement). The low δ^{15}N value (8.7 ‰) in December–January at Site C supports the idea of fish waste being unavailable there (south of the waste outfall) in summer.

Although we expected that the growth rates of *Gracilaria* would be higher at Site B throughout the summer and that δ^{15}N values from Site B would indicate uptake of far more fish-waste N than at Site A, this was not always the case. Except for the 2 months when cultivated *Gracilaria* died at Site A but grew well at Site B, there was no consistent difference in RGRs or in tissue C/N ratios between the two sites. There are several possible reasons. Water circulation patterns in Small Bay are not simple, and it is appears from our results that fish-waste N may be transported anywhere in the Bay at any particular time. More important, this waste clearly is spread widely enough to affect growth rates of cultivated *Gracilaria* throughout the north of the bay in summer, and even at its mouth in winter. Furthermore, Smit (unpubl.) found that fish-derived N is abundant in sediments throughout the north of Small Bay, and points out the likelihood of re-mineralization of N from these sediments, although it is not known how fast this N could enter the water column. However, re-mineralization and advection of sedimentary fish-N could explain why the δ^{15}N values of *Gracilaria* from Site A, for the four periods we measured them, were consistently higher than from Site B. Also, assumptions of nutrient concentrations from water temperatures should be treated with caution, as this study has shown that fish-waste nitrogen is widely distributed, and can strongly affect the growth of suspended seaweeds.

It is possible to estimate the overall amount of nitrogen that extensive *Gracilaria* farming in the bay would remove. Assuming a nitrogen content of 3.1% of dry weight (value from Smit, unpubl.), and a commercial net yield of 36 t ha^{-1} (from Anderson et al., 1996a), this gives a total nitrogen removal rate of 1.15 t ha^{-1} year^{-1}. In a commercial farm of 40 ha, 44.6 t of nitrogen would be removed annually, which is only about 7% of the 650 t introduced by the fish-factories. Substantial removal of nitrogen would therefore only be achieved by extensive *Gracilaria* farming.

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