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Algal and invertebrate bioindicators detect sewage effluent along the coast of Titahi Bay, Wellington, New Zealand

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Sewage effluent contains both dissolved and particulate matter that can affect coastal ecosystems and cascade through food webs. We used $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios to explore performance of a kelp (*Carpophyllum maschalocarpum*) and two invertebrates (a grazing isopod, *Amphoroidea media*, and a filter-feeding crab, *Petrolisthes elongatus*) as bioindicators along a gradient of sewage exposure. These species vary in trophic status and, consequently, are likely to assimilate sewage constituents differently into food webs. $\delta^{15}\text{N}$ values in *C. maschalocarpum* and *A. media* were highest near the sewage outfall and decreased with increasing distance from the outfall. $\delta^{13}\text{C}$ values in these two species did not appear to be reliable predictors of sewage concentrations in seawater. In contrast, $\delta^{13}\text{C}$ ratios for *P. elongatus* were consistently depleted at sites nearest the sewage outfall, indicating the consumption of ^{13}C -depleted sewage particulates. These results suggest there is value in multiple-isotope and multi-species bioindicator approaches both for detecting sewage dispersal patterns and understanding the incorporation of sewage-derived nutrients into food webs.

Keywords: nutrient enrichment; stable isotope ratios; $\delta^{15}\text{N}$; $\delta^{13}\text{C}$; marine; assimilation; macroalgae

Introduction

Coastal discharges of untreated and treated sewage effluent are a common feature of many populated coastlines worldwide. Sewage effluent can negatively affect marine biota directly (e.g. through the influence of organochlorines and heavy metals; Scanes 1996; Ajani et al. 1999; Echavarrri-Erasun et al. 2007) and indirectly (e.g. through ecosystem changes resulting from nutrient enrichment; GESAMP 2001; Bokn et al. 2002; Hauxwell et al. 2003; Karez et al. 2004; Tewfik et al. 2005). Detecting the effects of coastal sewage discharges is an important aim of applied ecologists and managers, but this can be hindered by natural variability in the local structure and composition of marine communities (Hewitt et al. 2005). Furthermore, recognition and detection

of the effects of sewage effluent may be inhibited by the limited understanding of sewage constituent uptake and assimilation into natural communities.

Bioindicators such as marine macroalgae and filter feeders provide a potential means of assessing the spatial extent and biological effects of coastal sewage discharges (e.g. Fong et al. 1998, 2004; Lin & Fong 2008). Sewage effluent often contains carbon and nitrogen that is isotopically distinct from background isotopic signatures found in unimpacted coastal marine ecosystems (Bedard-Haughn et al. 2003; Gordon & Goñi 2003; Savage & Elmgren 2004). Isotopic analysis therefore can be used for tracing terrestrial nutrient sources entering marine ecosystems (Fry & Sherr 1984; Fry & Wainright 1991). Isotopic signatures in the tissues of bioindicators

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are particularly valuable as measures of sewage impact because they act as time-integrative measures of sewage exposure, reflecting the contributions of available nutrient sources over the period of tissue generation and turnover (Gartner et al. 2002; Gaston & Suthers 2004; Perga & Gerdeaux 2005). Where effluent concentrations are variable in time, bioindicators may detect biologically important pulses of sewage discharge that may otherwise be missed by more standard water-column sampling programmes (Fong et al. 2004). However, given the diversity of species and life histories present in many coastal communities, the identification of appropriate bioindicators is potentially problematic.

Macroalgae seem particularly well suited as bioindicators because they assimilate the isotopic signatures of biologically available nitrogen in seawater (McClelland & Valiela 1998). Sampling the grazers of sewage-affected algal tissues may provide information on the propensity for pollutants to cascade through aquatic food webs (McClelland et al. 1997; Rogers 1999, 2003). Filter-feeding organisms such as bivalves appear to assimilate and concentrate the distinct $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of sewage particulate organic matter (SPOM; Rogers 2003; Dolenc et al. 2006; Piola et al. 2006; Forrest et al. 2007).

This study had two main objectives: (1) to examine how nitrogen and carbon derived from sewage are incorporated into a shallow coastal rocky shore food web in Titahi Bay, New Zealand; and (2) to evaluate the use of bioindicators for detecting the input of sewage effluents into coastal regions of New Zealand. Specifically, we focused on three potential bioindicator species with differing trophic status: a brown algae (*Carpophyllum maschalocarpum* (Turner) Greville 1830), a grazing isopod (*Amphoroidea media* Hurley & Jansen 1971) and a suspension-feeding crab (*Petrolisthes elongatus* H. Milne-Edwards 1840). We explored the potential of each species to indicate exposure to pollutants along a distance gradient from a sewage outfall. Furthermore, we explored the feasibility of using multi-species assessment approaches to indicate potential mechanisms of uptake, assimilation and

cascades of sewage-derived pollutants through marine communities.

Materials and methods

Three potential bioindicators across different trophic levels were selected for the study. *Carpophyllum maschalocarpum* is a brown alga (endemic to New Zealand) that inhabits rock pools and shallow channels and forms distinct zones along many open coasts (Adams, 1994). *Amphoroidea media* is an epifaunal isopod that commonly grazes on several algal species, including *C. maschalocarpum* (Taylor & Cole 1994). *Petrolisthes elongatus* (half crab or porcelain crab) is common in intertidal habitats along rocky shores (Morton & Miller 1968) and a particulate suspension feeder, whose diet typically includes phytoplankton and detritus (Morton & Miller 1968; Johnston & Freeman 2005). The geographical ranges of all three species extend throughout New Zealand.

The study was conducted near a sewage outfall of the Titahi Bay wastewater treatment plant (TWTP), Wellington, New Zealand (41°7'S, 174°49'E) (Fig. 1). This site is characterised by a shallow (c. 5 m) rocky reef running east-west and is sheltered from strong westerly swells by an offshore island (Mana Island). Prevalent currents flow in a predominantly westerly direction (Ridgeway 1961). The nearest major freshwater input in the area flows from a largely urban and agricultural catchment, entering coastal waters through Porirua Harbour, 3.5 km to the northwest.

We sampled tissue isotope concentrations of potential bioindicators at 11 sites, which were distributed so that five sites were east (generally 'upcurrent') of the outfall (E1–E5), five sites were west ('downcurrent') of the outfall (W1–W5) and one site was immediately offshore of the outfall (C) (Fig. 1). Sites were permanently marked with buoys along rocky reef habitat and spaced at intervals that ranged from 50 m (nearest to the outflow) to a maximum of 200 m (separating sites most distant from the outflow).

During the 6 months before the study, TWTP released an average of 237.91 of tertiary processed sewage per minute (range = 0–1048 l/min),

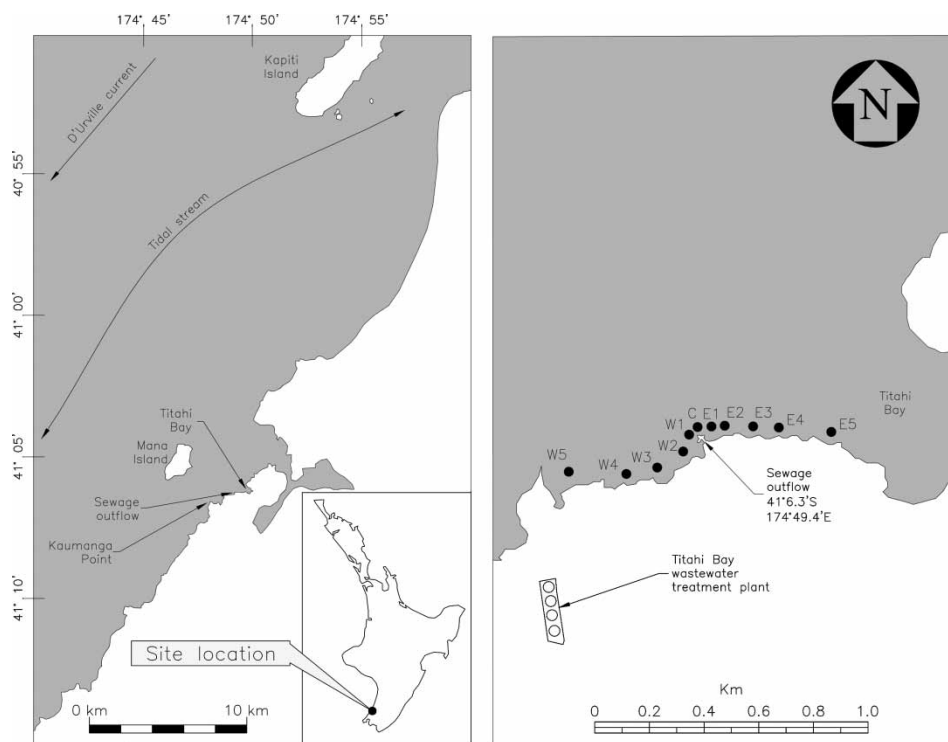


Fig. 1 Titahi Bay wastewater treatment plant (TWTP) with inset map of the North Island of New Zealand and the spatial arrangement of sampled sites located to the east (generally upcurrent, E1–E5) and west (downcurrent, W1–W5) of the TWTP outflow. ‘C’ reflects the position of the site centred immediately offshore of the discharge.

with an average DIN (dissolved inorganic nitrogen, composed of ammonium, nitrate and nitrite) concentration of 0.158 mmol ($n = 40$; $SE = 0.012$) (H. Bond, unpublished data). Effluent discharged from TWTP is generally tertiary processed, except during extremely high flow, when some overflow passes directly from the primary screening facility to the outflow (H. Bond, personal communication). The treatment train at the TWTP includes nitrification and denitrification reactions to remove nitrogen from the effluent. The discrimination against ^{15}N in treatment plants that use nitrification/denitrification treatment processes typically results in effluent containing ^{15}N -enriched dissolved nitrogen (Bedard-Haughn et al. 2003). The TWTP outflow releases effluent at the shoreline, and the release point is bordered to the northeast by a concrete

seawall to reduce movement of the effluent northeast towards Titahi Bay.

Sampling potential bioindicators

We collected three samples of each potential bioindicator (*C. maschalocarpum*, *A. media* and *P. elongatus*), at each sampling site, on each of three dates, at approximately monthly intervals, March–May 2005. Specimens of *C. maschalocarpum* were collected from a depth of *c.* 1 m, within 20 m of the shore and maintained on ice in the field (except during epifauna removal, see below) and subsequently frozen at -20°C until analysis. *Amphoroidea media* were collected from the *C. maschalocarpum* samples by briefly exposing *C. maschalocarpum* to heat shock (e.g. sampled algal tissues were contained within plastic bags without

water and exposed to direct sunlight for 30 min to facilitate removal and sampling of epifaunal communities). Individuals of *A. media* were selected haphazardly from these collections and subsequently frozen at -20°C until analysis. *Petrolisthes elongatus* were collected from beneath rocks around the low tide mark, within 20 m of where *C. maschalocarpum* was collected, maintained on ice in the field and subsequently frozen at -20°C .

Before analysis, samples of *C. maschalocarpum* were thawed, cleaned of any epiphytes, dried for 48 h at 70°C and ground to a fine powder. Algal tissue was collected from a homogenised sample of one entire macroalgal specimen (minus stipe and holdfast) to provide a standard relationship between consumer and producer isotope ratios. *Amphoroidea media* and *P. elongatus* samples were thawed, rinsed in fresh water, dried for 48 h at 70°C and ground to a fine powder. To generate sufficient tissue for isotope analysis of each sample, 10 individuals of *A. media* and *P. elongatus* were homogenised to form each replicate sample for each species, site and date. For each *A. media* sample, the 10 specimens were collected from the same host specimen of *C. maschalocarpum* (i.e. each specimen of *C. maschalocarpum* yielded its own sample of epifaunal *A. media*).

Inorganic carbon (e.g. from the exoskeletons of invertebrates, not derived from diet; Fry 1988) was removed by acidification. Dried and ground algal and epifaunal samples were acidified with 1 mol/l hydrochloric acid (HCl) using the drop-by-drop method following Jacob et al. (2005), re-dried at 70°C and re-ground before analysis.

All isotope samples were analysed using a Europa Geo 20/20 isotope ratio mass-spectrometer interfaced to an ANCA-SL elemental analyser. Duplicate samples of 1.8 mg of powder were loaded into tin capsules for analysis of organic carbon and nitrogen content and carbon and nitrogen isotopic composition. The standard analytical error between duplicate analyses is lower than $\pm 0.3\text{‰}$ for nitrogen and $\pm 0.1\text{‰}$ for carbon. Relative nitrogen isotopic concentrations are reported as $\delta^{15}\text{N}$ values relative to an air standard, where:

$$\delta^{15}\text{N}\text{‰} = \left(\frac{{}^{15}\text{N}/{}^{14}\text{N}_{\text{sample}} - {}^{15}\text{N}/{}^{14}\text{N}_{\text{air}}}{{}^{15}\text{N}/{}^{14}\text{N}_{\text{air}}} \right) \times 1000. \quad (1)$$

Relative carbon isotopic values are reported as $\delta^{13}\text{C}$ ratios relative to a Vienna Pee Dee belemnite standard (an international ${}^{13}\text{C}/{}^{12}\text{C}$ standard of cretaceous belemnite from the PeeDee formation in South Carolina, USA).

$$\delta^{13}\text{C}\text{‰} = \left(\frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{VPDB}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{VPDB}}} \right) \times 1000. \quad (2)$$

Sampling pollutants

To characterise undiluted effluent composition discharged from TWTP, we sampled both mill-screened (primary processed) and tertiary processed effluent at weekly intervals ($n=3$) in March 2005. Single samples were collected at 09:00 h to control for possible diel variation in sewage composition and/or processing. Effluent samples were refrigerated at $+4^{\circ}\text{C}$ on site, frozen within 3 h of collection and processed within 4 weeks. Samples were prepared for ${}^{15}\text{N}$ analysis of DIN according to Tozer et al. (2005). Filtered effluent samples were alkali distilled in the presence of Devarda's alloy to reduce nitrate to ammonium. Total N concentration was determined by back titration with standardised acid. Samples were prepared for mass spectrometry by drying the remaining solution in excess acid. Sewage particulates were collected by filtering effluent samples through Whatman 0.7- μm GFF glass fibre filters. The particulate portion was dried at 70°C for 48 h before carbon and nitrogen isotope analysis.

To assess general patterns of effluent dispersal, seawater samples were collected using a Niskin bottle at approximately monthly intervals (five dates between November 2004 and March 2005) by boat, and analysed for nutrient concentrations. Concentrations of ammonium/ammonia ($\text{NH}_4^+/\text{NH}_3$), nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) were measured in all seawater samples. Nutrient concentrations rather than $\delta^{15}\text{N}$ in seawater were used as a proxy for sewage dispersal owing to the

analytical difficulty in measuring $\delta^{15}\text{N}$ of dilute DIN (Gartner et al. 2002).

Samples were taken at the surface and at 4 m depth within 30 m of the shore at each of the 11 study sites. Samples were stored on ice in the field and subsequently frozen at -20°C until analysis. Ammonium and phosphate concentrations were estimated following Koroleff (1983). Nitrate and nitrite were estimated following Parsons et al. (1984).

We measured salinity using an RBR XR420 data logger on five runs over four dates between January and April 2005 at 0.5 m and 4.0 m depth. The data logger was programmed to record at 5-s intervals, and was deployed for 1 min at each location. Salinity estimates were generated by time-averaging data over the period of deployment at each location (1 min = 12 data acquisitions).

Statistical analysis

Spatial variation in tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was explored separately for each potential bioindicator using analysis of covariance (ANCOVA) models (Fisher 1935) where we included: (1) distance from the outfall as a covariate; (2) direction (east or west) from the outflow as a categorical variable; and (3) an interaction term. Data were assessed for normality using quantile–quantile plots (Quinn & Keough 2002) and homogeneity of variance by plotting residuals against adjusted group means (Quinn & Keough 2002). Where appropriate, dependent variables were \log_e transformed to improve homogeneity of variances. Samples of bioindicator tissue were averaged over time to obtain a single spatial measure for each site. The performances of bioindicators as measures of pollutant dispersal were examined using simple linear regression (Quinn & Keough 2002). Time averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each species were regressed against chemical proxies for sewage influence (time averaged DIN, PO_4^- and salinity) in surface waters at each site.

A single isotope, two-end linear mixing model was used to estimate the percentage contribution of sewage effluent to the tissue nitrogen of *C. maschalocarpum* individuals at each site. The model is of the form:

$$\delta^{15}\text{N}_x = X(\delta^{15}\text{N}_{\text{effluent}}) + (1 - X)(\delta^{15}\text{N}_y) \quad (3)$$

where X is the percentage contribution of sewage nitrogen, $\delta^{15}\text{N}_x$ is the $\delta^{15}\text{N}$ ratio of algae near the outflow, and $\delta^{15}\text{N}_y$ is the $\delta^{15}\text{N}$ ratio of background oceanic DIN (after Spies et al. 1989; modified by Wayland & Hobson 2001). The model assumes two sources of available nitrogen: background coastal DIN and sewage DIN. The model also assumes zero fractionation, or at least consistent fractionation across all sites, as DIN is assimilated into algal tissue, and hence the background coastal DIN $\delta^{15}\text{N}$ value can be estimated from the $\delta^{15}\text{N}$ values of *C. maschalocarpum* in clean coastal seawater. Only nitrogen isotope data were used in the model as the assumption of equal fractionation across sites was deemed less likely to be adequately met for carbon (Raven et al. 2002; Cornelisen et al. 2007). No measures of clean oceanic DIN $\delta^{15}\text{N}$ signatures were made during this study owing to the impractically large quantity of water required to get a signature measurement from oligotrophic waters (Gartner et al. 2002). The $\delta^{15}\text{N}$ of background DIN was estimated from the tissue $\delta^{15}\text{N}$ value of *C. maschalocarpum* taken from site E5. Seawater nutrient concentrations suggested that this site was unimpacted by sewage, and $\delta^{15}\text{N}$ values of *C. maschalocarpum* at this site were within the range of whole plant samples of *C. maschalocarpum* taken from unimpacted sites in northern New Zealand waters, between 5.3 and 7.6‰ (Poor Knights Islands, Mokohinau Islands and Leigh; N. Shears, unpublished data). The $\delta^{15}\text{N}$ value of sewage DIN was calculated from tertiary processed sewage (described above).

Previous dietary research suggests that *A. media* is herbivorous, and *C. maschalocarpum* is likely to form a significant part of its diet (Robbins 1990). To explore this potential dietary connection (and to evaluate how sewage-derived pollutants might cascade through a local system), we examined covariance in isotopic signatures between algal and consumer tissues using simple linear regression.

Results

Composition and dispersal of sewage effluent

Tertiary processed (final) effluent discharged from the TWTP contained DIN with $\delta^{15}\text{N}$ of $23.4 \pm 2.1\text{‰}$ (SE), $n=3$, and particulates with a $\delta^{15}\text{N}$ value of $6.8 \pm 1.2\text{‰}$, $n=3$. Particulates in final effluent had a $\delta^{13}\text{C}$ value of $-27.4 \pm 0.76\text{‰}$, $n=3$, and particulate $\delta^{13}\text{C}$ did not appear to be greatly affected by tertiary processing. Milliscreened effluent as would leave the TWTP in periods of high flow was comparatively depleted in ^{15}N ($\delta^{15}\text{N}$ of DIN $7.3 \pm 1.6\text{‰}$, $n=3$; $\delta^{15}\text{N}$ of particulates $5.1 \pm 1.8\text{‰}$, $n=3$). There was a trend towards higher concentrations of nitrogen and phosphate ions, and lower salinity in water samples collected to the west of the TWTP outflow (Fig. 2). The predominant east to west flow and resulting gradient of nutrient concentrations around the TWTP allowed examination of the influence of varying concentrations of pollutants on the three study species. Nitrites were comparatively low ($<1 \mu\text{M}$) throughout sample water, and

did not appear to vary systematically in relation to the sewage outflow.

Spatial variation $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures of potential bioindicators

Patterns of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in relation to distance gradients from the outfall (and measured gradients of sewage concentration) varied among the three bioindicator species. $\delta^{15}\text{N}$ values appeared to increase at sites to the west of the discharge point (receiving higher sewage concentrations) in all three species, whereas $\delta^{13}\text{C}$ values of *P. elongatus* tissue were generally decreased to the west of the discharge point (Fig. 3). To the west of the discharge, $\delta^{15}\text{N}$ values show a Gaussian plume pattern, with peak levels recorded at sites W2 and W3. $\delta^{15}\text{N}$ values recorded by bioindicators from the most westerly site (W5) suggest that pollutants may have extended beyond the spatial extent of sampling. In contrast, $\delta^{15}\text{N}$ values declined monotonically

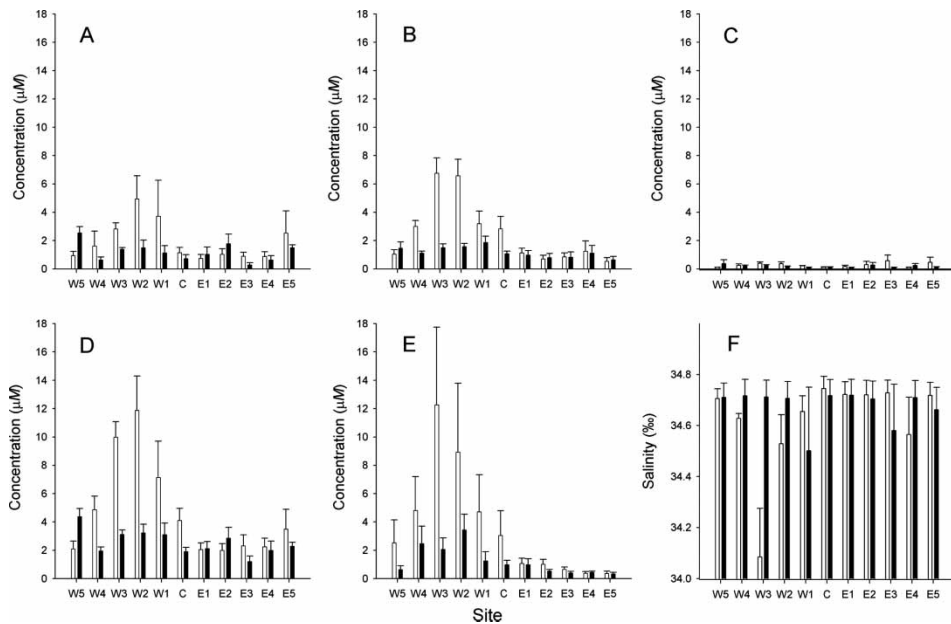


Fig. 2 Seawater concentrations (mean \pm SE, $n=5$ sampling times between November 2004 and March 2005) of (A) ammonium; (B) nitrate; (C) nitrite; (D) dissolved inorganic nitrogen; (E) phosphate and (F) salinity in water samples taken at the surface (unshaded bars) and at 4 m depth (shaded bars) for 11 sites near the TWTP. Sites are located to the east (generally upcurrent, E1–E5) and west (downcurrent, W1–W5) of the TWTP outflow. Site ‘C’ is immediately offshore from the discharge.

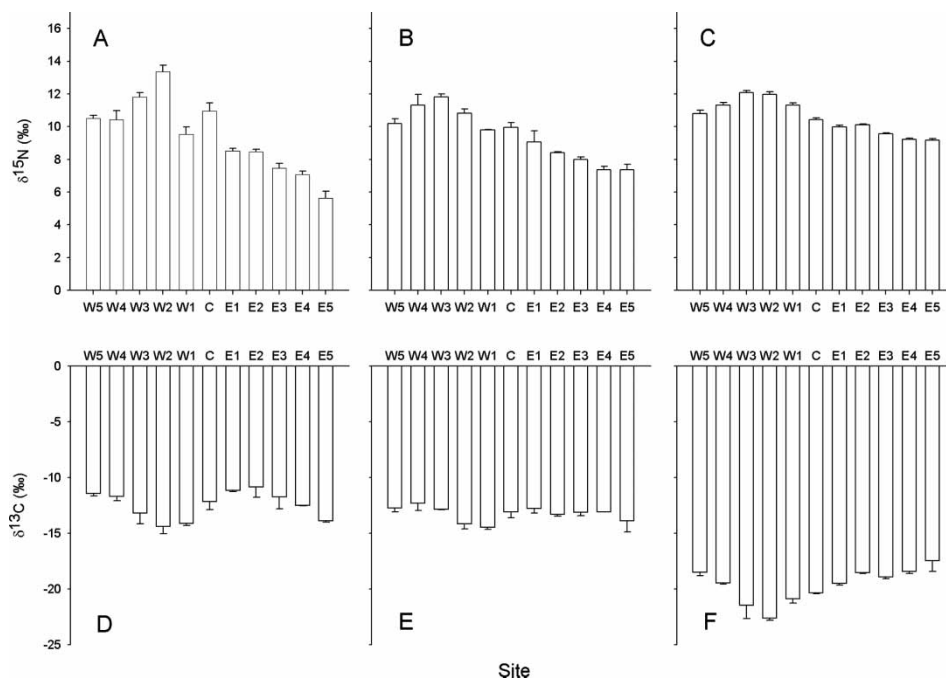


Fig. 3 Spatial variation in isotope signatures (time-averaged values \pm SE, $n = 3$). $\delta^{15}\text{N}$ values in (A) *Carpophyllum maschalocarpum*; (B) *Amphoroidea media*; and (C) *Petrolisthes elongatus*. $\delta^{13}\text{C}$ values in (D) *C. maschalocarpum*; (E) *A. media* and (F) *P. elongatus* (time-averaged values \pm 1 SE, $n = 3$). Sites are located to the east (generally upcurrent, E1–E5) and west (downcurrent, W1–W5) of the TWTP outflow. Site ‘C’ is immediately offshore from the discharge.

to the east of the discharge (Fig. 3A–C). $\delta^{13}\text{C}$ values recorded within the tissue of *C. maschalocarpum* and *A. media* appeared to exhibit spatial variation that was less clearly concordant with expected spatial variation in sewage concentrations (Fig. 3D,E). In contrast, $\delta^{13}\text{C}$ values from *P. elongatus* tissue mirrored spatial patterns indicated by $\delta^{15}\text{N}$ values (Fig. 3F,C).

A significant interaction in ANCOVA suggested that the reduction of $\delta^{15}\text{N}$ values with distance for *C. maschalocarpum* varied with direction from the discharge point ($F_{1,6} = 6.136$, $P = 0.047$; Fig. 3A). Similarly, the pattern of spatial variation in *A. media* $\delta^{15}\text{N}$ values differed between directions ($F_{1,6} = 6.854$, $P = 0.040$; Fig. 3B). $\delta^{15}\text{N}$ values for *P. elongatus* varied with direction from discharge ($F_{1,6} = 39.22$, $P < 0.001$) and declined with distance in both directions ($F_{1,6} = 8.91$, $P = 0.024$), with no significant interaction ($F_{1,6} = 2.499$, $P = 0.165$; Fig. 3C). Spatial variation in $\delta^{13}\text{C}$ values

exhibited interactions between distance and direction for *C. maschalocarpum* ($F_{1,6} = 6.12$, $P = 0.007$; Fig. 3D) and *A. media* ($F_{1,6} = 4.707$, $P = 0.073$; Fig. 3E). $\delta^{13}\text{C}$ values for *P. elongatus* varied with direction from discharge ($F_{1,6} = 6.063$, $P = 0.036$) and declined with distance ($F_{1,6} = 16.32$, $P = 0.007$), with a non-significant interaction ($F_{1,6} = 0.079$, $P = 0.788$; Fig. 3F).

$\delta^{15}\text{N}$ values of *P. elongatus* tissue showed a significant regression relationship with DIN concentrations in surface waters ($r^2 = 0.680$, $P = 0.002$), as did those of *C. maschalocarpum* ($r^2 = 0.540$, $P = 0.010$) and *A. media* ($r^2 = 0.456$, $P = 0.023$). Tissue $\delta^{15}\text{N}$ values of all three species were higher at sites with elevated surface water phosphate concentrations. *Petrolisthes elongatus* showed the closest relationship ($r^2 = 0.830$, $P < 0.001$), followed by *A. media* ($r^2 = 0.717$, $P = 0.001$) and *C. maschalocarpum* ($r^2 = 0.664$, $P = 0.002$). $\delta^{13}\text{C}$ values in *P. elongatus* were lower at sites with

higher seawater DIN concentration ($r^2=0.791$, $P<0.001$), as were $\delta^{13}\text{C}$ values of *C. maschalocarpum* ($r^2=0.554$, $P=0.009$), while those of *A. media* did not vary predictably in relation to seawater DIN ($r^2=0.174$, $P=0.202$). Only *P. elongatus* showed a significant regression relationship between tissue $\delta^{13}\text{C}$ and surface water phosphate ($r^2=0.708$, $P=0.001$), while surface water salinity was not significantly related to tissue $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values in any of the bioindicators used.

$\delta^{15}\text{N}$ values recorded within the tissues of *C. maschalocarpum* and associated *A. media* positively covaried ($r^2=0.845$, $P<0.001$), and there was no statistical relationship between $\delta^{13}\text{C}$ values ($r^2=0.243$, $P=0.123$).

Sewage nitrogen contributions to the total tissue nitrogen of *C. maschalocarpum* calculated using the mixing model peaked at 43% at site W2. This value declined in a westerly direction to 27% at site W5, and in an easterly direction to 8% at site E4. Sewage nitrogen contributions at sites C and W1, located directly offshore and around 20 m to the west of the outflow were 29% and 21%, respectively.

Discussion

$\delta^{15}\text{N}$ values in *C. maschalocarpum*, *P. elongatus* and *A. media* indicate that DIN from sewage forms a high proportion of total seawater DIN to the west of TWTP, and short distances to the east of TWTP. Sites inside Titahi Bay (i.e. E4, E5) received the lowest concentrations of sewage effluent. Spatial patterns of $\delta^{15}\text{N}$ values in all three species appeared consistent with patterns of sewage dispersal as predicted by elevated seawater nutrient concentrations and reduced salinity. Spatial patterns of $\delta^{13}\text{C}$ values in the filter feeder *P. elongatus* indicate sewage particulates from TWTP have similar patterns of dispersal to those of dissolved pollutants, whereas $\delta^{13}\text{C}$ values of *C. maschalocarpum* and *A. media* varied less predictably in relation to sewage dispersal patterns.

Elevated $\delta^{15}\text{N}$ values in *C. maschalocarpum* suggest that sewage DIN forms a significant portion of the nitrogen budget of this alga at short distances from the outflow. Macroalgae are primarily reliant on water

column DIN to meet their nitrogen requirements (Wallentinus 1984), and hence the dispersal of ^{15}N -enriched sewage DIN from the TWTP can be directly linked to macroalgal $\delta^{15}\text{N}$ values (McClelland & Valiela 1998; Rogers 2003). Marine-derived DIN from remineralised NH_4^+ and upwelling NO_3^- naturally available to coastal systems typically show $\delta^{15}\text{N}$ values of *c.* 6–8‰ (Miyake & Wada 1967; Wada et al. 1975; Liu & Kaplan 1989; Yoshida et al. 1989; Sigman et al. 1997, 2000), which is consistent with $\delta^{15}\text{N}$ values in temperate intertidal algae from unimpacted areas throughout New Zealand and abroad (Rogers 1999, 2003; Gartner et al. 2002; Cohen & Fong 2005; Barr 2007; Cornelisen et al. 2007; Savage 2009). Therefore, macroalgal $\delta^{15}\text{N}$ values in New Zealand coastal marine waters are likely to be broadly sensitive to the exposure of isotopically distinct terrestrial nitrogen inputs (i.e. above or below 6–8‰). The elevated levels of $\delta^{15}\text{N}$ values in *C. maschalocarpum* clearly reflect the dispersal of sewage DIN in Titahi Bay, despite potential differences in fractionation during nitrogen uptake that may occur across the concentration gradients of seawater nitrogen (Pennock et al. 1996; Waser et al. 1999; McKee et al. 2002), and fractionation effects of nitrification and denitrification reactions that may alter the $\delta^{15}\text{N}$ values of TWTP effluent following its release (Mariotti et al. 1984; Cifuentes et al. 1989). The observed pattern of $\delta^{13}\text{C}$ values in *C. maschalocarpum* is unlikely to be solely owing to the presence of sewage-derived carbon in seawater. Salinity and phosphate measurements indicate sewage was quickly diluted to the east of the outflow, and, based on its high $\delta^{13}\text{C}$ values, *C. maschalocarpum* is able to use both $\text{CO}_{2(\text{aq})}$ and HCO_3^- from the large marine dissolved inorganic carbon (DIC) pool (Raven et al. 2002). Therefore, the contribution of sewage DIC to the total pool of DIC available for uptake is likely to be small. $\delta^{13}\text{C}$ values of macroalgae tend to vary in response to turbulence (and hence flux of CO_2 ; Cornelisen et al. 2007) and light (Cornelisen et al. 2007; Wing et al. 2007), so that differences in light regime, wave action and currents might

contribute to the spatial pattern observed in *C. maschalocarpum* $\delta^{13}\text{C}$ values.

Carpophyllum maschalocarpum is perennial and common on open rocky coasts throughout New Zealand (Adams 1994); it has the potential to provide a regularly available and comparable measure of anthropogenic nitrogen input to open coastal reef systems. Notably, perennial brown algae tend to exhibit slower nitrogen uptake rates than fast-growing, foliose or filamentous genera (Wallentinus 1984). This characteristic makes *C. maschalocarpum* appropriate for providing a longer-term integration of seawater $\delta^{15}\text{N}$ values than commonly used bioindicator genera such as *Ulva* or *Gracilaria* (Gartner et al. 2002).

Elevated $\delta^{15}\text{N}$ values in the grazer *A. media* at sites with high sewage influence indicate a transfer of sewage nitrogen up the food chain from primary producers in these areas to this consumer. Many epifaunal species have been shown to be mobile (Martin-Smith 1994; Poore 2005), particularly between macroalgal plants when animals enter the water column at night (Alldredge & King 1980), or by crawling from plant to plant (Taylor 1998). However, the positive correlation in spatial patterns of $\delta^{15}\text{N}$ values between *A. media* and *C. maschalocarpum* within the sewage plume indicate that movement patterns and feeding ranges of this epifaunal species extend over sufficiently short distances that dispersal patterns of sewage nitrogen can be determined using $\delta^{15}\text{N}$ values of its tissues. The use of epifaunal grazers instead of or in conjunction with primary producers to measure sewage dispersal may be advantageous, as greater longevity and slower tissue turnover of consumers have been shown to result in lower seasonal variability in their $\delta^{15}\text{N}$ values than those of primary producers (Cabana & Rasmussen 1996).

Petrolisthes elongatus $\delta^{13}\text{C}$ values were generally depleted at sites with higher sewage influence, reflecting a higher consumption of SPOM carbon ($\delta^{13}\text{C} = -27.4\text{‰}$) in these areas. Owing to fractionation during metabolism, consumers organisms typically show isotopic enrichment, relative to food sources, of around 0–1‰ for $\delta^{13}\text{C}$ (DeNiro & Epstein 1976; Vander Zanden & Rasmussen 2001;

McCutchan et al. 2003) and 1.5–4‰ for $\delta^{15}\text{N}$ (DeNiro & Epstein 1981; Minagawa & Wada 1984; McCutchan et al. 2003; Vanderklift & Ponsard 2003). Marine particulate organic matter (POM) $\delta^{13}\text{C}$ values typically range from *c.* –19‰ to –24‰ (Rogers 1999; Cloern et al. 2002; Evans et al. 2006; Piola et al. 2006; Vizzini & Mazzola 2006). Tissue $\delta^{13}\text{C}$ values of around –17‰ to –18‰ at lower impact sites (e.g. E3, E4 and E5) suggest a primarily marine diet to the east of the outflow, and comparatively little consumption of SPOM. *Petrolisthes elongatus* $\delta^{15}\text{N}$ values mirrored spatial patterns of $\delta^{13}\text{C}$ values in the same species, but because the $\delta^{15}\text{N}$ values of SPOM nitrogen from the TWTP was similar to that of typical clean coastal and oceanic POM (Rogers 1999; Cloern et al. 2002; Gartner et al. 2002), $\delta^{15}\text{N}$ value increases in this animal could not be attributed to the consumption of sewage particulates. Instead, higher $\delta^{15}\text{N}$ values in *P. elongatus* suggests primary producers that have taken up nitrogen within the sewage plume form a major part of its diet, resulting in the transfer of ^{15}N -enriched sewage DIN (Piola et al. 2006). The contribution of sewage DIC to depleted $\delta^{13}\text{C}$ values in *P. elongatus* via the same pathway is likely to be minor in this study, given the low variation in salinity observed between sites. The distinct accumulation of ^{13}C -depleted sewage carbon in *P. elongatus*, together with its abundance and geographical dispersal around New Zealand (Schiel 2006), mark this species as a potentially valuable indicator of inputs of sewage particulates to rocky shores. This species may be useful for tracing sewage in areas where the use of sessile filter feeders is limited by their distribution, such as the Cook Strait shores (Helson et al. 2007).

Patterns of $\delta^{13}\text{C}$ in *P. elongatus*, and $\delta^{15}\text{N}$ in all three species were distinct at high sewage impact sites, reflecting the low values of $\delta^{13}\text{C}$ SPOM and high values of $\delta^{15}\text{N}$ DIN released from the treatment plant relative to natural marine nitrogen and carbon sources. The rapid loss of ^{14}N relative to ^{15}N , associated with volatilisation of ammonium during sewage processing, tends to increase the $\delta^{15}\text{N}$ ratio of the dissolved nitrogen remaining in sewage effluent (Bedard-Haughn et al. 2003). As a

result, the $\delta^{15}\text{N}$ value of TWTP DIN (23.4‰) is high relative to naturally available marine DIN. However, $\delta^{15}\text{N}$ values of sewage DIN and SPOM can vary between wastewater treatment plants (Rogers 1999; Gartner et al. 2002; Savage & Elmgren 2004; Piola et al. 2006; Dudley 2007), and sewage processing facilities can produce effluent with DIN and SPOM $\delta^{15}\text{N}$ values within the range of values normally associated with marine-derived DIN and POM (Bedard-Haughn et al. 2003; Dudley 2007). The usefulness of ^{15}N as a tracer of sewage dispersal is limited if the $\delta^{15}\text{N}$ value of sewage nitrogen is not well separated from nitrogen naturally available to bioindicators. The $\delta^{13}\text{C}$ value of POM from the TWTP (-27.4‰) is typical of domestic sewage from a C_3 plant-derived diet, and variability in $\delta^{13}\text{C}$ values of SPOM appears low across sewage plants and processing types, typically ranging from *c.* -25‰ to -28‰ (Rogers 1999, 2003; Piola et al. 2006; Dudley 2007). $\delta^{13}\text{C}$ values of marine POM have been shown to vary across this range under some conditions, particularly when influenced by terrestrial and estuarine-produced organic carbon (Chanton & Lewis 1999, 2002; Cloern et al. 2002; Forrest et al. 2007). Because either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of sewage effluent may overlap those of natural carbon or nitrogen sources in marine ecosystems, the use of a dual isotope (C and N), multi-species approach is likely to more reliably distinguish patterns of sewage dispersal than single species or single isotope analysis. The results of this study suggest *C. maschalocarpum*, *A. media* and *P. elongatus* are suitable bioindicators to detect sewage nitrogen dispersal in the form of DIN, and that *P. elongatus* is also a suitable measure of the dispersal of carbon from sewage particulates.

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