

## $\delta^{15}\text{N}$ AS A TOOL TO DEMONSTRATE THE CONTRIBUTION OF FISH-WASTE-DERIVED NITROGEN TO AN *ULVA* BLOOM IN SALDANHA BAY, SOUTH AFRICA

P. M. S. MONTEIRO\*, R. J. ANDERSON† and S. WOODBOURNE‡

This study uses stable isotope ratios of nitrogen ( $\delta^{15}\text{N}$ ) to test the hypothesis that a bloom of the green seaweed *Ulva lactuca*, which occurred in Saldanha Bay, South Africa, in summer 1993/94 was linked to an adjacent discharge of nitrogen from pelagic fish processing waste. It is suggested that only two significant sources of new nitrogen were available to the *Ulva*: the natural nitrate flux from coastal upwelling and the fish factory nitrogen effluent. A significant difference (1.9‰) was found in the mean  $\delta^{15}\text{N}$  values between *Ulva* samples from a control site at Langebaan Lagoon (8.9‰) and those from Saldanha Bay (10.8‰). The latter value, which is relatively enriched in  $^{15}\text{N}$ , is consistent with the view that the nitrogen taken up in Saldanha Bay originated from a trophic position corresponding to pelagic fish in the southern Benguela system. The  $\delta^{15}\text{N}$  values from *Ulva* at the control site are consistent with nitrogen originating from the natural oceanic nitrate pool. It is suggested that this stable isotope method is useful in linking the causes and effects of eutrophication.

“Marine eutrophication is the process of enrichment of marine systems with plant nutrients that stimulate primary production, and, in its most serious manifestation, leads to visible algal blooms and at times massive growth of macrophytes” (Vollenweider 1992, p. 3). Marine eutrophication has become a global phenomenon, usually associated with organic waste discharge from human activities such as food processing, sewage or agricultural fertilizer runoff. It is invariably linked to development pressure on the land-ocean boundary and often has serious economic implications (Menesguen 1992, IGBP 1993).

Bay systems have restricted circulation and, especially in developing countries, are subject to conflicting uses linked to intense urbanization rates. Even where sound coastal zone management plans exist, it is often difficult to establish the relationships between sources and impacts because the sources are too numerous or diffuse. As a result, abatement and control of problems tend to be slow and difficult to implement and monitor. Such is the case in Saldanha Bay (Fig. 1), where a large bloom of *Ulva lactuca* occurred in the early austral spring of 1993/94 (Anderson *et al.* 1996, Sea Fisheries Research Institute [SFRI], unpublished data). The bloom occurred after an unusually large pulse of highly concentrated fish factory waste (fish tissue) in early summer (Anderson *et al.* 1996). This nitrogen-rich waste was discharged in a plume into a protected shallow water environment (Fig. 1) which is typically oligotrophic in the summer (CSIR 1991). The *Ulva* bloom, which displaced the red alga *Gracilaria verrucosa*, severely affected the economic

viability of a local industry dependent on the harvesting of *Gracilaria* wash-ups (Anderson *et al.* 1989) and resulted in high disposal costs for the local authority.

Saldanha Bay (Fig. 1) forms a part of the Benguela upwelling system (Monteiro and Brundrit 1990), where eutrophication can occur in tandem with natural biogeochemical forcing (Shannon 1985, Chapman and Shannon 1985). In this system, synoptic-scale upwelling events introduce pulses of nitrate ( $\text{NO}_3\text{-N} \sim 20\text{--}25\mu\text{M}$ ) into the euphotic zone, giving rise to typically high productivity levels (Barber and Smith 1981, Brown and Hutchings 1987, Brown 1992). However, in most upwelling systems, including the Benguela, nitrogen is the limiting nutrient for primary productivity (Bailey and Chapman 1985, Brown and Hutchings 1987). Typically, the initial upwelling-driven nitrogen pulse is utilized within 4–5 days (Brown and Hutchings 1987). The system then switches to a dependence on lower concentrations of regenerated ammonium ( $\text{NH}_4^+\text{-N}$ , Probyn 1985, 1992) until a subsequent  $\text{NO}_3\text{-N}$  pulse is supplied by further upwelling. In Saldanha Bay, there are three potential land-based sources of additional nitrogen inputs: runoff from a sewage treatment plant, a demersal fish (mainly hake *Merluccius capensis* and *M. paradoxus*) processing plant and a pelagic fish (mainly anchovy *Engraulis capensis* and pilchard *Sardinops sagax*) processing plant (CSIR 1991). The role of the last source in the observed *Ulva* bloom was investigated using stable isotope ratios of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ).

The use of stable isotope ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ )

\* Council for Scientific and Industrial Research, P.O. Box 320, Stellenbosch 7599, South Africa; formerly Sea Fisheries Research Institute, Private Bag X2, Rogge Bay 8012, South Africa. E-mail: pmonteir@csir.co.za

† Sea Fisheries Research Institute, Private Bag X2, Rogge Bay 8012, South Africa. E-mail: anderson@botzoo.uct.ac.za

‡ Department of Archaeology, University of Cape Town, Rondebosch 7700, South Africa

and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) as tracers of trophic interactions is now well established (Owens 1987, Peterson and Fry 1987, Monteiro *et al.* 1991, Sholto-Douglas *et al.* 1991). However, their use to investigate the impact of human activities on the marine environment is largely limited to sewage (Sweeney and Kaplan 1980, Spies *et al.* 1989, Van Dover *et al.* 1992). Stable isotope ratios provide an integrated signal over the turnover period of the tissue (Tieszen *et al.* 1983), and hence a robust measurement of specific interactions. As has been pointed out previously (Monteiro *et al.* 1991), the use of small sample sets (typically one or two) in natural abundance stable isotope ratio studies precludes insight into a central characteristic of natural systems: their variability. Larger sample sets provide a more realistic reflection of how trophic connections interact with each other dynamically in space and/or time. This is the approach adopted in the present study.

This study examines the hypothesis that a link existed between the input of fish factory nitrogen into Saldanha Bay and the appearance and subsequent growth of an *Ulva* bloom during the austral summer of 1993/94. Some of the physical and biogeochemical characteristics of Saldanha Bay, as well as *Ulva* physiology which underpin this link, are discussed in detail elsewhere (Anderson *et al.* 1996). The hypothesis was tested by comparing the stable nitrogen isotope ratios in *Ulva lactuca* in Langebaan Lagoon, a control site, with that of plants at the site impacted by pelagic fish factory waste in Saldanha Bay (Fig. 1).

## MATERIAL AND METHODS

The circumstances of the *Ulva* bloom placed a methodological constraint on the study: the bloom was reported only after the initial, large effluent pulse, which was thought to have precipitated the event, whereas the sampling was undertaken some six months later (Anderson *et al.* 1996). At the time of sampling, the *Ulva* bloom was still well established and, although the stable isotope method could no longer link it to the initial pulse, it could demonstrate that it was still largely sustained by the ongoing factory input.

*Ulva* samples were collected by SCUBA divers on the same day from both sites in February 1994. Although both sites had 100% cover of *Ulva*, in Saldanha Bay the bloom appeared suddenly and covered 20 ha, whereas in Langebaan Lagoon (Fig. 1) the *Ulva* presence is continuous and covers an area of 0:16 ha (Anderson *et al.* 1996). At Saldanha Bay, 23 *Ulva* plants were collected at random from the impacted area. Similarly, a total of 10 plants was collected at random from the control population in Langebaan

Lagoon. Each plant was placed in a separate plastic bag in an insulated container and frozen within 3 h.

Processing of *Ulva* samples followed the method outlined by Sealy (1986). To prevent the destructive corrosion of the quartz combustion tubes, traces of sea salt were removed by briefly washing in de-ionized water before the samples were freeze-dried and ground into a powder. A 25 mg subsample was sealed ( $<10^{-2}$  torr) in a quartz tube with excess copper oxide (CuO), metallic copper and a piece of silver filing. Combustion was carried out at 800°C for 5 h followed by slow overnight cooling. The  $\text{CO}_2$  and  $\text{N}_2$  were separated and dried by cryogenic distillation. The  $\delta^{15}\text{N}$  results are reported in per mil (‰) relative to air standard (0‰), in the conventional notation:

$$\delta \text{ (‰)} = [(R_s - R_{std})/R_{std}] \times 1000 \quad ,$$

where  $R_s$  and  $R_{std}$  are the atomic ratios ( $^{15}\text{N}/^{14}\text{N}$ ) of the sample and air standard respectively. The  $\delta^{15}\text{N}$  values were obtained using a VG 602E Mass Spectrometer and referenced throughout with Gelatine (Merck,  $\delta^{15}\text{N} = 7.50\text{‰}$ ) internal laboratory standard.

Some of the samples were too small for the direct inlet. These samples were introduced via a "cold finger" containing activated charcoal. However, the activated charcoal fractionates the sample gas, with the degree of fractionation being a function of the sample size. The nitrogen data are therefore the result of two methods of sample introduction:

- (i) Samples not requiring the use of the "cold finger". Standards run by this method fell within a standard deviation ( $SD = \pm 0.2\text{‰}$ ), and a constant correction of +0.15‰ was applied to all samples to bring the values in line with the accepted value of the standard.
- (ii) Samples that required the use of the "cold finger". Standard samples fell in an unacceptably low precision range ( $SD = \pm 0.48\text{‰}$ ), but they showed a predictable dependence on the inlet pressure. A linear regression relationship ( $r^2 = 0.87$ ) between the inlet pressure and the standard values was used to correct samples that were introduced by this method. This correction brought all the standards to within the acceptable precision ( $SD = \pm 0.2\text{‰}$ ) of the reference value.

## RESULTS

The mean  $\delta^{15}\text{N}$  values for *Ulva* from Saldanha Bay (10.8‰) and Langebaan Lagoon (8.9‰, Fig. 2), were significantly different ( $t$ -test,  $p = 0.005$ ,  $df = 31$ ). For a

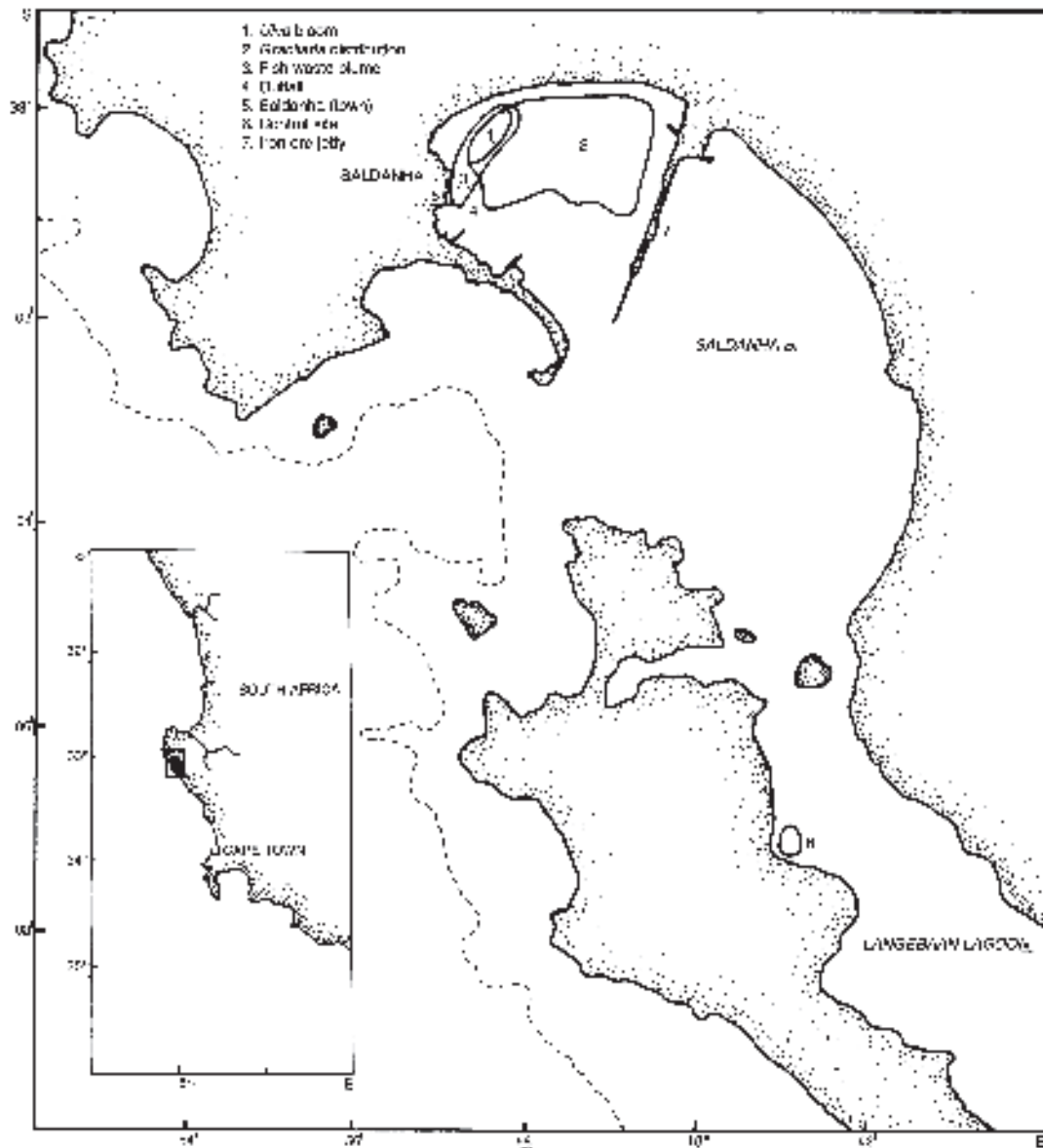


Fig. 1: Map of the study area, showing the geographical location (inset), range of the *Gracilaria* beds, site of the *Ulva* bloom and its association with the land-based, nitrogen-rich plume, and the control site

fuller comparison, additional literature values for a range of other seaweed genera are also shown in Figure 2 (Miyake and Wada 1967, Macko 1981, Sealy *et al.* 1987), marine nitrate (Miyake and Wada 1967, Cline and Kaplan 1975) and phytoplankton and anchovy

muscle nitrogen from the southern Benguela system (Sholto-Douglas *et al.* 1991). Figure 2 shows that Saldanha Bay *Ulva* samples were relatively enriched in  $^{15}\text{N}$  ( $\delta^{15}\text{N} = 10.8\text{‰}$ ), compared to both Langebaan Lagoon *Ulva* ( $\delta^{15}\text{N} = 8.9\text{‰}$ ) and various literature val-

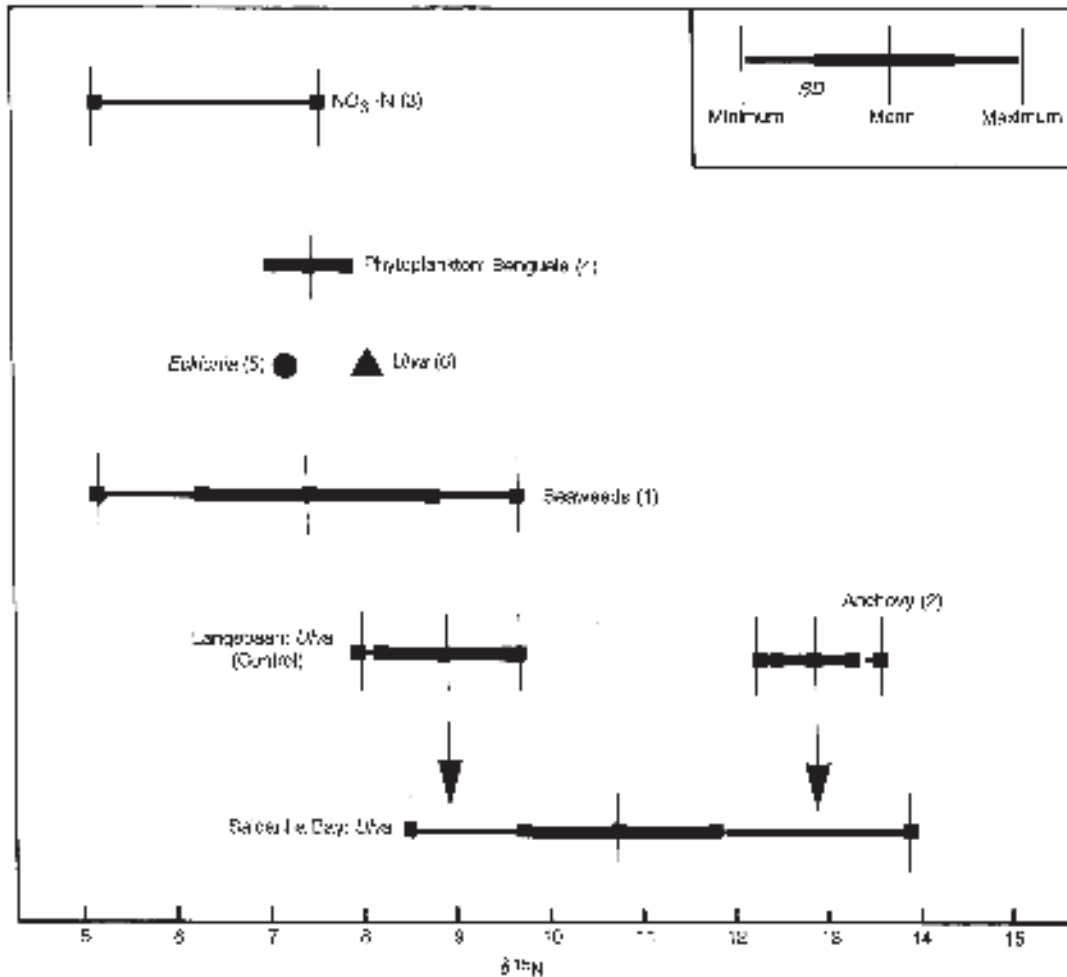


Fig. 2: A comparative display of the  $\delta^{15}\text{N}$  means, ranges (thin line) and standard deviations (thick line) of the impacted (Saldanha Bay) and control site (Langebaan Lagoon) *Ulva* samples, and the  $\delta^{15}\text{N}$  of potential N sources (anchovy from the southern Benguela system and  $\text{NO}_3^-$  from deep eastern Pacific Ocean). Published  $\delta^{15}\text{N}$  values of Benguela phytoplankton and selected seaweeds are as follows: (1) Miyake and Wada (1967), Macko (1981); (2) Sholto-Douglas *et al.* (1991); (3) Cline and Kaplan (1975), Miyake and Wada (1967); (4) Sholto-Douglas *et al.* (1991); (5) Sealy *et al.* (1987) and (6) Macko 1981

ues for a range of seaweeds (mean  $\delta^{15}\text{N} = 7.5\text{‰}$ ), including *Ulva* ( $\delta^{15}\text{N} = 8.1\text{‰}$ , Macko 1981). Values of anchovy muscle tissue nitrogen (mean  $\delta^{15}\text{N} = 12.9\text{‰}$ , Sholto-Douglas *et al.* 1991), which made up most of the waste from the fish factory (CSIR 1991), lie on the upper end of the Saldanha Bay *Ulva*  $\delta^{15}\text{N}$  range. The  $\delta^{15}\text{N}$  values derived from anchovy muscle nitrogen (Sholto-Douglas *et al.* 1991) were used because  $\text{NH}_4^+$  remineralized from anchovy tissue waste is one of the extreme values, the other being a natural  $\text{NO}_3^-$  input.

No fish from the factory was sampled because Sholto-Douglas *et al.* (1991) provided sufficient temporal and spatial coverage of the variability in anchovy  $\delta^{15}\text{N}$  from the southern Benguela system.

Histograms of the  $\delta^{15}\text{N}$  data are shown in Figure 3. One of the important aspects of multi-pathway trophic interactions is that no one pathway is always dominant. In order to define the relative magnitude of the main nitrogen pathways using this approach, two factors are essential. First, the sample size should be

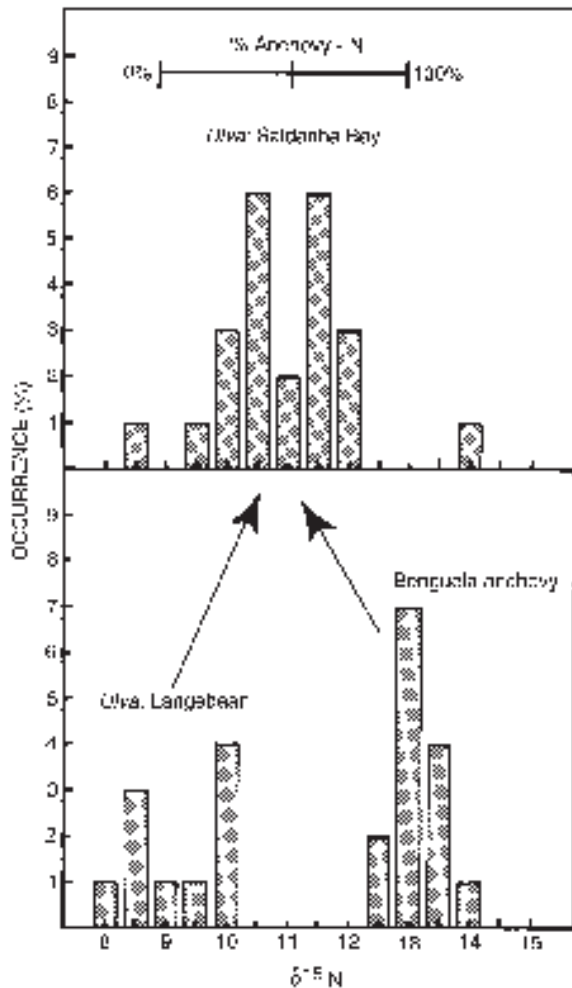


Fig. 3: Distribution of  $\delta^{15}\text{N}$  values from the Saldanha Bay *Ulva* bloom, the Langebaan control samples and anchovy (Sholto-Douglas *et al.* 1991). The intermediate distribution of the  $\delta^{15}\text{N}$  values of *Ulva* from Saldanha Bay is consistent with the derivation of its nitrogen requirements from both fish factory and natural inputs. The scale (0–100%) provides an approximate indication of the variability in the relative contribution of nitrogen from the fish factory effluent

large enough to reflect the effects of spatial and temporal variability. The stable isotope signal integrates temporal variability in the source of the N on a time-scale of the tissue turnover (Tieszen *et al.* 1983). Large sample sets, where thalli of different ages and exposure periods are randomly collected, allow the

temporal variability signal to be defined. Second, the data have to be modelled in such a way that the contribution of individual data values to the sample set can be discerned. This important indicator of variability can be lost with the use of means and standard deviations. The modes in a histogram, when sufficiently well resolved, can then be used to quantify the magnitudes of the pathways (Monteiro *et al.* 1991). In this study, the  $\delta^{15}\text{N}$  histogram (Fig. 3) confirms that the data from Saldanha Bay are distributed in the  $\delta^{15}\text{N}$  range between the  $\delta^{15}\text{N}$  extremes of the Langebaan control samples and the southern Benguela anchovy values.

## DISCUSSION

This discussion centres on four issues:

- (i) whether the Langebaan Lagoon site served as a valid and suitable control, representative of the natural nitrate input;
- (ii) whether it is possible to support the view that one can consider only reduced N from the fish factory and ignore all other sources of N;
- (iii) whether  $\delta^{15}\text{N}$  values from anchovy collected in the southern Benguela system provide suitable estimates of those in the fish factory waste;
- (iv) assessment of the relative magnitudes of the fish factory and natural nitrogen input pathways.

### Validation of the control site

The Langebaan Lagoon site was chosen because it was the closest alternative source of *Ulva lactuca* not influenced by land-based sources of nitrogen, and it was sufficiently far from Saldanha Bay for the impact of fish waste to be extremely unlikely (Fig. 1). This species of *Ulva* only grows in very sheltered environments on the South African west coast (Anderson *et al.* 1996). The Langebaan Lagoon site lies in the semi-arid West Coast National Park, where there is a very low human presence, no sewage discharge, no agricultural activity, and very little drainage runoff. The mean value for *Ulva*  $\delta^{15}\text{N}$  at that site is within the range expected from nitrogen-limited marine systems (5–9‰, Owens 1987).

One of the main sources of variability in  $\delta^{15}\text{N}$  data for marine primary producers is the dynamics of the isotope fractionation factor associated with the proportion of the nitrogen pool which is consumed (Owens 1987), and to a lesser extent internal metabolic fractionation (Hare *et al.* 1991). This was recently high-

lighted by the very depleted  $\delta^{15}\text{N}$  values obtained from phytoplankton samples along a meridional section across the Subtropical Convergence (Altabet and Francois 1994). Samples from sub-antarctic surface waters, where  $\text{NO}_3\text{-N}$  is not limiting, are more depleted ( $\delta^{15}\text{N}$  c.  $-2$  to  $-4\text{‰}$ ) than those in the oligotrophic waters farther north ( $6\text{--}8\text{‰}$ ), where nitrogen is both low and limiting. Published  $\delta^{15}\text{N}$  values for oceanic  $\text{NO}_3\text{-N}$  range from  $5.2\text{--}7.6\text{‰}$  (Fig. 2, Miyake and Wada 1967, Cline and Kaplan 1975). Although taken from a very small dataset, it is clear that, in nitrogen-limited systems, the  $\delta^{15}\text{N}$  ratio of primary producers is a good proxy for the  $\delta^{15}\text{N}$  of the source nitrogen. Therefore, for this type of fractionation dynamics, the more limiting the nitrogen supply, the more the values of  $\delta^{15}\text{N}$  for the natural  $\text{NO}_3\text{-N}$  supply and the plant tissue converge. Generally, it is expected that, where the N supply is growth-limiting and exclusively of natural marine origin, as in Langebaan Lagoon, the plant tissue  $\delta^{15}\text{N}$  will be close to the measured  $\text{NO}_3\text{-N}$  range given above. The most plausible explanation for the positive excursion by some of the Langebaan values is that the samples come from a sheltered area some distance from the mouth of Saldanha Bay (Fig. 1). The water advected into this area would have had some of its nitrate taken up by phytoplankton which, because of the nature of the same fractionation kinetics, would preferentially remove the lighter fraction (Owens 1987). This would result in the advection of  $\text{NO}_3\text{-N}$  enriched to some degree in  $\delta^{15}\text{N}$ , which would be a function of the proportion of  $\text{NO}_3$  removed (Owens 1987). This convergence of values and the geographical location of the Langebaan site suggest that it is suitable as a control, and that it provides  $\delta^{15}\text{N}$  values from *Ulva* which reflect the range for natural  $\text{NO}_3\text{-N}$  inputs.

The single  $\delta^{15}\text{N}$  value for the Benguela macroalga *Ecklonia maxima* (Sealy *et al.* 1987) is in the same range as those other macroalgae such as *Gelidium crinale* ( $\delta^{15}\text{N} = 7.9\text{‰}$ ) and *Fucus nodosum* [*sic.*] ( $\delta^{15}\text{N} = 8.1\text{‰}$ ), all from sites in Texas or Maine (Macko 1981). Sholto-Douglas *et al.* (1991) report similar values for pelagic phytoplankton in the southern Benguela system (mean  $\delta^{15}\text{N} = 7.5\text{‰}$ , Fig. 2). All of these values fall within the range  $6\text{--}8\text{‰}$ , supporting the contention that low  $\delta^{15}\text{N}$  values are consistent with nitrogen-limited systems. Published seaweed data support the view that there is a predictable  $\delta^{15}\text{N}$  range for seaweeds growing in waters characterized by natural oceanic  $\text{NO}_3\text{-N}$  inputs. The similarity between such seaweed and  $\text{NO}_3\text{-N}$   $\delta^{15}\text{N}$  values suggests that the former are suitable and reliable indicators of the variability in the isotopic characteristics of the nitrogen source.

#### Potential sources of $\delta^{15}\text{N}$ variability besides fish waste

The  $\delta^{15}\text{N}$  values from the Saldanha Bay *Ulva* bloom (Fig. 2) differ in two respects from those from the control site. The mean  $\delta^{15}\text{N}$  ( $10.8\text{‰}$ ) is enriched in  $^{15}\text{N}$  by  $1.9\text{‰}$  relative to that of the control site. Similarly, the range between the highest and lowest values ( $1.7\text{‰}$ ) is smaller in Langebaan Lagoon relative to the more variable value of  $5.4\text{‰}$  in Saldanha Bay. This indicates that the nitrogen source in Langebaan Lagoon is also more uniform. The difference in mean  $\delta^{15}\text{N}$  values suggests a  $^{15}\text{N}$  enriched source which is not available to the *Ulva* population in Langebaan Lagoon. On the other hand, the larger range of  $\delta^{15}\text{N}$  values for the *Ulva* population in Saldanha Bay suggests a dynamic interaction between the two sources with differing  $^{15}\text{N}$ . There is no correlation between  $\delta^{15}\text{N}$  and depth or distance from the outfall. The positive direction of the isotope shift excludes the possibility of an input of human sewage, because its  $\delta^{15}\text{N}$  values are typically in the range  $-1\text{--}2\text{‰}$  (Sweeney and Kaplan 1980, Spies *et al.* 1989, Van Dover *et al.* 1992, SFRI unpublished data from Table Bay, Cape Town).

The relatively depleted  $\delta^{15}\text{N}$  signal in sewage is linked to the fact that terrestrial plants obtain most of their nitrogen by fixation of atmospheric nitrogen, which has a reference value of  $0\text{‰}$  (Owens 1987). In some circumstances a strong positive shift in  $\delta^{15}\text{N}$  can be found in farm animal sewage after prolonged exposure to the atmosphere (Heaton 1986). This is linked to kinetically controlled fractionation, which accompanies direct loss of  $\text{NH}_3\text{-N}$  to the atmosphere rather than reflecting a natural signal (Heaton 1986). This mechanism is not expected to play any role in the treated sewage from the town of Saldanha Bay. The calculated flux rate of sewage-derived nitrogen ( $50 \times 10^{-3} \mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) from the effluent of the treatment plant into Saldanha Bay is also seven orders of magnitude smaller than the nitrogen flux value of  $4 \times 10^5 \text{ m}^2$  calculated for the pelagic fish factory over an equivalent area (Table I, SFRI, unpublished data). Furthermore, discharge is into the north-east corner of this region of the bay, about 2 km from the *Ulva* bloom, and the prevailing clockwise circulation in the bay (Weeks *et al.* 1990) transports the sewage input away from the affected *Ulva* zone. Therefore, isotope characteristics would transport the low nitrogen flux rate, and circulation patterns rule out sewage as a cause of the *Ulva* bloom.

Similarly, N from river-borne agricultural fertilizer can be discounted on account of the semi-arid nature of the local environment. This is particularly so because this study was carried out in summer, the dry season in

Table I: Fluxes of the main potential sources of nitrogen in Saldanha Bay

Potential sources of nitrogen	Nitrogen output ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ )
Natural input [ $\text{NO}_3^-$ ]	1 000
Pelagic fish waste	2 000; 285*
Demersal fish waste	200
Sewage	$5 \times 10^{-3}$

\*Values reflect the "normal" output, where the first value assumes the N load to be distributed within the plume ( $4 \times 10^5 \text{ m}^2$ ) and the second value assumes it to be distributed over the Bay ( $70 \times 10^6 \text{ m}^2$ ). The pulse in August 1993 that induced the *Ulva* bloom was between 20 000 and 50 000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ , and it lasted for approximately two weeks

the southern Benguela. Furthermore, fertilizer may also be excluded because, by virtue of its atmospheric nitrogen origin through the Haber Process, it is also very depleted ( $\delta^{15}\text{N} < 0\text{‰}$ , Heaton 1986). The isotope shift at the Saldanha site is in the wrong direction to imply that fertilizer was involved.

#### The use of anchovy $\delta^{15}\text{N}$ values to characterize the $\delta^{15}\text{N}$ of fish factory effluent utilized by *Ulva*

The fish factory to which the impact is ascribed is known to process mainly (>90%) anchovy into fishmeal (CSIR 1991). A large set of  $\delta^{15}\text{N}$  values for this species is provided by Sholto-Douglas *et al.* (1991), which reflects their expected range of temporal and spatial distribution off the South African west coast. This dataset, which comprises  $\delta^{15}\text{N}$  values for both muscle and bone tissues, likely includes the range of  $\delta^{15}\text{N}$  variability characterizing the fish processed by this factory over a year. The daily waste discharged by the plant in the fishing season (normally January–August, but extended to December in 1993) is constituted by mainly small, macerated pieces of anchovy soft tissue (muscle), produced as a result of the mechanical and heat damage to the fish. The bulk of the nitrogen is discharged in an organic (protein) form (CSIR 1991), which would be expected to be rapidly remineralized into  $\text{NH}_4^+$  and taken up by, *inter alia*, the thalli of the *Ulva*. Based on accumulated evidence from the literature on this type of nitrogen uptake (Harlin *et al.* 1981), the  $\delta^{15}\text{N}$  values of this remineralized inorganic N would reflect the values of the source material (anchovy muscle) rather than the isotopically lighter "natural"  $\text{NO}_3^-$ - $\delta^{15}\text{N}$ . This assumption is the basis for the use of stable isotopes as tracers for specific inputs such as sewage (Sweeney and Kaplan 1980, Spies *et al.* 1989, Van Dover *et al.* 1992) and of trophic pathways (Sholto-Douglas *et al.* 1991).

#### Fish processing waste inputs

The remaining possible N sources for *Ulva* in Saldanha Bay are waste from the factories processing either the pelagic fish and/or the demersal fish. Both have  $\delta^{15}\text{N}$  values (Sholto-Douglas *et al.* 1991) which, given a sufficiently large flux, should result in a  $^{15}\text{N}$  enriched *Ulva*  $\delta^{15}\text{N}$ . However, it has been shown previously that the total N flux (Kjeldahl-N) from the pelagic factory discharge is one to two orders of magnitude larger than that from the demersal fish processing (Anderson *et al.* 1996, Table I). This is supported by the isotope data (Fig. 3). The highest *Ulva*  $\delta^{15}\text{N}$  value (14) corresponds to a scenario where 100% of the nitrogen requirements of that sample were provided by the upper range of the anchovy processing waste input. In contrast, hake  $\delta^{15}\text{N}$  values range between 15 and 17‰ (C. Parkins, University of Cape Town, pers. comm), for which there are no corresponding *Ulva*  $\delta^{15}\text{N}$  values.

Pelagic fish processing waste from physical damage during offloading, fishmeal processing and canning is primarily of soft tissue origin. Two features are evident in Figure 2: first, the mean value (12.89 ‰) is at the upper range of the Saldanha Bay *Ulva*  $\delta^{15}\text{N}$ ; second, the maxima of the two (13.6 and 13.9 ‰) are comparable. Variability in anchovy  $\delta^{15}\text{N}$  is a function of the trophic position of the organism, but the range encountered is small (1.3‰) (Monteiro *et al.* 1991, Sholto-Douglas *et al.* 1991). The  $\delta^{15}\text{N}$  values used in this study to characterize fish factory waste are from a spatially and temporally variable historical data set of  $\delta^{15}\text{N}$  from anchovy caught in the southern Benguela (Sholto-Douglas *et al.* 1991). These data, rather than a few samples taken directly from the effluent, are considered to represent better the full range of  $\delta^{15}\text{N}$  values of the fish which make up the bulk (>90%) of the waste. The fact that this  $\delta^{15}\text{N}$  contribution is constrained by such a large range of values, rather than by a few samples, strengthens the argument that much of the N in the *Ulva* came from the fish waste.

The effluent constitutes both a particulate and dissolved load of nitrogen from anchovy soft tissue: the reduced nitrogen (mostly tissue protein) is remineralized to  $\text{NH}_4^+$  in the water column and benthos after discharge (CSIR 1991), and taken up in this form by the *Ulva* (Harlin and Thorne-Miller 1982). Its isotope characteristics are linked to the characteristics of the source material (fish protein), which is more enriched in  $^{15}\text{N}$  (Sholto-Douglas *et al.* 1991) than in naturally derived  $\text{NO}_3^-$  (Cline and Kaplan 1975). There are no published  $\delta^{15}\text{N}$  values for  $\text{NO}_3^-$  in the southern Benguela system. However, given that its natural nitrogen ( $\text{NO}_3^-$ -N) inputs are driven by the shelf scale upwelling of South Atlantic Central Water

(SFRI, unpublished data), its values will not be very different to those from equivalent oceanic environments elsewhere (Cline and Kaplan 1975). *Ulva* can take up both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , but it is particularly efficient in the use of  $\text{NH}_4^+$ , especially in comparison to the local endemic rhodophyte *Gracilaria verrucosa* (Anderson *et al.* 1996).

The distribution of the  $\delta^{15}\text{N}$  values of the Saldanha Bay *Ulva* samples presented in Figure 3 is consistent with variable inputs of nitrogen from both natural sources and fish factory effluent. The magnitudes of the fluxes from these two sources to the *Ulva* bloom area (Table I) shows that, although the fish factory nitrogen flux is higher ( $2\,000\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  cf.  $\text{NO}_3^-$  flux:  $1\,000\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ), they are both of the same order of magnitude. This is in good agreement with the distribution of the  $\delta^{15}\text{N}$  values from the Saldanha Bay *Ulva* samples, which showed that the highest occurrences ( $>5$ ) were in the  $\delta^{15}\text{N}$  range, corresponding to a 40–60% contribution from nitrogen derived from anchovy waste.

### CONCLUSION

The results of this study support the hypothesis that the output of the pelagic fish factory provided a significant proportion of the nitrogen requirements which sustained the *Ulva* bloom. These isotope data, in conjunction with effluent discharge figures discussed elsewhere, provide strong support for the hypothesis that fish waste not only caused the bloom but also sustained it while the favourable summer conditions of low turbidity prevailed. The problems associated with fish factory discharges in Saldanha Bay are long-standing (Newman and Pollock 1973, Monteiro *et al.* 1990). Historically, there has been little evidence to link it with specific environmental impacts, mainly because of the high levels of natural eutrophication (SFRI, unpublished data). The present results are a start to changing this status. Modelling of these interactions assists in constraining the boundaries of variability attributable to the natural and anthropogenic biogeochemical driving mechanisms and aids in quantifying their relative impact.

### ACKNOWLEDGEMENTS

This work forms part of the Saldanha Bay Mariculture-Environment Research Programme, coordinated by the Sea Fisheries Research Institute Working Group: Interactions between mariculture and

the environment. We thank our colleagues at the Sea Fisheries Research Institute, Mr G. J. Levitt, for assisting in the sample collection, and Dr T. A. Probyn, for constructive discussions. The analyses were carried out in the Archaeometry Laboratory, University of Cape Town.

### LITERATURE CITED

- ALTABET, M. A. and R. FRANCOIS 1994 — Sedimentary nitrogen isotopic ratio as a recorder for surface ocean nitrate utilization. *Global biogeochem. Cycles* **8**(1): 103–116.
- ANDERSON, R. J., MONTEIRO, P. M. S. and G. J. LEVITT 1996 — The effect of localised eutrophication on competition between *Ulva lactuca* (Ulvaceae, Chlorophyta) and a commercial resource of *Gracilaria verrucosa* (Gracilariaceae, Rhodophyta). *Hydrobiologia* **326/327**: 291–296.
- ANDERSON, R. J., SIMONS, R. H. and N. G. JARMAN 1989 — Commercial seaweeds in southern Africa: a review of utilization and research. *S. Afr. J. mar. Sci.* **8**: 277–299.
- BAILEY, G. W. and P. CHAPMAN 1985 — The nutrient status of the St Helena Bay region in February 1979. In *South African Ocean Colour and Upwelling Experiment*. Shannon, L. V. (Ed.). Cape Town; Sea Fisheries Research Institute: 125–145.
- BARBER, R. T. and R. L. SMITH 1981 — Coastal upwelling ecosystems. In *Analysis of Marine Ecosystems*. Longhurst, A. R. (Ed.). London; Academic Press: 31–68.
- BROWN, P. C. 1992 — Spatial and seasonal variation in chlorophyll distribution in the upper 30 m of the photic zone in the southern Benguela/Agulhas ecosystem. In *Benguela Trophic Functioning*. Payne, A. I. L., Brink, K. H., Mann, K. H. and R. Hilborn (Eds). *S. Afr. J. mar. Sci.* **12**: 515–525.
- BROWN, P. C. and L. HUTCHINGS 1987 — The development and decline of phytoplankton blooms in the southern Benguela upwelling system. 1. Drogue movements, hydrography and bloom development. In *The Benguela and Comparable Ecosystems*. Payne, A. I. L., Gulland, J. A. and K. H. Brink (Eds). *S. Afr. J. mar. Sci.* **5**: 357–391.
- CHAPMAN, P. and L. V. SHANNON 1985 — The Benguela ecosystem. 2. Chemistry and related processes. In *Oceanography and Marine Biology. An Annual Review* **23**. Barnes, M. (Ed.). Aberdeen; University Press: 183–251.
- CLINE, J. D. and I. R. KAPLAN 1975 — Isotopic fractionation of dissolved nitrate during denitrification in the eastern tropical north Pacific Ocean. *Mar. Chem.* **3**: 271–299.
- CSIR 1991 — Evaluation of the discharge of selected fish factory effluents and the impact on the adjacent marine environment. *Rep. S. Afr. Coun. scient. ind. Res.* **EMA-C 91171**: 49 pp. + Appendices and Figures.
- HARE, P. E., VOGEL, M. L., STAFFORD, T. W., MITCHELL, A. D. and T. C. HOERING 1991 — The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *J. archaeol. Sci.* **18**: 277–292.
- HARLIN, M. M. and B. THORNE-MILLER 1981 — Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. *Mar. Biol.* **65**: 221–229.
- HEATON, T. H. E. 1986 — Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chem. Geol.* **59**: 87–102.
- IGBP 1995 — Land-Ocean Interactions in the Coastal Zone. Implementation plan. Pernetta, J. C. and J. D. Milliman (Eds). Stockholm; IGBP: 215 pp. (*IGBP Global Change*



- Rep. 33).
- MACKO, S. A. 1981 — Stable nitrogen isotope ratios as tracers of organic geochemical processes. Ph.D. thesis, University of Texas: 181 pp.
- MENESGUEN, A. 1992 — Modelling coastal eutrophication: the case of French *Ulva* mass blooms. In *Marine Coastal Eutrophication*. Vollenweider, R. A., Marchetti, R. and R. Viviani (Eds). Amsterdam; Elsevier: 979–992.
- MIYAKE, Y. and E. WADA 1967 — The abundance ratio of  $^{15}\text{N}/^{14}\text{N}$  in marine environments. *Rec. oceanogr. Wks Jap.* **9**(1): 37–53.
- MONTEIRO, P. M. S. and G. B. BRUNDRIT 1990 — Interannual chlorophyll variability in South Africa's Saldanha Bay system, 1974–1979. *S. Afr. J. mar. Sci.* **9**: 281–287.
- MONTEIRO, P. M. S., JAMES, A. G., SHOLTO-DOUGLAS A. D. and J. G. FIELD 1991 — The  $\delta^{13}\text{C}$  trophic position isotope spectrum as a tool to define and quantify carbon pathways in marine food webs. *Mar. Ecol. Prog. Ser.* **78**: 33–40.
- MONTEIRO, P. M. S., MCGIBBON, S. and J. L. HENRY 1990 — A decade of change in Saldanha Bay: natural or anthropogenic? *S. Afr. J. Sci.* **86**(7–10): 454–456.
- NEWMAN, G. G. and D. E. POLLOCK 1973 — Organic pollution of the marine environment by pelagic fish factories in the Western Cape. *S. Afr. J. Sci.* **69**(1): 27–29.
- OWENS, N. J. P. 1987 — Natural variations in  $^{15}\text{N}$  in the marine environment. *Adv. mar. Biol.* **24**: 389–451.
- PETERSON, B. J. and B. FRY 1987 — Stable isotopes in ecosystem studies. *A. Rev. Ecol. Syst.* **18**: 293–320.
- PROBYN, T. A. 1985 — Nitrogen uptake by size-fractionated phytoplankton populations in the southern Benguela upwelling system. *Mar. Ecol. Prog. Ser.* **22**(3): 249–258.
- PROBYN, T. A. 1992 — The inorganic nitrogen nutrition of phytoplankton in the southern Benguela: new production, phytoplankton size and implications for pelagic foodwebs. In *Benguela Trophic Functioning*. Payne, A. I. L., Brink, K. H., Mann, K. H. and R. Hilborn (Eds). *S. Afr. J. mar. Sci.* **12**: 411–420.
- SEALY, J. C. 1986 — Stable carbon isotopes and prehistoric diets in the south-western Cape Province. South Africa. *Br. Archaeol. Rep. intl Series* **293**: 150 pp. (Cambridge Monographs in African Archaeology 15).
- SEALY, J. C., VAN DER MERWE, N. J., LEE THORP, J. A. and J. L. LANHAM 1987 — Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. *Geochim. cosmochim. Acta* **51**: 2707–2717.
- SHANNON, L. V. 1985 — The Benguela ecosystem. 1. Evolution of the Benguela, physical features and processes. In *Oceanography and Marine Biology. An Annual Review* **23**. Barnes, M. (Ed.). Aberdeen; University Press: 105–182.
- SHOLTO-DOUGLAS, A. D., FIELD, J. G., JAMES, A. G. and N. J. VAN DER MERWE 1991 —  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  isotope ratios in the southern Benguela Ecosystem: indicators of food web relationships among different size-classes of plankton and pelagic fish; differences between fish muscle and bone collagen tissues. *Mar. Ecol. Prog. Ser.* **78**: 23–31.
- SPIES, R. B., KRUGER, H., IRELAND, R. and D. W. RICE 1989 — Stable isotope ratios and contaminant concentrations in a sewage-distorted food web. *Mar. Ecol. Prog. Ser.* **54**: 157–170.
- SWEENEY, R. E. and I. R. KAPLAN 1980 — Tracing flocculent industrial and domestic sewage transport on San Pedro shelf, southern California, by nitrogen and sulphur isotope ratios. *Mar. environ. Res.* **31**: 215–224.
- TIESZEN, L. L., BOUTTON, T. W., TESDAHL, K. G. and N. A. SLADE 1983 — Fractionation and turnover of stable carbon isotopes in animal tissues: implications for  $\delta^{13}\text{C}$  analysis of diet. *Oecologia* **57**: 32–37.
- VAN DOVER, C. L., GRASSLE, J. F., FRY, B., GARRITT, R. H. and V. R. STARCZAK, 1992 — Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. *Nature, Lond.* **360**: 153–156.
- VOLLENWEIDER, R. A. 1992 — Coastal marine eutrophication: principles and control. In *Marine Coastal Eutrophication*. Vollenweider, R. A., Marchetti, R. and R. Viviani (Eds). Amsterdam; Elsevier: 1–20.
- WEEKS, S. J., BOYD, A. J., MONTEIRO, P. M. S. and G. B. BRUNDRIT 1991 — The currents and circulation in Saldanha Bay after 1975 deduced from historical measurements of drogues. *S. Afr. J. mar. Sci.* **11**: 525–535.