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Use of $\delta^{15}N$ signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal

A. Gartner[1,*], P. Lavery[1,], A. J. Smit[2]**

[1]Centre for Ecosystem Management, Edith Cowan University, 100 Joondalup Drive, Joondalup, Western Australia 6027, Australia
[2]Botany Department, The University of Cape Town, Rondebosch 7700, South Africa

ABSTRACT: We examined whether $\delta^{15}N$ levels of marine biota with different nutrient uptake characteristics can be used to trace the dispersal of sewage effluent in highly mixed, nitrogen-limited waters, and whether they can reveal the dispersal of sewage over different timescales. We hypothesised that macroalgal species with fast uptake rates would display a spatial pattern in $\delta^{15}N$ levels reflecting recent sewage dispersal while those with slower rates would provide a signal integrated over a longer time period. Filter-feeding sponges and ascidians were also sampled to see if they reflected patterns in the dispersal of sewage particulate organic matter (POM). A laboratory experiment was performed to test whether the $\delta^{15}N$ level of 3 macroalgal species (*Ulva australis*, *Vidalia* sp. and *Ecklonia radiata*) and 2 filter-feeding species (*Clathria* sp. and *Pyura australis*) was altered after cultivation in sewage nitrogen. We then sampled each organism along transects radiating away from the outlet of a wastewater treatment plant north of Perth, Western Australia, to determine spatial patterns in $\delta^{15}N$. *U. australis* and *Vidalia* sp. developed higher isotopic signatures when exposed to low concentrations of sewage nitrogen (1:500 dilution in seawater) for 7 d in the laboratory. *U. australis* and *Vidalia* sp. showed an increase of 1.7 and 1.4 ‰ in treatments respectively. In the field, macroalgae sampled north and south of the sewage outlet generally had higher $\delta^{15}N$ levels than those sampled west of the outlet and at the reference site, and algae within 500 m of the outfall tended to have lower values than at 1000 m or more from the outfall. These trends are consistent with our current knowledge of plume dynamics: a predominantly northerly drift of effluent as a buoyant plume that tends not to be fully mixed in the water column for the first 500 m. The results confirmed that the $\delta^{15}N$ signature of macroalgae could be used to trace sewage disposed in well-mixed waters. The strength of the spatial trends varied between algae, with *E. radiata*, the species with the lowest nutrient uptake rates and affinity, having the least spatial variability. We interpret this as reflecting a wider regional dispersal of sewage in the longer time frame, but a strong northerly drift in the short term, which was reflected in the $\delta^{15}N$ values of the species with the fastest nitrogen uptake rates. The results were consistent with our hypothesis and are suggestive of a relationship between algal functional form and isotopic signatures that can be applied to determine the dispersal of sewage over different timescales. The $\delta^{15}N$ values of benthic filter feeders did not provide strong evidence to suggest that they can be used to represent the dispersal of sewage POM, but trends found in the field experiment for *Clathria* sp. warrant further investigation.

KEY WORDS: Stable isotopes · $\delta^{15}N$ · Nitrogen · Sewage · Marine macroalgae · Reefs · Western Australia

INTRODUCTION

Throughout the world, the loads of sewage discharged into marine waters have steadily increased, matching the growth of coastal urbanisation. The impacts of sewage disposal on benthic biota have been examined and include reduced species richness and abundance (Brown et al. 1990, Hardy et al. 1993, Munda 1993, Kelly 1995), with the nutrient enrichment effect of sewage pollution often implicated as a major factor. In many cases however, it can be difficult to separate the impacts of sewage on marine biota from natural variability in species diversity and abundance (Carballo et al. 1996, Roberts 1996)

*Present address: Department of Environmental Protection, PO Box 822, Perth, Western Australia 6842, Australia
**Corresponding author. E-mail: p.lavery@ecu.edu.au

making it difficult to perform classic control-impact studies.

Traditional methods for monitoring sewage dispersal around sewage outlets often use spot sampling of physiochemical tracers, such as nutrients and salinity, or phytoplankton biomass. These methods are laborious, time-consuming and costly, and often only provide a generalised pattern of effluent dispersal. In well-mixed environments the signal of these tracers is rapidly lost as the pollutant is diluted and, at best, they provide only an instantaneous view of sewage dispersal. Short pulses of sewage pollution in areas that would not receive sewage under 'normal' circumstances are likely to be missed, although these events may be ecologically significant. The need exists therefore for a technique which can provide an integrated picture of effluent plume dispersal over specific time-periods and which is sensitive enough to detect subtle differences in nutrient concentrations. Recent work indicates that stable isotopic signatures of aquatic macrophytes may indicate the dispersal of sewage nitrogen (Handley & Raven 1992, Udy & Dennison 1997, McClelland & Valiela 1998).

Often the marine environment can be nitrogen-limited, and algae will assimilate nitrogen additions from alternative sources, such as sewage effluent (Dhargalkar 1986, Lyngby & Mortensen 1994). As sewage generally has a significantly higher $\delta^{15}N$ value than seawater (Owens 1987, Aravena et al. 1993, Sigman et al. 1997), the natural abundance of stable isotopes can confirm the source of nutrients that primary producers are assimilating. Udy & Dennison (1997), McClelland & Valiela (1998) and Fry et al. (2000), have used the $\delta^{15}N$ levels of seagrasses and mangroves to infer the dispersal of sewage effluent. Recent studies have used the $\delta^{15}N$ levels of marine macroalgae to demonstrate the contribution of anthropogenic-derived N to macroalgal blooms at sites in the vicinity of a fish-processing waste outlet (Monteiro et al. 1997, Anderson et al. 1999). While these studies did not provide a detailed, time-integrated picture of effluent dispersal, they support the notion that macroalgal $\delta^{15}N$ levels could be used for this purpose. Tucker et al. (1999) also used stable nitrogen isotopes of macroalgae to demonstrate widespread evidence of sewage-derived materials in Massachusetts Bay, USA. We hypothesised that macroalgae would be potentially more useful in tracing sewage dispersal than either seagrasses or mangroves for 2 reasons. First, their widespread dispersal and reliance on water column dissolved inorganic nitrogen (DIN) makes them strong candidates; second, most macroalgal assemblages will include species with a wide range of nutrient uptake characteristics. We hypothesised that this facet could be used to reveal not only the dispersal of sewage effluent, but also provide information on the timescale over which a site was affected by sewage.

Macroalgae depend predominantly on DIN from the water column to meet their nitrogen requirements (Wallentinus 1984). Littler (1980) classified the macroalgae into functional groups according to morphology, and Wallentinus (1984) showed that their nutrient uptake characteristics are related to these functional groupings. Foliose and filamentous species have high nutrient uptake rates, and so on exposure to a pulse of nitrogen they should have the ability to assimilate a relatively large amount of that source and for this to be reflected in their isotopic signature. In comparison, species with slow uptake rates should only exhibit a shift in their isotope ratio after exposure to an alternative source of nitrogen for a longer period of time. Likewise, filter-feeding organisms such as sponges and ascidians that assimilate particulate organic matter (POM) from the water offer the potential to trace the dispersal of sewage-derived POM, as opposed to assuming it has the same dispersal as sewage DIN.

The objectives of this study were to test whether the $\delta^{15}N$ levels of marine macroalgae and filter-feeders can be used to determine the spatial and temporal patterns of sewage effluent dispersal around a sewage outlet. To do this, 2 procedures were used. First, a laboratory experiment was used to test the capacity of a range of algae and filter-feeders to develop a $\delta^{15}N$ signal when exposed to sewage effluent. The algae included species with different nitrogen uptake rates. We then sampled the same organisms *in situ* at sites radiating away from a sewage outlet to see whether they displayed spatial patterns in $\delta^{15}N$ values which corresponded to the known spatial and temporal dispersal pattern of the effluent plume.

MATERIALS AND METHODS

Study area. The study was conducted off Ocean Reef, Western Australia (31° 52′ S, 115° 44′ E: Fig. 1), in the vicinity of the sewage outlet for the Beenyup Wastewater Treatment Plant (WWTP). The outlet lies 1.8 km offshore in a shallow (<15 m) body of water, semi-enclosed by a high-relief reef system running parallel to the coast, which has helped form a coastal lagoon. Approximately 140 ml d^{-1} (total N of approximately 32 mg l^{-1}) of secondary treated sewage is discharged into the Ocean Reef lagoon (Lord & Hillman 1995). Oceanic swells from the west and southwest ensure that the lagoon is well mixed (Searle & Semeniuk 1985).

The lagoon contains patchy high- and low-relief reefs with diverse algal assemblages (Phillips et al. 1997, Kendrick et al. 1999). *Ecklonia radiata* and *Sargassum* sp., form canopies underlain by a highly diverse understorey. Sponge and ascidian assemblages

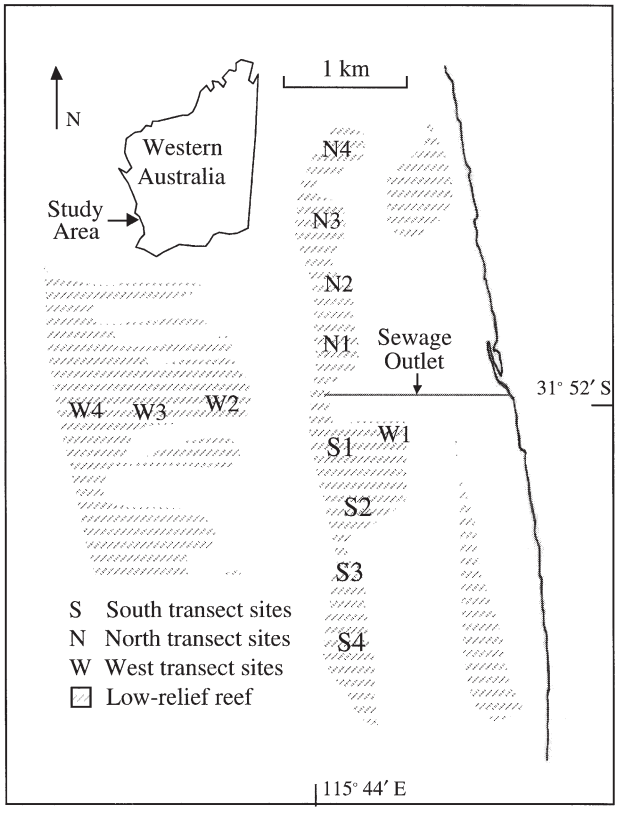

Fig. 1. Location of the Beenyup Wastewater Treatment Plant (WWTP) outlet, showing the 12 sampling sites (S1–4, N1–4, W1–4) located on 3 transects around the sewage outlet. Hatched areas are low-relief reef (reef periodically covered with sand)

dominate the reef in areas where insufficient light precludes algal growth.

Test species. Three morphologically distinct macroalgae were selected for their expected differences in nutrient uptake rates (Littler 1980, Wallentinus 1984). *Ulva australis* Areschoug (Sonder), a foliose chlorophyte, has a high nutrient-uptake capacity. *Vidalia* sp., a corticated foliose rhodophyte, was chosen to represent species with a moderate uptake rate, and the leathery kelp *Ecklonia radiata* (C. Agardh) was chosen to represent macroalgae with a low nutrient uptake rate (Wallentinus 1984). Two filter-feeders were sampled to test whether distinct patterns in POM dispersal could be detected. These were *Clathria* sp. (Porifera), and *Pyura australis* (Quoy Gainard) (Ascidia).

Laboratory experiment. A laboratory experiment was performed to confirm that the test organisms were capable of developing an altered nitrogen isotopic signature when exposed to low concentrations of sewage nitrate. Organisms used in the experiment were collected randomly from a reef, approximately 100 m offshore, in an area not known to be affected by sewage effluent. To prevent stress, organisms were collected and bagged underwater with as little disturbance as possible, and then transported to the laboratory in a cooler with ice before being introduced into aquaria.

Individuals of each test species were grown under controlled conditions for 7 d in either the presence (treatment) or absence (control) of sewage effluent. Four independent replicates of each treatment or control were established. Each replicate comprised an aquarium containing 1 individual organism. Each aquarium was operated as a flow-through system fed by sand-filtered seawater through header tanks at 2600 ± 10 ml min^{-1}. Individual peristaltic pumps (Masterflex®, L/S) delivered 5.20 ± 0.05 ml min^{-1} of secondary treated sewage effluent from the Beenyup WWTP to each of the treatment tanks, yielding a 1:500 dilution of sewage. This dilution was similar to that found within a 1 km radius of the Beenyup sewage outlet (Lord & Hillman 1995). Controls received no sewage effluent. Artificial light (HPM CAT Series 605, 500 W) was used to provide 45 to 50 µmol m^{-2} s^{-1} at the surface of each aquarium on a 12:12 h cycle. Aquarium air pumps and water pumps (MINI-JET®, Aquarium Systems) were also fitted to all aquaria to ensure oxygenation and mixing of the water. Temperature was maintained at 18 to 22°C.

After 7 d the individuals were harvested and processed for nitrogen-stable isotope analysis. The $\delta^{15}N$ levels of treatment and controls were compared by a Student's *t*-test to determine whether significant differences had developed. As the aim of this experiment was simply to test that the test organisms could develop an altered $\delta^{15}N$ signature after 7 d exposure to sewage, the $\delta^{15}N$ levels of the sewage and seawater were not determined. However, the charecteristics of these sources were determined in the field study (see following subsections).

Field sampling strategy. The test organisms were collected from low-relief reefs along 3 transects, running north, south and west of the diffuser (Fig. 1), and from a reference site 18.5 km north-northwest of the outlet at Alkimos reef. Each transect was approximately 2 km in length, covering the reported extent of the sewage plume radius (Lord & Hillman 1995). Four sites were located at approximately 500 m intervals along each transect (Fig. 1). All sites had similar reef habitat in terms of rugosity, depth, aspect and algal communities.

Where possible, 3 replicate samples of each organism were randomly collected from each site for stable isotope analysis. The $\delta^{15}N$ content of each species was compared between sites to test for spatial variability. Data were tested for normality and homogeneity of

variance using Cochran's test and transformed using logarithms if they failed the test. A 1-factor analysis of variance (ANOVA) was used to test differences in mean $\delta^{15}N$ between sites for each test species. Tukey's post hoc test was used for pair-wise comparisons.

Replicate samples of sewage effluent, seawater and groundwater were taken to ensure that potential sources of DIN and PON were isotopically distinct. Seawater was collected from Sites W1 to W4 and above the outlet, at a depth of approximately 1.0 m. Groundwater was sampled from 3 piezometers located between 500 m and 2.5 km inland. Secondary-treated sewage effluent was obtained from the Beenyup WWTP. Three replicate samples were collected in November 1999 and again in March 2000.

Collection and preparation of macroalgae, sponge and ascidian samples. SCUBA divers collected macroalgae randomly from each site in November, 1999. Thalli and filter-feeders were dried at 65°C until dry, and ground to a fine powder using a mortar and pestle. For *Clathria* sp., the outer test was removed and the internal tissue used for isotope analysis. For *Pyura australis*, the stem tissue was discarded. Homogenised tissue was analysed for $\delta^{15}N$ on a Europa Scientific ANCA-GSL 20/20 mass spectrometer. Precision for replicate tissue samples was ±0.2‰. $\delta^{15}N$ values are reported relative to atmospheric nitrogen.

Collection and preparation of seawater, groundwater and sewage. Seawater, groundwater and sewage were collected in pre-washed plastic containers (5% HCl), stored on ice in the absence of light, and returned to the laboratory. Samples were vacuum-filtered (0.45 µm pre-combusted glasfibre filters) and stored frozen until ready for distillation. To collect sufficient nitrogen for 3 replicate isotope analyses (about 200 µg), it was necessary to distill 9 l of seawater, 3 l of groundwater and 1.5 l of sewage.

The distillation procedure followed the methods of Bremner & Edwards (1965), with modifications taken from Cline & Kaplan (1975) and Velinsky et al. (1989). Sample aliquots of 500 ml were added to the distillation flask, followed by 5.0 mg of Devarda's alloy to reduce nitrate to ammonia. A sufficient volume of 40% NaOH was added to raise the pH to 9.5. Over 35 to 40 min, 250 ml of distillate was collected per sample in a sealed ammonia recovery flask containing 10 ml of 0.003 M HCl and 30 mg zeolite (W-85 molecular sieve). The distillate was stirred for 1 h to ensure the complete adsorption of ammonium onto the zeolite, and the zeolite slurry was collected onto a silica acetate filter membrane by vacuum. The filter membrane plus zeolite was dried for 48 h at 50°C, before being introduced to a mass spectrometer.

Collection of oceanic and sewage POM. Filter papers used for filtering oceanic and sewage POM (see above) were dried at 65°C and stored whole in sealed, pre-washed containers before being introduced to the mass spectrometer. Samples were analysed as for ground tissue. The precision for replicate sewage N and oceanic POM samples was 0.2‰.

Calculation of proportion of sewage-derived N for macroalgae. The contribution of sewage-derived nitrogen to the macroalgal $\delta^{15}N$ signal at each site was calculated using a single isotope, 2 end-member linear mixing model (Balasdent & Mariotti 1996):

$$f_A = \frac{\bar{\delta}_M - \bar{\delta}_A}{\bar{\delta}_A - \bar{\delta}_B} \quad (1)$$

where $\bar{\delta}_M$, $\bar{\delta}_A$ and $\bar{\delta}_B$ are the mean $\delta^{15}N$ value of macroalgae, sewage DIN and background oceanic DIN respectively. Variance (and, subsequently, standard deviation) estimates were made according to Taylor (1982):

$$\sigma^2_{f_A} = \frac{1}{\left(\bar{\delta}_A - \bar{\delta}_B\right)^2}\left(\sigma^2_{\bar{\delta}_M} + f_A^2\,\sigma^2_{\bar{\delta}_A} + \left(1 - f_A\right)^2\sigma^2_{\bar{\delta}_B}\right) \quad (2)$$

where $\sigma^2_{\bar{\delta}_M}$, $\sigma^2_{\bar{\delta}_A}$ and $\sigma^2_{\bar{\delta}_B}$ represent variances of the mean isotopic signatures for the macrolagae, the sewage and background seawater respectively.

A 2 end-member model was appropriate as the estimated groundwater stable isotope signature was indistinguishable from background seawater, and so these were pooled as a single source. Only 1 isotope was used (nitrogen) as analytical constraints reduced our confidence in applying the carbon isotope data in the model (see 'Results' and 'Discussion' sections). In order to apply this model we assumed no fractionation effects by the test organisms, or at least consistent fractionation across all sites, and that the background oceanic DIN can therefore be estimated as the $\delta^{15}N$ value of macroalgae growing at the reference site. Since the 3 species of macroalgae had different $\delta^{15}N$ values at the reference site, we applied a different background DIN $\delta^{15}N$ value in the model for each species. This variability could be due to several sources and this, as well as other constraints to the application of mixing models, is discussed later.

RESULTS

Secondary-treated sewage DIN ($NH_4^+ + NO^{3-}$) had mean (±SD) $\delta^{15}N$ values ranging from 25.3 ± 1.4‰ in November to 13.5 ± 0.6‰ in March (Table 1). POM from the same sewage had a mean $\delta^{15}N$ value of 9.2 ± 2.2‰ in November 1999. The mean $\delta^{15}N$ value for oceanic POM collected at the reference site was 7.0 ± 0.03‰, similar to the value for POM collected from directly above the Beenyup wastewater outlet (7.2 ± 0.1‰; Table 1).

Table 1. $\delta^{15}N$ values for the major sources of DIN in the study area. WWTP: wastewater treatment plant. Values for oceanic (background) DIN were derived from the literature (Miyake & Wada 1967, Cline & Kaplan 1975, Kon-Kee & Kaplan 1989, Sigman et al. 1997)

Source	Mean $\delta^{15}N$ (‰)
Sewage DIN	
November	25.33 ± 1.45
March	13.47 ± 0.63
Sewage POM	
Beenyup WWTP	9.17 ± 2.17
Oceanic DIN	
Background nitrogen	6.7 (estimated)
Oceanic POM	
Above outlet	7.23 ± 0.12
Reference site	7.02 ± 0.03

There was insufficient DIN in the samples of seawater and groundwater to permit isotope analysis (DIN values ranged from 3.4 to 11.8 µg l^{-1}). Consequently, values for seawater and groundwater were estimated from the literature.

Laboratory experiment

Ulva australis and *Vidalia* sp. grown in the presence of sewage had higher $\delta^{15}N$ levels than the controls (Fig. 2, Table 2). Both displayed similar differences between treatment and control tanks (1.7 and 1.4‰ respectively), the differences being statistically significant in both cases ($t = -2.96$, p = 0.03 and $t = -2.87$, p = 0.03, for *U. australis* and *Vidalia* sp. respectively). The $\delta^{15}N$ values of *Ecklonia radiata* grown in the presence of sewage was not significantly different from controls ($t = -0.81$, p = 0.45). Both the filter-feeders, *Clathria* sp. and *Pyura australis*, had significantly higher $\delta^{15}N$ values after exposure to sewage effluent, compared with controls ($t = -3.15$, p = 0.03 and $t = -3.03$, p = 0.04 respectively).

Table 2. Results of Student's *t*-test (0.05 significance level) for differences in $\delta^{15}N$ levels of organisms grown in the presence of sewage effluent (treatments) and those grown in seawater without effluent (controls)

Species	Control (‰)	Treatment (‰)	*t*	df	p
Ulva australis	7.79	9.33	−2.96	6	0.03
Vidalia sp.	7.88	9.28	−2.87	6	0.030
Ecklonia radiata	9.79	10.24	−0.81	6	0.450
Clathria sp.	10.65	13.07	−3.15	4	0.030
Pyura australis	6.60	10.44	−3.03	4	0.040

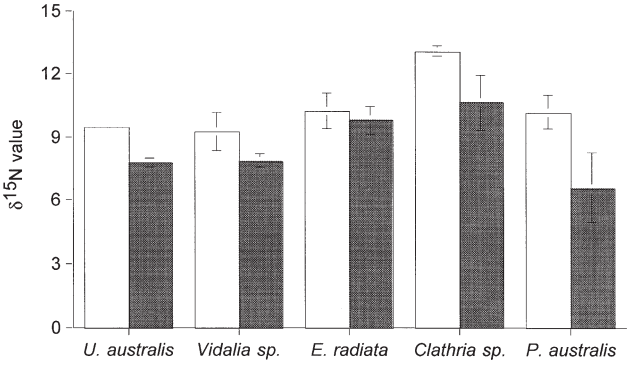

Fig. 2. Mean $\delta^{15}N$ levels (‰) of organisms grown in a 1:500 dilution of secondary-treated sewage effluent (treatment: white bars) and in seawater (controls: shaded bars). Values are means ± SE (n = 4). Differences were significant for all species except *Ecklonia radiata*. Full specific names in Fig. 4

Spatial patterns of $\delta^{15}N$ in macroalgae

The mean $\delta^{15}N$ levels of *Ulva australis* at sites around the outlet varied by 4‰, from 8.8‰ at Site W3 to 12.8‰ at Site N3 (Fig. 3). There was significant variation in $\delta^{15}N$ among the sites around the outlet (Fig. 3) and between all of these sites and the reference site (6.1‰). Around the outlet, sites from the northern transect tended to have higher $\delta^{15}N$ values than other sites,

Table 3. Results of 1-factor ANOVA testing for differences in $\delta^{15}N$ levels in organisms collected from different sites around the Beenyup WWTP outlet

Species	SS	df	MS	*F*	p
Macroalgae					
Ulva australis					
Between	12.18	12	10.348	74.656	<0.001
Within	3.60	26	0.139		
Total	127.78	28			
Vidalia sp.					
Between	61.54	11	5.594	16.286	<0.001
Within	7.90	23	0.344		
Total	69.44	34			
Ecklonia radiata					
Between	48.98	12	4.081	4.084	0.001
Within	24.99	25	0.999		
Total	73.96	37			
Filter feeders					
Clathria sp.					
Between	11.91	7	1.701	15.015	<0.001
Within	1.81	16	0.113		
Total	13.72	23			
Pyura australis					
Between	19.44	9	2.16	0.575	0.8020
Within	75.16	20	3.758		
Total	94.59	29			

and along this transect values increased with increasing distance from the outlet to a maximum of about 12.5‰ at 1500 m. Thalli collected from western transect showed the opposite trend, a decrease in $\delta^{15}N$ with distance from the outlet, with the westernmost sites, W3 and W4, significantly lower than all other sites ($p < 0.05$). The southern transect had no discernible trend.

The mean $\delta^{15}N$ levels for *Vidalia* sp. at sites around the outlet varied by 3.9‰, from 6.3‰ at Site W4 to 10.2‰ at N4. The mean $\delta^{15}N$ of plants collected from the reference site (6.5‰) was significantly lower than at sites S3, S1, N4 and W1 around the outlet (Fig. 3, Table 3). Like *Ulva australis*, mean $\delta^{15}N$ values decreased with increasing distance from the outlet along the western transect, while those at sites along the northern transect (N3, N4) had comparatively high values. While the trend was similar to that for *U. australis*, the magnitude of differences among sites was less.

Trends in the $\delta^{15}N$ levels of *Ecklonia radiata* sampled around the outlet were similar to, but less pronounced than, those of *Ulva australis* and *Vidalia* sp. North and south of the outlet, mean $\delta^{15}N$ levels increased with increasing distance from the outlet, and values along the western transect were generally lower. The reference site had values generally lower than sites around the outlet. However, there was a high degree of variability within each sample site, and few of the differences among sites were significant (Table 3). In particular, the reference site showed a high degree of variability, which accounts for the lack of difference between it and the sites around the outlet; only Sites N3, S3 and W1 had significantly higher $\delta^{15}N$ values than the reference site.

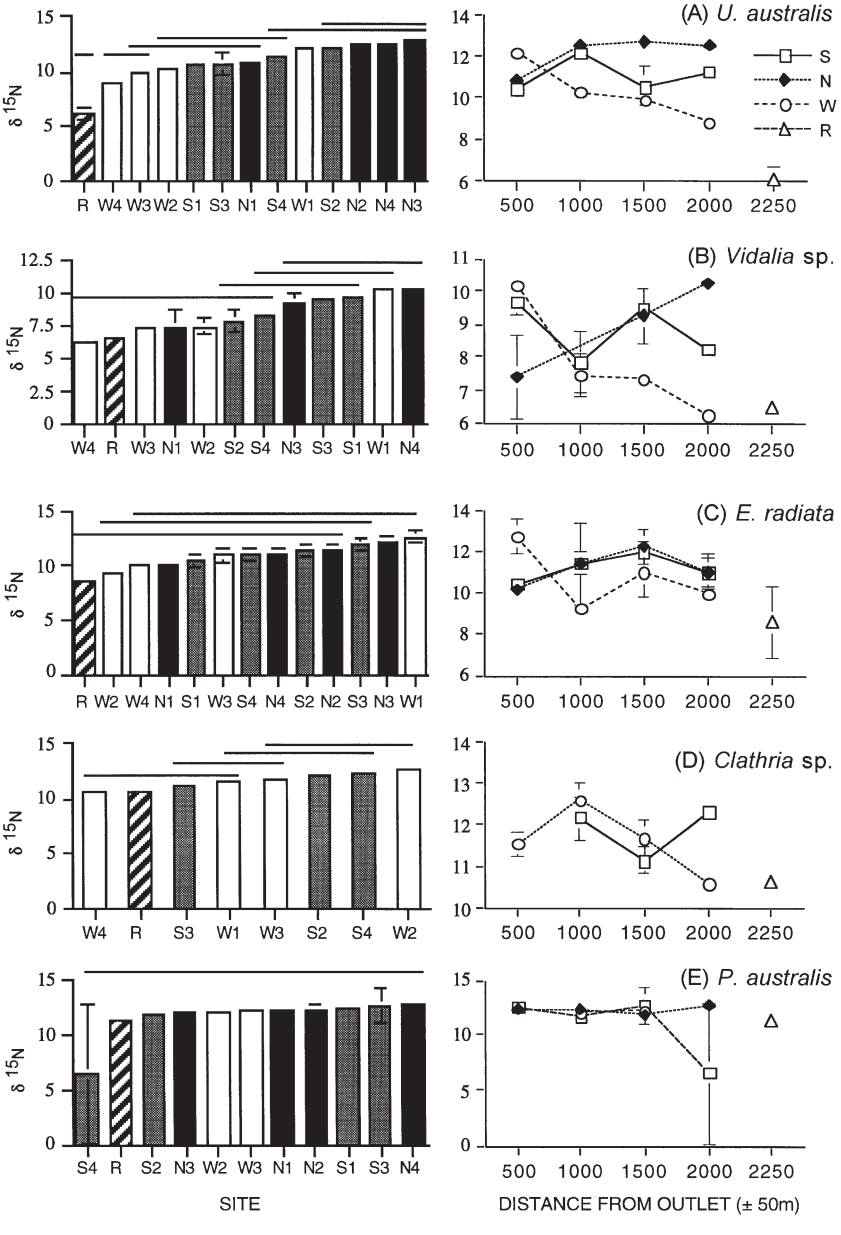

Fig. 3. Mean $\delta^{15}N$ levels (‰) of organisms at the 12 sampling sites around the Beenyup WWTP outlet (labelled as in Fig. 1) and the reference site (R). All values are means ±SE (n = 3). Bar graphs on left show sites in order of increasing $\delta^{15}N$ with shading indicating the transect (black: north; grey: south; striped: reference site; white: west site. Shared horizontal lines above bars indicate no significant difference ($p > 0.05$: Tukey's test). Line graphs on right show values along the 3 sampling transects (S, N, W) and at R. Full specific names in Fig. 4

Spatial patterns of $\delta^{15}N$ in filter feeders

The sponge *Clathria* sp. was not found at any site on the north transect or at Site W1. The spatial pattern for $\delta^{15}N$ levels at the remaining sites was similar to that for *Ulva australis* and *Vidalia* sp. Along the western transect, mean $\delta^{15}N$ values decreased with increasing distance from the outlet, and samples collected from the reference site were significantly lower than those from several sites around the outlet (S4, S2, W2, W3: Fig. 3, Table 3). The spatial patterns expressed in *Clathria* sp. $\delta^{15}N$ values were weaker than those expressed in *U. australis* and *Vidalia* sp. For *Pyura*

australis, there was no significant spatial variability in $\delta^{15}N$ among the sites.

Proportion of sewage DIN assimilated by macroalgae

Since sewage DIN showed significant temporal variability in $\delta^{15}N$ (13.5‰ as observed in March, or 25.3‰ as observed in November) and as mixing models are sensitive to the values of the sources used, the model was solved with both values. In both cases, the model results indicated that macroalgae derived significant proportions of their assimilated N from sewage-derived DIN, even as far as 2000 m north of the diffuser (Fig. 4).

Thalli north of the diffuser were estimated to more consistently assimilate higher proportions of DIN from sewage effluent. The model suggests that *Ulva australis* assimilated 25 to 90% of its nitrogen from sewage effluent, depending on the site and the sewage DIN value used (Fig. 4). *Vidalia* sp. thalli collected north of the outlet were estimated to have assimilated between 5 and 53% of their nitrogen from sewage DIN, and *Ecklonia radiata* between 9 and 74% (Fig. 4). Organisms sampled from the 3 westernmost sites (W2, W3 and W4) and from the reference site generally assimilated a much lower proportion of their DIN from sewage effluent: between 14 and 57% for *U. australis*, 0 to 15% for *Vidalia* sp. and 0 to 48% for *E. radiata*, with a decreasing trend westwards.

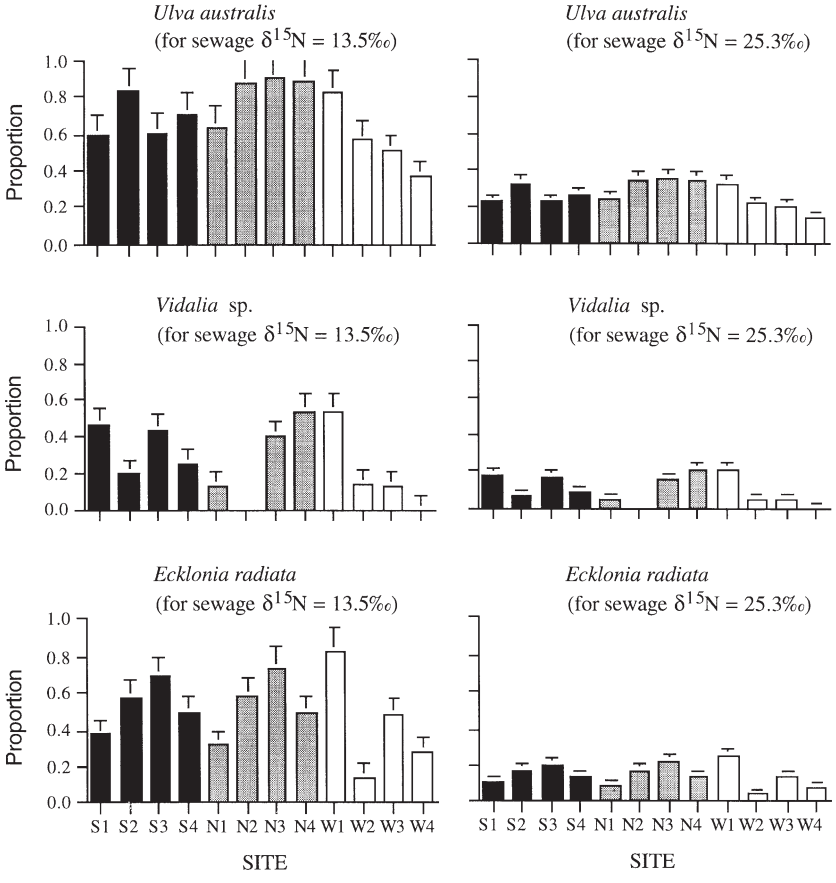

Fig. 4. Estimates of the percentage of nitrogen acquired from sewage DIN for the 3 species of macroalgae at each sampling site, based on March and November sewage DIN $\delta^{15}N$ values of 13.5‰ (left) and 25.3‰ (right) respectively. Bar shading indicates the transect on which sites were located (black: south; grey: north; white: west). Values for the reference site are not presented as it was assumed there was 0% sewage-derived nitrogen at this site

DISCUSSION

The laboratory experiment confirmed that the species of macroalgae tested can assimilate sewage-derived DIN and display a measurably altered $\delta^{15}N$ level after as little as 7 d exposure to sewage effluent. *Ulva australis* and *Vidalia* sp. developed significantly higher values, suggesting that these species respond rapidly to nutrient additions and are capable of acquiring measurably altered values at timescales of less than 7 d. *Ecklonia radiata* on the other hand failed to develop any signal after 7 d.

Ulva australis is an opportunistic, foliose alga, with a high nitrogen uptake rate (Wallentinus 1984, Lavery & McComb 1991) that allows it to respond quickly to additional nutrients in the water column (Lyngby & Mortensen 1994). As we have shown, it can assimilate significantly sufficient amounts of DIN from the effluent over a comparatively short period for its $\delta^{15}N$ content to strongly reflect nutrient sources it has assimilated in the recent past. Although unexpected, the similar response of *Vidalia* sp. exposed to sewage suggests that it too is capable of responding over this time scale. The smaller surface area to volume ratio of corticated foliose algae such as *Vidalia* sp. compared to foliose algae was expected to result in lower nitrogen uptake rates and higher half-saturation constants. This would reduce the ability to acquire nitrogen during a pulse event and a greater dilution of the new nitrogen by nitrogen already contained within the more substantial thallus. However, while the functional forms of

algae broadly relate to differences in physiological attributes, there can be significant variability within a group (Littler & Arnold 1982, Wallentinus 1984, Padilla & Allen 2000), and it may be that these 2 species have similar physiological attributes with respect to DIN uptake.

Ecklonia radiata typically has a very low nitrogen uptake rate and large amounts of structural biomass (Wallentinus 1984), and so would require longer periods of exposure to assimilate sufficient new nitrogen to alter the average $\delta^{15}N$ signature of its thallus. This may account for the failure of *E. radiata* to acquire a measurably different signal over the experimental period. Phaeophycean algae have often been cited in the literature as declining in abundance and diversity around sewage outlets as they are known to be sensitive to sewage (Munda 1993, Peckol & Rivers 1996). It is possible that *E. radiata* were simply too stressed to accumulate nitrogen. However, the trends observed in the field-sampling programme suggest that *E. radiata* was assimilating sewage-derived nitrogen near the outfall.

The time required for thalli to develop measurable changes in their nitrogen isotopic signature following exposure to sewage would depend on more than just physiological attributes. The concentration and speciation of the DIN source, and environmental conditions such as irradiance and hydrodynamics, could affect the magnitude of fractionation, and hence signal acquired, over a given period of exposure (Montoya 1990 as cited in Goericke et al. 1994). However, the results of this study indicate that the relative magnitude of any response appears related to the functional form of the algae; *Ulva australis* and *Vidalia* sp. may vary in the time necessary to acquire a signal, but it is reasonable to assume that they will respond more quickly than *Ecklonia radiata*.

Spatial patterns in sewage dispersal

If we accept that the isotopic signatures of these species provide a representation of nutrient sources integrated over different time periods, then we should be able to interpret the spatial patterns in $\delta^{15}N$ signatures in terms of the timescales of effluent dispersal in a region. How then do we interpret the results for the 3 macroalgae sampled here?

The dispersal of effluent revealed by the macroalgal $\delta^{15}N$ signals is entirely consistent with the known dispersion pattern. The higher $\delta^{15}N$ of *Ulva australis* and *Vidalia* sp. sampled north of the outlet indicate a generally northerly drift of the plume. West of the outlet there was a decline in $\delta^{15}N$ for both species, indicating little westerly drift of the plume. These trends conform with models of the plume dispersal (Pattiaratchi & Knock 1995, Kinhill unpubl. data) and with Lord & Hillman's (1995) conclusion that sites north of the outlet are most often exposed to sewage effluent at the time of this study. The relative strengths of the trends in the 2 species reflect the timescale of this sewage dispersal. The *U. australis* and *Vidalia* sp. results imply that the plume had undergone a predominantly northerly drift in the preceding few days. The weaker differences between the northern and other sites for *Ecklonia radiata* can be interpreted as reflecting a generally northerly drift over a longer timescale (weeks to months) but with a broader regional dispersal at intervals during that period.

The lack of any statistical significance in the spatial pattern of *Ecklonia radiata* $\delta^{15}N$ may indicate that all sites, including the reference site, are equally influenced, or not influenced, by sewage nitrogen. However, this is inconsistent with the data for *Ulva australis* and *Vidalia* sp. More likely there is a fundamental biological difference between the species. The variability in $\delta^{15}N$ of *E. radiata* at the control site is exceptionally large compared to the variability in the other species and sites, and could arise from several sources. It is possible that plants of different ages were sampled, although there was an equal probability of this occurring at the other sites too. Other studies have reported variability in the stable isotope values of different tissues in marine plants (Stephenson et al. 1986, Shearer & Kohl 1989, Boyce et al. 2001). *E. radiata* has a higher degree of tissue differentiation than either *U. australis* or *Vidalia* sp., and while we attempted to control for this, it is possible that we captured variability between parts of the thallus blade. However, recent sampling (P. Lavery unpubl. data) indicates no significant variation in $\delta^{15}N$ levels among non-holdfast tissue in *E. radiata*. Despite the lack of statistical support, it is clear that *E. radiata* shows the same underlying trends in the spatial pattern of $\delta^{15}N$. This suggests that at relatively long timescales (greater than weeks), the dispersal of sewage is widespread through out the region, although still generally with a northerly and southerly dispersal and little westerly movement. Clearly there is a need to more fully understand the cause of the significant variability in the $\delta^{15}N$ signature of *E. radiata*, and in any species which might potentially be used for monitoring purposes.

The $\delta^{15}N$ levels recorded for *Ulva australis* indicate that stable isotope analysis may provide a more sensitive tool for tracking sewage dispersal than conventional methods. Lord & Hillman's (1995) model suggested that detectable differences in nutrient concentrations due to sewage discharge abate between 1000 to 2000 m from the outlet. If the plume dissipated at 1000 m, it could be expected that $\delta^{15}N$ levels for *U. australis* at Sites N3 and N4 (1500 and 2000 m north of the diffuser respectively) would be comparable to

those at the reference site. However, *U. australis* had elevated $\delta^{15}N$ levels as far as 2000 m north of the outlet, indicating that sewage nitrogen was carried and assimilated by biota at least that far away.

The isotope values also revealed finer-scale dispersal patterns. With the exception of *Vidalia* sp. at Site S1, mean $\delta^{15}N$ levels of for all 3 macroalgae at 500 m north and south of the outlet were at least 1.5‰ lower than at sites 1000 m from the diffuser. The effluent plume is known to have a buoyant stage, whereby it is carried by surface currents before becoming fully mixed with the water column (Lord & Hillman 1995). The macroalgal $\delta^{15}N$ data are consistent with the plume remaining buoyant for the first 500 m north and south of the diffuser. The lower $\delta^{15}N$ values in this zone suggest less exposure to sewage-derived N than at sites further north and south. Similar trends in $\delta^{15}N$ signals for macroalgae have been found around a wastewater outlet in Saldanha Bay, South Africa (Anderson et al. 1999, Smit unpubl. data).

Spatial patterns in dispersal of sewage POM

A comparison of values between POM collected from the reference site and that collected from filtered sewage revealed little difference in the $\delta^{15}N$ values of these sources (7.1 and 9.16‰ respectively). Owens (1987) also reported values for POM for similar sources (ocean mean range 4.6 to 9‰; sewage mean range 2.3 to 7.2‰), and concluded that the $\delta^{15}N$ signatures of organisms assimilating POM is unlikely to be useful in distinguishing which sources were assimilated.

However, the trends in the $\delta^{15}N$ signature of *Clathria* sp. were similar to trends displayed by macroalgae species. *Clathria* sp. grown in treatment tanks in the laboratory had higher $\delta^{15}N$ than individuals grown in control tanks, there was a decrease in $\delta^{15}N$ west of the outlet, and samples from the reference site had lower $\delta^{15}N$ values than at several of the sites around the outlet. Tipping (unpubl. data) sampled phytoplankton that had been exposed to sewage DIN and observed elevated $\delta^{15}N$ values. A potential explanation for the results, then, is that *Clathria* sp. selectively assimilated phytoplankton, bacteria or some other component of the total POM which could have elevated the $\delta^{15}N$ derived from assimilation of sewage DIN. Different filter-feeders are known to target different components of the POM on which they feed. For example, sponges generally ingest smaller size fractions than other suspension-feeders (Reiswig 1975, Stuart & Klumpp 1984). However, not enough is known about the feeding biology of either of the filter-feeders used here, or the $\delta^{15}N$ values of different fractions of POM, to confirm this hypothesis, and further research is warranted.

Contribution of sewage nitrogen to plant requirements

The range of $\delta^{15}N$ values (13.5 to 25.3‰) marginally extends the range of values reported for sewage effluent in the literature (Aravena et al. 1993, Paerl & Fogel 1994, McClelland & Valiela 1998). The value for oceanic DIN could not be obtained by distillation due to the low nitrogen concentrations in seawater, and an estimate was made based on values provided by Miyake & Wada (1967), Cline & Kaplan (1975), Kon-Kee & Kaplan (1989), Sigman et al. (1997), and the values recorded for each species of macroalgae at the reference site. The literature sources suggest that oceanic waters have levels in the order of 6.7‰. Macroalgae at the references site had $\delta^{15}N$ values between 6 and 8.5‰. While it is reasonable to expect some fractionation of DIN during uptake by primary producers (Fogel & Cifuentes 1993), there is no published literature providing any indication of likely fractionation factors of macroalgae. We therefore assumed no fractionation, and for each species of macroalgae assumed that the $\delta^{15}N$ levels at the reference site reflected the value of background marine DIN. The large distinction between oceanic and sewage $\delta^{15}N$ values makes it possible to differentiate between these 2 sources for tracer studies. The only other potentially significant source of DIN to the region is groundwater (Johannes & Hearn 1985). Groundwater typically has $\delta^{15}N$ values between –2 and 8‰ (Kreitler et al. 1978, Aravena et al. 1993, Macko & Ostrom 1994), making it difficult to distinguish it from oceanic nitrogen sources, but still allowing any signal from sewage to be detected. This number of assumptions requires that any outputs from the mixing model be interpreted with great caution. At this point in time, there is insufficient background information on the fractionation effects associated with macroalgal uptake of DIN to permit this source of variability to be reduced. Consequently, the outputs cannot be interpreted with any degree of statistical confidence, but do serve as a rough estimate of likely nitrogen sources to macroalgae at each site. This also emphasises the need for further research into fractionation effects during macroalgal uptake and assimilation of nitrogen.

Depending on the $\delta^{15}N$ value of the sewage used, *Ulva australis* north of the outlet was modelled to have assimilated between 25 and 90% of its nitrogen from sewage DIN. These estimates indicate that sewage effluent contributes a significant proportion of the nitrogen to this alga. Several studies have reported a decline in the number and abundance of red and brown macroalgae species (Coles & Ruddy 1995, Peckol & Rivers 1996) and increased abundance of

opportunistic Chlorophyta (Dhargalkar 1986) in the vicinity of sewage outlets. The isotope values established in this study demonstrate that the sites around the outlet and at distances exceeding those previously thought to be affected by the sewage plume receive sewage effluent. While the results do not imply any adverse impact resulting from this exposure, they do indicate that stable isotope data provide a means of identifying, with high spatial precision, potential 'impact' and 'control' sites for assessment of sewage-related effects.

Conclusions

We conclude that the $\delta^{15}N$ signatures of marine macroalgae can provide a useful means of tracing sewage dispersal in well-mixed ocean conditions, where conventional methods may fail to reveal the extent of sewage dispersal. In addition, the $\delta^{15}N$ signals of macroalgal species with different nutrient uptake characteristics may provide an integrated picture of the dispersal of sewage over different timescales. On the other hand, the results were ambiguous with respect to the usefulness of filter-feeder isotopic signatures for tracing particulate sewage dispersal. Our experimental design did not replicate functional groups of macroalgae, and so we cannot establish conclusive relationships between functional form and the propensity for algal isotopic signatures to reflect timescales of sewage dispersal in the environment. This would require an experimental and field sampling design with replicate species of each functional form. However, the data we have presented here are entirely consistent with our hypothesis, and can be interpreted as suggestive of a relationship between functional form and isotopic signatures that can be applied to reflect timescales of sewage dispersal. In this respect, the choice of species and a sound understanding of their biology with respect to stable isotopes would be required. This technique could complement conventional plume-tracking techniques and permit control-impact-type studies by confirming with greater certainty whether the algal or other biotic assemblages at a site have been exposed to sewage over different time periods.

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