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Carbon and nitrogen stable isotope analysis of three types of oyster tissue in an impacted estuary

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Abstract

The stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) of the muscle, ctenidia and viscera of the Sydney rock oyster, *Saccostrea glomerata*, showed the dilution and assimilation of tertiary treated sewage along an estuarine gradient. The enriched ^{15}N values of oyster ctenidia and viscera from within 50 m of the sewage outfall indicated the use of ^{15}N-enriched tertiary treated sewage effluent ($16 \pm 2.3‰$) as a nutrient source. The effect of sewage nitrogen on oyster δ^{15}N was localised, with oysters 5 km upstream and downstream of the outfall not significantly enriched. Viscera δ^{15}N was most sensitive to sewage nutrients and δ^{13}C significantly defined an ocean-to-estuarine gradient. High variance in isotope ratios of viscera compromised its use as an indicator of anthropogenic nutrients, and this also reduced the utility of whole-body stable isotope ratios. Ctenidia was the most useful indicator tissue of sewage discharge at the scale of this study, being consistently and significantly enriched in δ^{15}N close to the sewage outfall and δ^{13}C clearly defined an estuarine gradient with less internal variability than viscera. Muscle δ^{15}N was least sensitive to sewage effluent and showed the least variability, making it more suited to investigations of anthropogenic nutrient enrichment over larger spatio-temporal scales.

Keywords: South East Australia; carbon; nitrogen; δ^{13}C; δ^{15}N; sewage; outfalls; *Saccostrea glomerata*

1. Introduction

Sewage effluent is a rich source of dissolved nutrients and particulate organic matter that can disrupt the natural nutrient balance of estuarine and coastal systems, altering algal (Brown et al., 1990), seagrass (Udy and Dennison, 1997; Jones et al., 2001), benthic invertebrates (van Dover et al., 1992; Roberts, 1996; Waldron et al., 2001; Savage et al., 2002) and fish assemblages (Russo, 1989; Smith and Suthers, 1999). The biodiversity of macrobenthic communities has been shown to decrease with proximity to a marine deepwater outfall (Roberts, 1996). In estuarine environments, the abundance of macrobenthic taxa is negatively correlated with annual sewage nitrogen loads (Savage et al., 2002). The abundance and mean length of hulafish, *Trachinops taeniatus*, have been shown to be lower at sewage outfall locations compared to control locations (Smith and Suthers, 1999). Even when sewage treatment is improved, ecosystem responses such as these are slow to reverse due to lag effects associated with the 'nutrient memory' of sediments (Krovang et al., 1999).

The stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) can trace the movement and assimilation of nutrient and organic matter sources, such as sewage effluent, within food chains of coastal systems (Fry and Sherr, 1984; Owens, 1987; Fry, 1988; Hobson and Welch, 1992; Hansson et al., 1997). Bivalves are particularly sensitive bio-indicators of anthropogenic nutrients (Feinstein et al., 1996; Yelenik et al., 1996; Fry, 1999; Lake et al., 2001; McKinney et al., 2001a,b), with δ^{15}N indicating human-derived waste and/or effluent (Yelenik et al., 1996; McKinney et al., 2001b). This is because bivalves incorporate chemicals and elements into their tissues at levels significantly above those found in the

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surrounding environment, from the large volumes of water they filter for feeding (Alexander and Young, 1976; Viarengo and Canesi, 1991; Phillips and Rainbow, 1993; Scanes, 1996). The Sydney rock oyster, *Saccostrea glomerata*, occurs in Australian estuaries from Victoria to Queensland and is of great economic importance, forming the foundation of a multi-million dollar oyster cultivation industry. *Saccostrea glomerata* has proven to be a useful indicator of organochlorins in sewage, with the New South Wales Department of Environment and Conservation ''Oyster Watch'' monitoring program detecting significant decreases in concentrations in *S. glomerata* tissues after sewage outfalls were relocated offshore (Scanes, 1996).

Stable isotope studies of oysters as indicators of nutrient loads have so far used whole-body analysis (Riera and Richard, 1996; Riera, 1998). Isotope ratios of the whole body can vary depending on the health and condition of the animal (Gannes et al., 1997). Whole-body isotope ratios can also vary due to different rates of isotopic turnover for specific tissues. This is attributed to differing metabolic rates and biochemical processes among tissues, resulting in more metabolically active tissues reflecting changes in dietary nutrient sources more rapidly than less metabolically active tissues (Tieszen et al., 1983; Hobson and Clark, 1992a). By analysing the stable isotope composition of specific tissues with differing isotope turnover rates, tracking the movement of nutrient sources within a system over varying temporal and spatial scales may be possible.

In this study the carbon and nitrogen stable isotope ratios of muscle, ctenidia and viscera of *Saccostrea glomerata* are used as short- and long-term indicators of tertiary treated sewage effluent in an estuary. Oysters were deployed at the sewage discharge point and at sites 5 km apart upstream and downstream of this point. Primary nutrient sources, including estuarine and sewage particulate organic matter (POM) were examined to determine potential nutrient signatures. The sensitivity and ability of muscle, ctenidia and viscera to provide information on sewage-derived and naturally occurring nutrient loads over varying temporal and spatial scales are assessed.

2. Materials and methods

2.1. Study area

The Manning River is situated 350 km north of Sydney on the mid-north coast of New South Wales, Australia (Fig. 1). The river has a number of tributary creeks and branch channels and two ocean entrances. The main entrance is at Harrington and the secondary entrance is 12 km to the south. The catchment is the fourth largest in NSW (8320 km^2) with a waterway area of 25.4 km^2 (GTCC, 1997). Approximately 5 ML of tertiary treated sewage effluent is discharged into the Manning River daily from the Dawson sewage treatment plant (STP).

Oysters were deployed at four sampling sites approximately 5 km apart (Sites 1–4) along the Manning River, with Site 1 near Harrington being the most oceanic and Site 4 the furthest upstream near Taree (Fig. 1). Site 3 was within 50 m of the Dawson STP discharge point.

Water temperatures in the Manning River seasonally increased from November to December 2002, followed by a decrease in February 2003 (Fig. 2a). Salinity ranged from 33 at the oceanic Site 1, to 26 at the upstream Site 4. Daily rainfall at Taree was low from October to early December, with a large rainfall event between 10 and 12 December 2002 when 125 mm of rain fell over a 72 h period (Fig. 2c; NSW Bureau of Meteorology). Lower temperature and salinity observations following the rainfall event were evident at all sampling sites in January compared to those recorded for the earlier dry weather period (Fig. 2a, b). Salinity recovered in the estuary by February and was similar to November measurements (Fig. 2b).

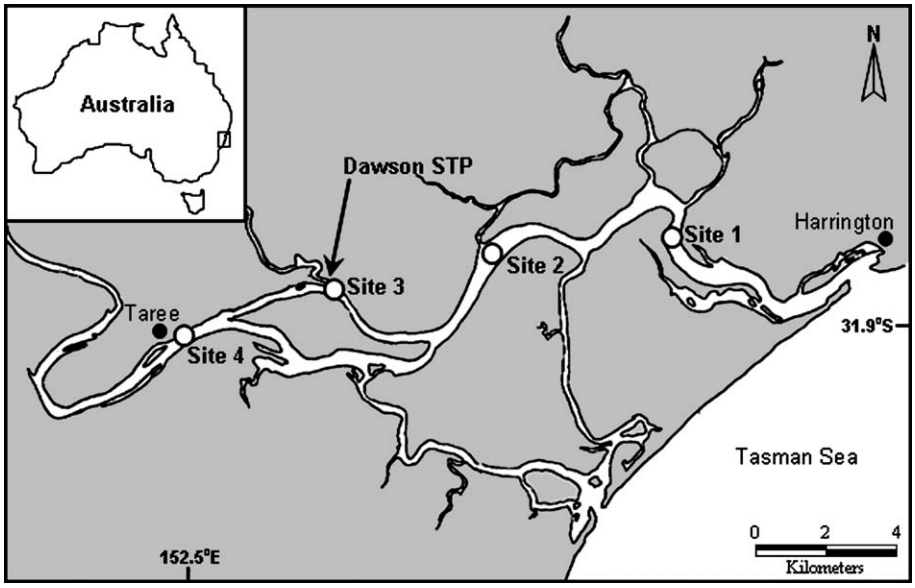

Fig. 1. The Manning River estuary, indicating the four oyster deployment sampling sites and the location of the Dawson sewage treatment plant (STP) discharge point.

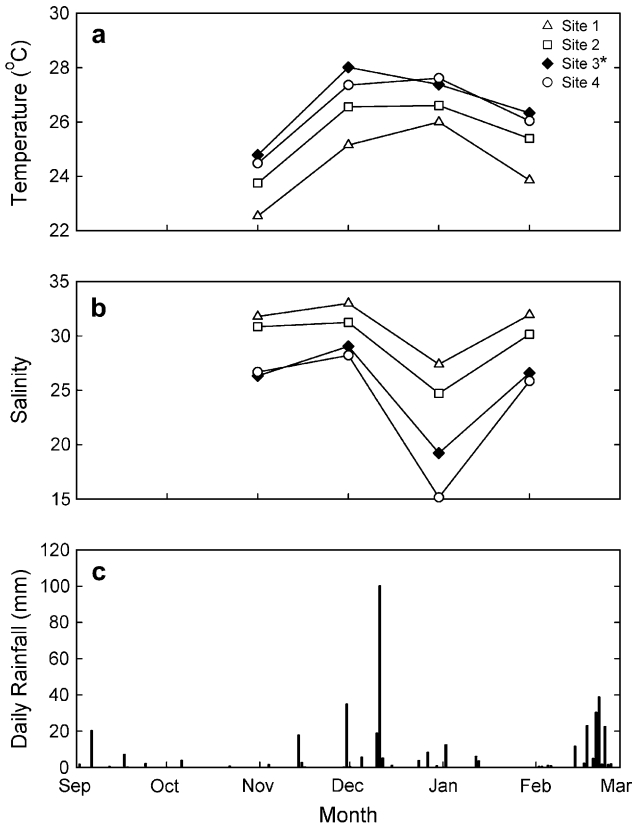

Fig. 2. Physico-chemical data for the Manning River estuary. (a) Temperature and (b) salinity recorded at the four sampling sites over four months, with Site 3* being the location of the sewage outfall. (c) Daily rainfall readings for the area between September 2002 and February 2003.

2.2. Sample collection and processing

Oysters (30–40 g wet-weight each) were obtained from commercial oyster leases in Wallis Lake, New South Wales, and deployed in oyster farming trays 50 cm above the substrate in 1 m of water at low tide. Two replicate trays (or plots) were deployed at each site 15–20 m apart, each containing 50–60 oysters. Trays were deployed in August 2002 and the oysters were allowed three months to acclimatise to the new environment. Four oysters of a similar size were collected on 21 November, 3 December, 6 January and 3 February, and were immediately frozen until analysis. Two oysters from each experimental plot were removed from their shells, rinsed in distilled water and the adductor muscle and ctenidia tissue dissected out for tissue specific stable isotope analysis. A portion of the remaining tissue, which we term as viscera, was dissected from the posterior end of each oyster for isotope analysis. The remaining visceral tissue was retained to calculate total dry weight of the entire oyster. The condition of all oysters was determined from the ratio of dry weight of oyster meat to volume of internal cavity (Lawrence and Scott, 1982).

Water samples for suspended POM and chlorophyll *a* (chl *a*) analysis were collected at each site at the time of oyster sampling in 2 L light-proof jars. POM samples were filtered onto pre-combusted Advantec glass fibre filter papers (GC-50; 47 mm ∅) under low vacuum and frozen. Magnesium carbonate (2 mL) was added prior to filtration of chl *a* samples onto Advantec glass fibre filter papers (GC-50; 47 mm ∅) under low vacuum. Filter papers were chopped finely and chl *a* extracted in 5 mL of 90% acetone. Samples were centrifuged and the supernatant analysed at 630, 647 and 664 nm using a Beckman DU-640 spectrophotometer and chl *a* content determined as per Hallegraeffe and Jeffrey (1975). Additional POM and chl *a* samples were taken one and two weeks following the December rainfall event on 19 and 26 December.

One litre of tertiary treated sewage effluent was collected monthly from the Dawson STP from November 2002 to February 2003 for stable isotope analysis of sewage particulate organic matter (SPOM). Samples were filtered onto pre-combusted Advantec glass fibre filter papers (GC-50; 47 mm ∅) under low vacuum and frozen.

Mangrove (*Avicennia marina*), seagrass (*Zostera capricorni*) and seagrass epiphyte samples were collected for stable isotope analysis in November 2002. Mangrove leaves were sampled from the growing tips of individual trees growing on the river bank at Site 4. Young seagrass leaves and seagrass epiphytes were collected from beds located near oyster plots at Site 2. Only fresh mangrove and seagrass samples were collected as it has been shown that there is almost no change in the $\delta^{13}C$ values of mangrove and seagrass leaves during decomposition (Zieman et al., 1984). Mangrove and seagrass leaves were rinsed in distilled water to remove detritus. Epiphytes were obtained from seagrass leaves by gently scraping with a scalpel blade and rinsing in distilled water and frozen.

All samples for stable isotope analysis were freeze dried to a constant weight using a Dynavac FD3 freeze drier unit. Oyster and plant samples were weighed and homogenised into a fine powder and transferred into tin capsules for isotope analysis. For POM and SPOM samples, particulate matter was scraped from the surface of filter papers using forceps and sealed in tin capsules for isotope analysis. Analysis of carbon and nitrogen stable isotope ratios and C and N contents was carried out using an elemental analyser (Eurovector EA 3000) and mass spectrometer (Isoprime). Ratios of ^{13}C:^{12}C and ^{15}N:^{14}N are expressed as the relative per mil (‰) between the sample and the conventional international standards (atmospheric nitrogen for $\delta^{15}N$ and Pee Dee Belemnite for $\delta^{13}C$):

$$\delta X = \left(R_{\text{sample}}/R_{\text{standard}} - 1\right)1000$$

where $X = {}^{13}C$ or ^{15}N and $R = {}^{13}C$:^{12}C or ^{15}N:^{14}N. Measurements were to a precision of approximately 0.2‰ for ^{13}C:^{12}C and ^{15}N:^{14}N.

2.3. Potential food sources

To establish the potential nutrient sources of the oysters, a "composite" whole-oyster stable isotope value was calculated

for each individual oyster sampled based upon the relative isotope values of individual tissues using:

$$\delta\text{Whole} = [\delta X_m \times \%\text{DW}_m] + [\delta X_c \times \%\text{DW}_c] + [\delta X_v \times \%\text{DW}_v]$$

where δWhole is the composite whole-oyster isotope value for each individual oyster, δX is the δ^{13}C or δ^{15}N isotope value for individual tissues and %DW is the percentage dry weight of the individual tissue (mg; m = muscle; c = ctenidia; v = viscera) with respect to the dry weight of the entire oyster.

Most consumers are only slightly enriched in δ^{13}C ($\sim$1‰) in comparison to their diet while ^{15}N-enrichment of consumers relative to their diet is generally much greater ($\sim$3‰) (Peterson and Fry, 1987). Since the fractionation values of carbon and nitrogen isotopes from diet to oysters is unknown, potential food sources for oysters were considered to be those primary sources with a mean δ^{13}C composition within the range of 2‰ less than or 1‰ greater than the mean oyster composition and with a mean δ^{15}N composition within the range of 1–5‰ (Bunn and Boon, 1993).

Carbon–nitrogen ratios (C:N) were used to characterise the nutritional quality of potential food sources. This technique has been successfully used to investigate the diets and quality of food sources for aquatic snails (Sheldon and Walker, 1997) and decapod crustaceans (Burns and Walker, 2000). Most animals use food sources with C:N ratios <4 (Burns and Walker, 2000), with lower C:N values generally equating to increased nutritional quality.

2.4. Data analysis

Significant differences in each tissue type and each isotope for oysters were tested using a three-factor analysis of variance (ANOVA) with the first two factors (Month, 4 levels and Site, 4 levels) fixed and orthogonal, and the third factor (Plot) nested within Sites ($n = 2$). Orthogonal, fixed two factor ANOVAs were used to test for significant differences in oyster condition, oyster size (dry weight) and POM samples. Rainfall event POM samples were excluded from the analysis of the regular monthly POM samples. One-way ANOVAs for δ^{13}C and δ^{15}N were used to test for significant difference in SPOM samples. The within-tissue variability of δ^{13}C and δ^{15}N values was calculated as the variance component for each tissue across all sampling months and compared using F-tests.

3. Results

3.1. Oyster tissues

The average stable isotope values of muscle, ctenidia and viscera over all sampling occasions ranged from −27 to −21‰ for δ^{13}C and from 5.5 to 10.0‰ for δ^{15}N (Table 1). Tissues had distinct isotope ratios (Fig. 3), with muscle consistently enriched in ^{13}C by 2.1–2.7‰ and ^{15}N by 0.9–1.1‰ compared to ctenidia, and in ^{13}C by 3.2–4.1‰ and ^{15}N by 3.0‰ compared to viscera. Within each tissue type grouping, samples from the outfall site (Site 3) were consistently enriched in ^{15}N by between 0.3 and 1.9‰ and depleted in ^{13}C by between 0.07 and 1.5‰ compared to Sites 1, 2 and 4 (Fig. 3). This provides a relative measure of the sewage effect among sites, with the effect greatest at the outfall and decreasing moving away from the outfall. An ocean-to-upstream gradient is evident for Site 1, 2 and 4 within each tissue type, with ^{13}C values decreasing as site locations move further upstream from the ocean (Fig. 3).

Of the three tissues analysed, muscle exhibited the least variance in δ^{13}C and δ^{15}N values across all sites (Table 2), with significantly lower variability than ctenidia and viscera samples (Table 2). Muscle isotope values ranged from −20.6 to −23.9‰ and 8.5 to 10.4‰ for δ^{13}C and δ^{15}N, respectively. Muscle δ^{13}C was depleted at the outfall Site 3 and an ocean-to-upstream gradient was evident for Sites 2 and 4; however, these trends were not significant when averaged over the four-month sampling period (Fig. 3). There was no significant difference in muscle δ^{15}N other than some variation between plots at Site 3 (SNK post hoc comparison, ANOVA, $P \leq 0.05$; Table 3).

Ctenidia ranged from −22.8 to −26.9‰ and 6.9 to 10.1‰ for δ^{13}C and δ^{15}N, respectively. The variance in ctenidia δ^{13}C and δ^{15}N values was significantly greater than muscle and significantly less than viscera (Table 2). February δ^{13}C values for ctenidia were significantly depleted in comparison to all other months (ANOVA, $P \leq 0.05$; Table 3). There was some variation in δ^{13}C between replicate plots at Site 3 (ANOVA, $P \leq 0.05$; Table 3). Ctenidia δ^{15}N was significantly different among months and sites with a significant month by site interaction (ANOVA, $P \leq 0.01$; Table 3). The outfall location (Site 3) was significantly enriched in δ^{15}N with respect to all other sites for each sampling occasion (Fig. 4).

Viscera δ^{13}C and δ^{15}N values were the most variable of all the tissues at each site with variance in δ^{13}C values significantly greater than muscle and δ^{15}N values significantly greater than muscle and ctenidia (Table 2). Isotopic values ranged from −23.0 to −28.3‰ and 4.6 to 8.7‰ for δ^{13}C and δ^{15}N, respectively. Viscera δ^{13}C was significantly different among months (ANOVA, $P \leq 0.05$; Table 3) with November samples significantly enriched compared to February samples. An ocean-to-upstream gradient was evident with viscera δ^{13}C from Site 1 significantly depleted compared to Sites 2, 3 and 4, and Site 2 samples significantly depleted compared to Sites 3 and 4 (Fig. 5a). Viscera samples from the outfall location (Site 3) were significantly enriched in δ^{15}N compared to other sites (ANOVA, $P \leq 0.01$; Table 3; Fig. 5b).

The condition indices of oysters were not significantly different among months or sites (ANOVA; Table 3). There was a significant difference in the oyster size among months (ANOVA, $P \leq 0.001$; Table 3) with January and February oysters having a significantly greater dry weight than November and December oysters consistent with growth over the four-month period. There was no significant difference in the size of oyster among sites within any given month (ANOVA; Table 3).

Table 1
Mean stable isotope ratios for $\delta^{13}C$ and $\delta^{15}N$ for oysters and major primary sources sampled from sites on the Manning River over four months. Values for oysters are separated into individual tissues (muscle, ctenidia and viscera) and whole. Values indicate mean ± SE, where $n = 4$ for all oysters samples; $n = 2$ for POM, Rain-POM and SPOM; $n = 3$ for mangroves, epiphytes and seagrass. The symbol asterisk (*) indicates the location of the sewage outfall

	November		December		January		February	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Muscle								
Site 1	−21.9 ± 0.5	9.0 ± 0.2	−22.3 ± 0.2	9.1 ± 0.2	−22.2 ± 0.2	9.3 ± 0.2	−21.8 ± 0.1	9.0 ± 0.3
Site 2	−22.4 ± 0.2	9.8 ± 0.2	−22.5 ± 0.1	8.8 ± 0.1	−22.0 ± 0.2	9.3 ± 0.2	−22.4 ± 0.2	9.1 ± 0.1
Site 3*	−22.9 ± 0.1	9.8 ± 0.2	−22.7 ± 0.4	10.0 ± 0.1	−22.9 ± 0.3	9.9 ± 0.1	−22.7 ± 0.4	9.6 ± 0.3
Site 4	−22.0 ± 0.1	9.8 ± 0.1	−22.7 ± 0.3	9.7 ± 0.2	−22.1 ± 0.2	9.1 ± 0.3	−22.7 ± 0.3	9.4 ± 0.2
Ctenidia								
Site 1	−24.2 ± 0.3	7.6 ± 0.3	−23.7 ± 0.4	7.7 ± 0.2	−23.6 ± 0.3	8.6 ± 0.2	−25.0 ± 0.1	8.2 ± 0.3
Site 2	−24.4 ± 0.5	8.3 ± 0.1	−24.5 ± 0.5	8.0 ± 0.2	−24.4 ± 0.2	8.9 ± 0.2	−25.2 ± 0.3	8.3 ± 0.1
Site 3*	−25.7 ± 0.4	9.1 ± 0.2	−25.6 ± 0.3	9.6 ± 0.1	−25.7 ± 0.3	9.7 ± 0.2	−25.7 ± 0.6	9.1 ± 0.1
Site 4	−25.0 ± 0.5	8.5 ± 0.2	−25.1 ± 0.7	8.9 ± 0.1	−25.2 ± 0.5	8.8 ± 0.1	−25.1 ± 0.4	8.1 ± 0.3
Viscera								
Site 1	−25.8 ± 0.4	5.8 ± 0.4	−25.0 ± 0.6	5.7 ± 0.3	−25.5 ± 0.5	6.3 ± 0.5	−24.7 ± 0.5	6.4 ± 0.4
Site 2	−26.3 ± 0.2	6.6 ± 0.6	−25.8 ± 0.4	6.3 ± 0.4	−25.8 ± 0.3	6.8 ± 0.5	−25.0 ± 0.7	6.6 ± 0.3
Site 3*	−26.7 ± 0.7	7.5 ± 0.3	−26.7 ± 0.6	8.2 ± 0.4	−26.6 ± 0.4	8.2 ± 0.1	−25.9 ± 0.1	7.9 ± 0.1
Site 4	−27.0 ± 0.3	6.6 ± 0.5	−27.2 ± 0.6	6.4 ± 0.5	−25.8 ± 0.4	6.0 ± 0.6	−25.7 ± 0.8	7.1 ± 0.2
Whole								
Site 1	−24.9 ± 0.3	6.6 ± 0.3	−24.5 ± 0.5	6.4 ± 0.3	−24.7 ± 0.4	7.1 ± 0.3	−24.2 ± 0.3	7.0 ± 0.3
Site 2	−25.3 ± 0.2	7.4 ± 0.5	−25.2 ± 0.3	6.9 ± 0.3	−25.1 ± 0.2	7.4 ± 0.4	−24.8 ± 0.6	7.0 ± 0.2
Site 3*	−26.0 ± 0.5	8.0 ± 0.2	−26.0 ± 0.6	8.7 ± 0.2	−26.1 ± 0.4	8.5 ± 0.1	−25.6 ± 0.1	8.1 ± 0.1
Site 4	−25.6 ± 0.3	7.6 ± 0.4	−26.1 ± 0.5	7.3 ± 0.3	−25.3 ± 0.4	6.7 ± 0.4	−25.4 ± 0.7	7.3 ± 0.2
POM								
Site 1	−25.1 ± 0.1	9.0 ± 0.6	−23.9 ± 0.3	7.8 ± 0.3	−29.7 ± 0.5	5.7 ± 0.2	−24.9 ± 0.1	4.0 ± 0.2
Site 2	−25.4 ± 0.1	9.4 ± 0.7	−26.0 ± 0.1	7.6 ± 0.3	−23.3 ± 1.9	5.2 ± 0.1	−25.3 ± 0.1	4.4 ± 0.1
Site 3*	−25.7 ± 0.2	9.5 ± 0.6	−25.7 ± 0.1	7.8 ± 0.2	−29.9 ± 2.7	6.3 ± 0.2	−25.7 ± 0.1	4.6 ± 0.5
Site 4	−25.6 ± 0.2	9.9 ± 0.2	−25.3 ± 0.0	7.2 ± 0.1	−31.5 ± 0.7	5.5 ± 0.1	−25.9 ± 0.2	5.3 ± 0.3
Rain-POM								
Site 1	–	–	−27.9 ± 3.2	5.3 ± 0.5	–	–	–	–
Site 2	–	–	−23.2 ± 1.4	5.3 ± 0.7	–	–	–	–
Site 3*	–	–	−24.7 ± 2.7	4.4 ± 0.2	–	–	–	–
Site 4	–	–	−25.9 ± 2.1	3.2 ± 0.1	–	–	–	–
SPOM	−24.9 ± 0.9	15.3 ± 0.0	−26.3 ± 0.5	6.6 ± 0.3	−26.6 ± 0.2	18.7 ± 0.0	−27.9 ± 0.3	23.5 ± 0.0
Mangrove	−27.3 ± 0.5	3.6 ± 0.3	–	–	–	–	–	–
Epiphytes	−24.3 ± 0.4	4.8 ± 0.2	–	–	–	–	–	–
Seagrass	−11.4 ± 0.2	4.1 ± 0.3	–	–	–	–	–	–

3.2. Primary sources and potential food sources

The $\delta^{13}C$ values of estuarine POM ranged from −18.9 to −35.4‰. This large range was predominantly driven by the large variability among January samples following the December rainfall event (Table 1), with the mean $\delta^{13}C$ value for POM being −26.2 ± 0.5‰ (±SE, $n = 32$). POM $\delta^{13}C$ values were not significantly different among sites in November, December and February (Fig. 6a). The $\delta^{15}N$ values for POM were highly variable ranging from 2.9 to 10.1‰ (Table 3; Fig. 6b), with a mean value of 6.8 ± 0.4‰ (±SE, $n = 32$). There was a significant depletion in $\delta^{15}N$ values over the four months of sampling for each site (ANOVA, $P \leq 0.001$; Table 4); however, there were no significant differences in $\delta^{15}N$ values among sites within any one sampling month (Fig. 6b).

The mean value for $\delta^{13}C$ and $\delta^{15}N$ of Rain-POM collected one and two weeks after the rainfall event was −25.4 ± 1.2‰ and 4.6 ± 0.3‰, respectively (±SE, $n = 16$). This represents a 2.2‰ depletion in the ^{15}N composition of POM in the weeks following the rainfall event compared to monthly estuarine POM samples collected at the time of oyster collection. The log $\delta^{15}N$ value of POM was highly and negatively correlated with log chl *a* concentrations ($r = 0.75$, $P < 0.01$, $n = 24$, Fig. 7). Chl *a* concentrations in the weeks following the rainfall event are up to 16.7 mg m^{-3} above values recorded in November and early December (Fig. 8). Chl *a* concentrations in January and February were similar to levels observed before the rainfall event (Fig. 8).

The mean value for $\delta^{13}C$ and $\delta^{15}N$ of SPOM over the sampling period was −26.4 ± 0.4‰ and 16.0 ± 2.3‰, respectively (±SE, $n = 8$). This is considerably enriched in ^{15}N compared to estuarine POM by 9.2‰, but has similar $\delta^{13}C$ values. The mean monthly $\delta^{13}C$ values for SPOM ranged from −24.9 to −27.9‰ (Table 1) with no significant difference between months. Mean SPOM $\delta^{15}N$ values were more variable than that of POM, ranging from 6.6 to 23.5‰

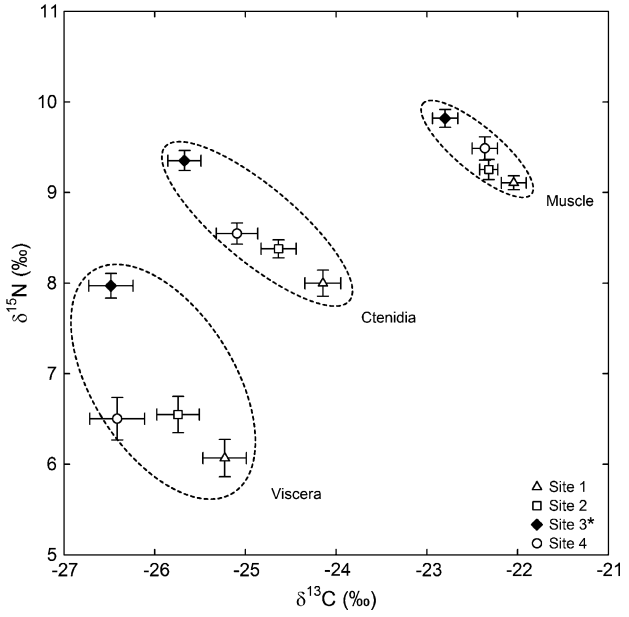

Fig. 3. Comparison of mean (±SE, $n = 16$) carbon and nitrogen isotope values for oyster muscle, ctenidia tissue and viscera sampled from four sites on the Manning River over four months. The symbol asterisk (*) indicates location of the sewage outfall.

($n = 2$, Table 1). The δ^{15}N values were significantly different over sampling months (ANOVA, $P \leq 0.001$; Table 4) with the lowest value (6.6‰) occurring in December following the rainfall event.

Mangrove leaves were the most depleted in mean δ^{13}C of all nutrient sources (−27.5‰) and were depleted in ^{15}N compared to estuarine POM by 3.2‰ (Table 1; Fig. 9). Epiphytic algae had a mean δ^{13}C value of −24.3‰ and were depleted in ^{15}N compared to estuarine POM by 2‰ (Table 1; Fig. 9). The mean δ^{13}C value of seagrass (−11.4‰) was considerably enriched compared to other primary sources and was depleted in ^{15}N compared to estuarine POM by 2.7‰ (Table 1; Fig. 9).

The mean isotope values for whole oysters ranged from −24.6 to −25.9‰ and 6.9 to 8.3‰ for δ^{13}C and δ^{15}N, respectively ($n = 16$, Table 1; Fig. 9). As with individual tissues, whole oysters from the outfall site (Site 3) were significantly enriched in ^{15}N and depleted in ^{13}C compared to oysters from all other sites (ANOVA, $P = 0.002$; Table 3; Fig. 9). SPOM was most enriched in ^{15}N compared to all other potential food sources (Fig. 9). Due to the influence of sewage effluent on the oyster isotope values at the outfall site, only oysters from Sites 1, 2 and 4 were used to assess potential food sources.

The mean ^{13}C values of POM sampled at the time of oyster collection and Rain-POM sampled one and two weeks after the rainfall event are consistent with possible organic carbon sources for oysters. However, only the Rain-POM showed the corresponding enrichment in δ^{15}N (of 2.2–2.6‰; Fig. 9) consistent with shifts in δ^{15}N from primary producer to primary consumer (1–5‰) as stated by Bunn and Boon (1993). The largest observed shift in δ^{15}N from regular POM to oysters was only 0.4‰ (Fig. 9). The carbon and nitrogen isotope values of mangrove and seagrass epiphytes indicate that they could not be contributors of organic carbon (Fig. 9). Mangroves and seagrass epiphytes were sampled only in November and more thorough sampling may help in elucidating their relative contribution to oyster diet. Seagrass isotope signatures were too depleted in ^{13}C to be a likely source of organic carbon for oysters (Fig. 9).

C:N ratios for POM and Rain-POM were 7.14 and 5.57, respectively, indicating that Rain-POM was more nutritious that regular POM, (Table 5). The Rain-POM C:N ratio was closest to the ratios for foods used by most animals (<4, Burns and Walker, 2000). The C:N ratio for SPOM (8.5) was similar to values recorded for regular POM samples. Mangroves (79.0), seagrass epiphytes (26.1) and seagrass (23.1) had the highest C:N ratios and were least nutritious of the possible food sources measured (Table 5).

Table 2
Summary of the (a) variability of δ^{13}C and δ^{15}N values within oyster muscle, ctenidia and viscera samples from four sites on the Manning River over four months ($n = 64$) and (b) the results of F-test comparisons of this variability in δ^{13}C and δ^{15}N values between oyster muscle, ctenidia and viscera (where $A > B$ indicates tissue A is more variable than tissue B). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; ns no significant difference

	δ^{13}C	δ^{15}N
(a) Variance component		
Muscle	0.33	0.24
Ctenidia	0.94	0.46
Viscera	1.27	1.11
(b) Comparison of variance components		
Ctenidia > muscle	*** ($F = 2.81$)	** ($F = 1.90$)
Viscera > muscle	*** ($F = 3.81$)	*** ($F = 4.59$)
Viscera > ctenidia	ns ($F = 1.36$)	*** ($F = 2.42$)

4. Discussion

4.1. Isotope signatures of oyster tissues

Oyster tissue δ^{15}N values indicated the localised use of sewage-derived nutrients at the outfall Site 3. This is due to the enriched δ^{15}N values in ammonium and oxidised nitrogen from treated wastewater sources, which can influence entire downstream estuarine food chains (McClelland and Valiela, 1998). Previous studies examining the influence of wastewater inputs to lakes and estuaries have also reported enriched δ^{15}N values of sediments, phytoplankton, macroalgae, invertebrates and fish (Hobbie et al., 1990; Wainright et al., 1996; Hansson et al., 1997; McClelland et al., 1997). The enriched δ^{15}N values recorded for sewage effluent in this study (mean values of 16‰ for SPOM) indicate that it is the only ^{15}N-enriched source that could influence oyster tissue δ^{15}N values.

The enrichment in δ^{15}N of ctenidia and viscera from the outfall site (Site 3) was significantly higher than other sites, indicating that sewage nutrients were being used by oysters at this site. The relatively depleted δ^{15}N values of ctenidia and viscera at sites 5 km upstream and downstream of the outfall indicate that the influence of the Dawson STP effluent is quite

Table 3
Results of analysis of variance (ANOVA) of oysters sampled from four sites on the Manning River over four months. Each site contained two replicate plots from which oysters were sampled. Separate analyses are presented for (a) δ^{13}C and (b) δ^{15}N values for oysters muscle, ctenidia, viscera and whole ($n = 2$) and (c) oyster condition and size ($n = 4$). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns, no significant difference

Source of variance	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P
(a) δ^{13}C values		Muscle			Ctenidia			Viscera			Whole		
Month − MO	3	0.29	1.39	ns	0.99	4.72	*	3.68	4.26	*	0.64	1.21	ns
Site − SI	3	1.55	2.32	ns	6.72	3.24	ns	5.87	22.82	**	5.82	9.42	*
Plot − PL (SI)	4	0.67	2.60	ns	2.08	3.10	*	0.26	0.22	ns	0.61	0.73	ns
MO × SI	9	0.27	1.29	ns	0.45	2.12	ns	0.46	0.53	ns	0.19	0.37	ns
MO × PL (SI)	12	0.21	0.81	ns	0.21	0.31	ns	0.87	0.75	ns	0.52	0.63	ns
Residual	32	0.26			0.67			1.15			0.84		
(b) δ^{15}N values		Muscle			Ctenidia			Viscera			Whole		
Month − MO	3	0.31	1.69	ns	1.31	24.17	***	0.52	0.77	ns	0.04	0.08	ns
Site − SI	3	1.57	5.54	ns	5.22	29.15	**	10.82	54.77	**	6.97	41.69	**
Plot − PL (SI)	4	0.28	2.68	*	0.18	1.07	ns	0.20	0.28	ns	0.17	0.46	ns
MO × SI	9	0.34	1.86	ns	0.33	6.02	**	0.46	0.68	ns	0.54	1.23	ns
MO × PL (SI)	12	0.18	1.73	ns	0.05	0.32	ns	0.68	0.96	ns	0.44	1.20	ns
Residual	32	0.11			0.17			0.71			0.36		
(c) Condition and size		Condition index			Dry weight[a]								
Month − MO	3	23.57	2.30	ns	1.87	12.34	***						
Site − SI	3	26.11	1.88	ns	0.94	4.60	ns						
Plot − PL (SI)	4	13.87	1.12	ns	0.20	1.59	ns						
MO × SI	9	4.07	0.40	ns	0.17	1.10	ns						
MO × PL (SI)	12	10.24	0.83	ns	0.15	1.18	ns						
Residual	96	12.38			0.13								

[a] Denotes data that was ln(x) transformed to meet Cochran's test of homogeneity of variances.

localised and either rapidly diluted to ambient river concentrations or selectively removed by other biological processes.

Observed δ^{13}C values showed an ocean-to-estuarine gradient and distinguished distance of the site from the ocean. The δ^{13}C values of viscera over all months showed significant depletion in ^{13}C from Sites 1–4 with distance upstream (Fig. 3). This trend was also clearly visible in ctenidia tissue within each month (Fig. 3), though not at a significant level. One possibility for this estuarine gradient is changes in the ^{13}C values of primary food sources from an enriched marine-influenced signature (Gearing et al., 1984; Peterson and Fry, 1987) to a depleted terrestrial-dominated signature (Richard et al., 1997; Dittmar et al., 2001), consistent with the mixing of river and ocean waters along this gradient. Previous research on estuarine food webs, however, has shown that consumer dependence on terrestrially derived organic matter is minimal, even in upper areas of the estuary where its availability is highest (Deegan and Garritt, 1997). A more likely explanation for the change in oyster ^{13}C values is the consumption of phytoplankton with differing ^{13}C isotope values reflecting the source of dissolved inorganic carbon (DIC) used for photosynthesis. DIC values typically change along an estuarine gradient, from a depleted signature ($\sim -12‰$) in the upper freshwater end to an enriched signature ($\sim 0‰$) at the marine end, and phytoplankton ^{13}C values have been shown to reflect this change (Deegan and Garritt, 1997). Primary consumers of phytoplankton, such as oysters, are also likely to integrate this varied estuarine ^{13}C signature. Oyster tissues were the most depleted in δ^{13}C at the outfall Site 3 indicating the localised use of sewage-derived nutrients and, therefore, did not conform to this gradient.

4.2. Oyster tissue sensitivity and specificity

The distinct isotope characteristics observed in different oyster tissues may be of importance in the application of tissue-specific isotope analyses of bio-indicators. Viscera showed the greatest temporal and spatial response of the three tissues examined. Viscera was the most sensitive to sewage nutrients, with samples from the outfall site enriched in ^{15}N by an average of 1.5‰, and to the estuarine gradient. However, viscera exhibited the most variation in δ^{13}C and δ^{15}N, not only among sites but also within sites. This variability may in part be because viscera is not one discrete tissue. A large component of the visceral mass in mature oysters is gonad, with the stomach and digestive system comprising the remainder (Beesley et al., 1998). The actual amount of gonad present in viscera can vary substantially based upon time of year, condition of the individual, physical parameters, and both natural and physically induced spawning events. *Saccostrea glomerata* may spawn naturally several times over the course of one summer (Mason and Nell, 1995), but are also known to spawn in response to large, rapid changes in water temperature and salinity. Gonad tissue in oysters can be resorbed by the organism and used as an energy source during period of poor condition. Either of these scenarios during the course of an extended study may produce variable isotopic values and confound results for visceral mass samples. Since viscera constitutes

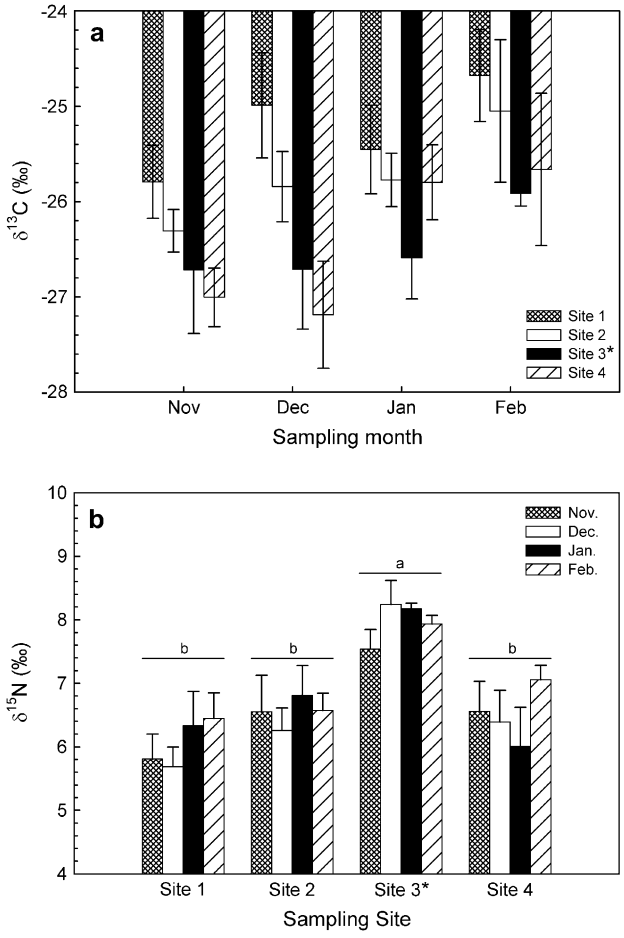

Fig. 4. Ctenidia δ^{15}N values for oysters from four sampling sites on the Manning River over four months. Values represent means ± SE ($n = 4$). Columns with different letters are significantly different according to Tukey's post hoc test ($\alpha = 0.05$). The symbol asterisk (*) indicates location of the sewage outfall.

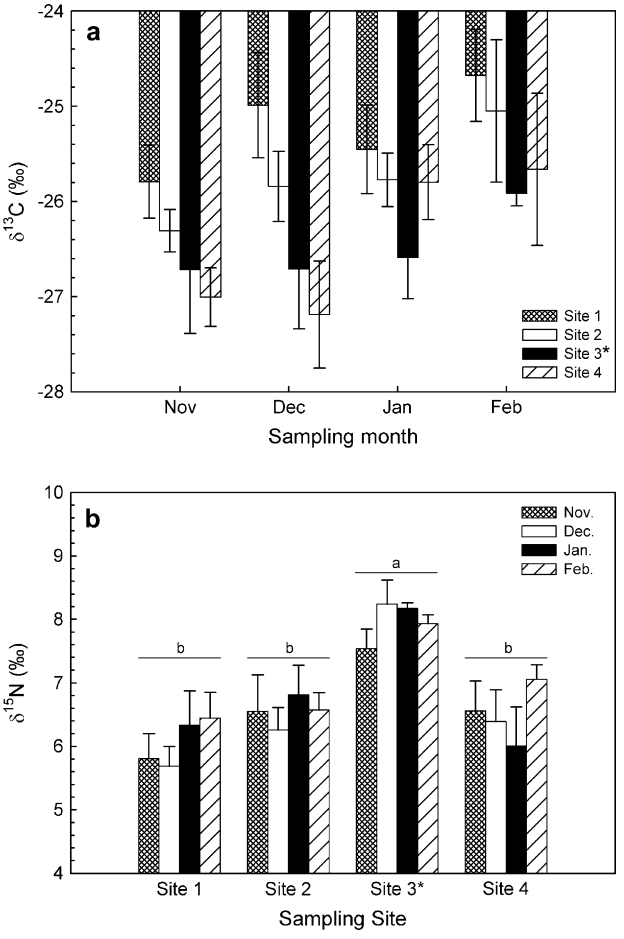

Fig. 5. Viscera (a) δ^{13}C and (b) δ^{15}N values for oysters from four sampling sites on the Manning River over four months. Values represent means ± SE ($n = 4$). Columns with different letters are significantly different according to SNK post hoc test ($\alpha = 0.05$). The symbol asterisk (*) indicates location of the sewage outfall.

75% of the entire *S. glomerata* body mass based on dry weight measurements in this study, the variability in visceral isotope values could mask the results of whole-oyster samples in long-term stable isotope studies.

In contrast to viscera, muscle showed the least temporal and spatial variability in stable isotope values of the three tissues; however, it was also the least responsive tissue showing no significant differences in δ^{13}C or δ^{15}N among sites or months. Unlike ctenidia and viscera, muscle failed to show significant ^{15}N-enrichment at the outfall site compared to other sites. The estuarine gradient in δ^{13}C was also poorly defined for some sampling months. When muscle samples were averaged over the four-month sampling period, the estuarine gradient and the elevated ^{15}N levels in outfall site oysters was apparent but not at any significant level. The isotopically static nature of muscle may be because of its slower turnover rate compared to other tissue types such as viscera. This has been reported for several fish species (Gaston, 2004). The ability of oyster muscle isotope values to behave independently of small-scale temporal and spatial changes may prove useful in long-term studies or for assessments over large spatial scales, such as between estuaries or catchment regions.

Ctenidia ^{13}C and ^{15}N were depleted compared to muscle and enriched compared to viscera. The sensitivity of ctenidia was similar to that of viscera, with the outfall site samples consistently enriched in ^{13}C and ^{15}N for each month, and the estuarine gradient clearly defined for three of the four months. Of the three tissues examined in this study ctenidia exhibited less within site variability in δ^{15}N than viscera. This low variability is likely due to ctenidia being one discrete tissue, resulting in more consistent isotope values and making it the preferred tissue for a study of this type. Tissue specific studies such as this needs further work on the turnover rates and isotopic fractionation values for different oyster tissue types under controlled conditions. This would allow assessments to be made over more highly resolved temporal scales with less intensive sampling effort and with distinct advantages over whole-body analyses.

4.3. Primary sources and potential food sources

POM δ^{13}C values in this study were depleted compared to values found in other estuarine habitats (Ogawa and Ogura,

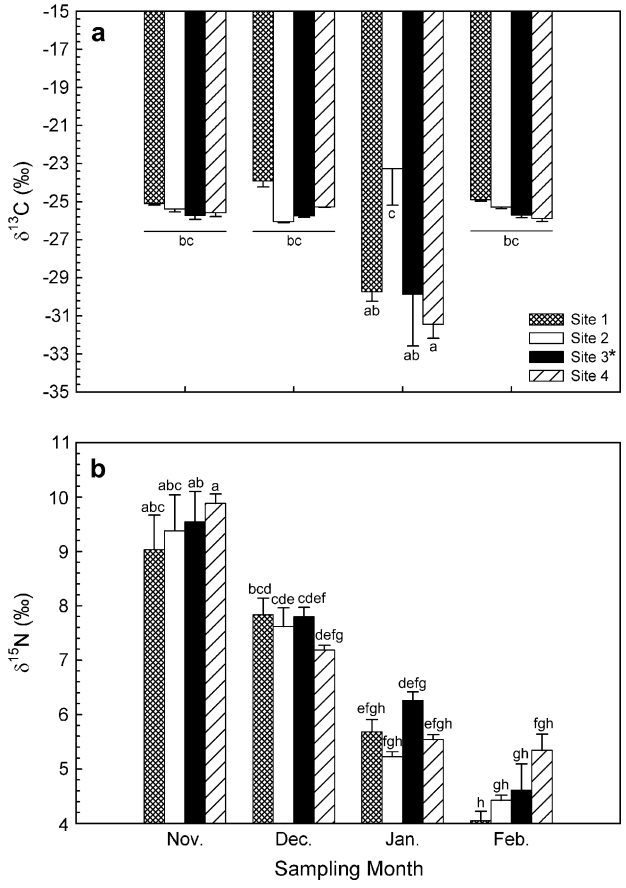

Fig. 6. Particulate organic matter (POM) (a) carbon and (b) nitrogen values from four sampling sites on the Manning River over four months. Columns with different letters are significantly different according to Tukey's post hoc test ($\alpha = 0.05$). Values represent means ± SE ($n = 2$). Approximately 100 mm of rain was recorded on 10 and 12 December 2002. The symbol asterisk (*) indicates location of the sewage outfall.

1997; Sauriau and Kang, 2000; Melville and Connolly, 2003), suggesting that it is largely derived from terrestrial sources (Simenstad and Wissmar, 1985; Riera and Richard, 1996, 1997) and almost certainly in the form of detritus (Haines, 1977; Mann, 1988; Riera, 1998). Oysters are selective filter

Table 4
Results of analysis of variance (ANOVA) of δ^{13}C and δ^{15}N for POM ($n = 2$) sampled from four sites on the Manning River over four months and SPOM ($n = 2$) sampled from the Dawson STP over four months. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; ns, no significant difference

Source of variance	DF	δ^{13}C[a]			δ^{15}N		
		MS	*F*	*P*	MS	*F*	*P*
POM							
Month − MO	3	0.17	12.84	***	37.16	156.04	***
Site − SI	3	0.06	4.61	*	0.36	1.50	ns
MO × SI	9	0.07	4.94	**	0.34	1.41	ns
Residual	32	0.01			0.24		
SPOM							
Month	3	3.14	5.69	ns	99.54	953.47	***
Residual	4	0.55			0.013		

[a] Denotes data that did not meet Cochran's test ($P < 0.05$) for homogeneity of variances after transformation.

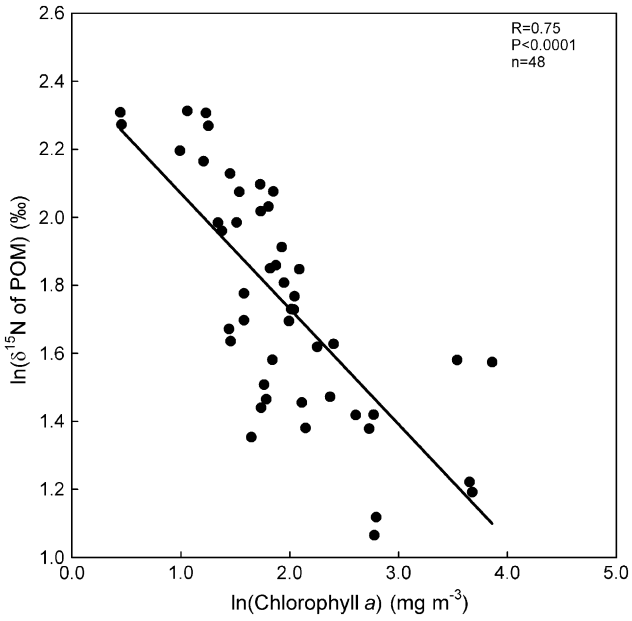

Fig. 7. Correlation between ln ^{15}N isotope composition of particulate organic matter (POM) and ln chlorophyll *a* content in water samples from four sampling sites on the Manning River over four months ($r = 0.75$, $P < 0.01$, $n = 24$). Values represent means ± SE ($n = 2$).

feeders able to select food based upon size, quality and quantity (Loret et al., 2000) and have been shown to target certain organic matter components of suspended POM (Newell and Jordan, 1983; Riera and Richard, 1996). Phytoplankton is assumed to be the main food source within POM for bivalves (Smaael and van Stralen, 1999) in addition to labile detritus (e.g. seagrass and mangrove, van der Enden, 1994) and microphytobenthos (Webster et al., 2002). The δ^{13}C of POM in this study was within the isotopic range to be a major food source. There was no corresponding 1–5‰ enrichment in ctenidia ^{15}N over POM ^{15}N as expected from primary source to primary consumer (Peterson and Fry, 1987). Oysters may be targeting the phytoplankton component within POM, with the ^{15}N signature of this phytoplankton component masked by the large

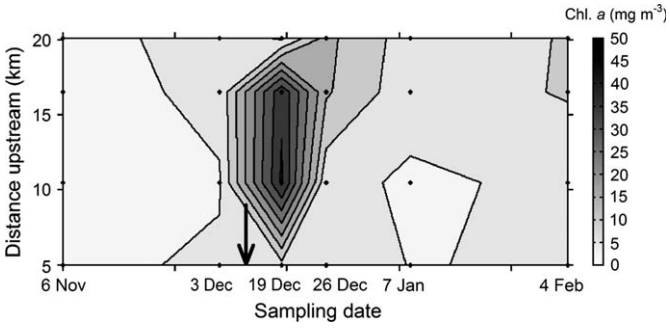

Fig. 8. Contour plot showing chlorophyll *a* concentrations in the Manning River estuary based on water samples from four sites sampled over four months. The 19 and 26 December sampling dates occurred one and two weeks after the 10 and 12 December rainfall event (arrow), respectively. Dots within plot area indicate the sampling sites (1–4) sampled at each time period, with Site 1 the furthest downstream (5 km from estuary entrance) and Site 4 furthest upstream (20 km from estuary entrance).

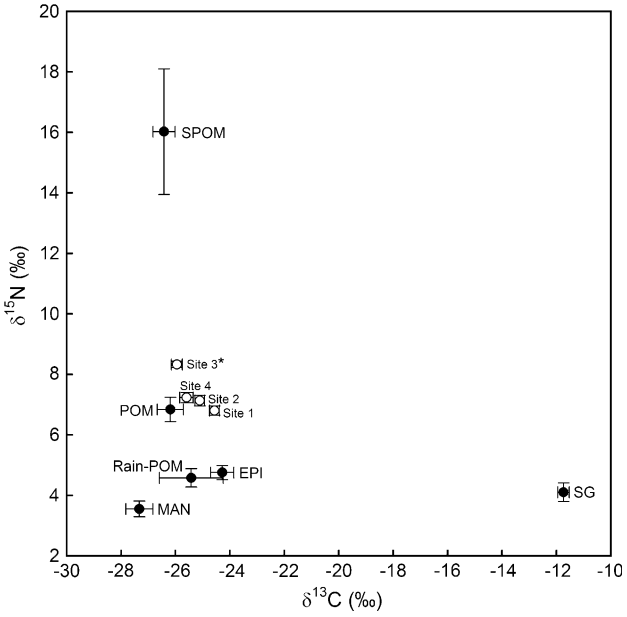

Fig. 9. Comparison of carbon and nitrogen isotope values for whole oysters (open symbols) and primary sources (closed symbols) from sampling sites on the Manning River over four months (sewage particulate organic matter – SPOM; particulate organic matter – POM; rainfall event particulate organic matter – Rain-POM; mangroves – MAN; seagrass epiphytes – EPI; seagrass – SG). Values represent means ± SE ($n = 16$ for oysters; $n = 8$ for POM and SPOM; $n = 3$ for mangroves, epiphytes and seagrass). The symbol asterisk (*) indicates location of the sewage outfall.

quantities of detrital matter. Rain-POM observed one and two weeks after the December rainfall event showed chl *a* concentrations up to 45 mg m^{-3}, indicating a significant increase in phytoplankton biomass. This observed increase is strongly correlated with a decrease in $\delta^{15}N$ values for POM observed at this time, suggesting that the $\delta^{15}N$ values of the phytoplankton component of the POM is masked by detrital matter and only apparent at high concentrations. Consequently we see the predicted 1–5‰ enrichment in oyster tissue ^{15}N over Rain-POM ^{15}N, indicating that the phytoplankton component within the POM is within the correct isotopic range of a primary food source.

The relatively lower C:N ratio of Rain-POM compared to regular POM also suggests that it is more nutritious due to the increased component of phytoplankton biomass. This further supports the hypothesis that an isotopically ''hidden'' phytoplankton component exists within the POM pool, and it is this component that oysters are deriving most of their nutrient requirements from.

Table 5
Mean C:N ratios of potential food sources sampled from sites on the Manning River over four months. Sources are particulate organic matter (POM, $n = 32$), post-rainfall event particulate organic matter (Rain-POM, $n = 16$), sewage particulate organic matter (SPOM, $n = 8$), mangrove leaves (*Avicennia marina*, $n = 3$), seagrass epiphytes ($n = 3$) and seagrass (*Zostera capricorni*, $n = 3$)

Primary source	Ratio	SE
POM	7.1	0.2
Rain-POM	5.6	0.1
SPOM	8.5	0.4
Mangroves	79.0	6.6
Seagrass epiphytes	26.1	1.4
Seagrass	23.1	0.6

The masking of $\delta^{15}N$ values of the phytoplankton component of dry weather POM by detrital matter is consistent with the lack of enrichment of POM from the outfall Site 3. Benthic and pelagic algae will take up ^{15}N-rich wastewater nitrogen, such as that in tertiary treated sewage effluent, and become enriched themselves (Fry, 1999). Outfall site oysters may be selectively feeding upon an ^{15}N-enriched phytoplankton component at this site that is not reflected in the $\delta^{15}N$ signature of POM. This finding is exciting in that it raises the possibility of using the isotope values of bivalves, such as oysters, to identify anthropogenic nitrogen used by phytoplankton from an otherwise noisy background of particulates. Masking of phytoplankton isotopic signatures by excessive detritus would also explain the lack of an estuarine gradient in $\delta^{13}C$ values of POM. It is possible, however, that detrital matter forms some minor component of Manning River oyster diet with the transfer of carbon from detritus being facilitated by bacterial decay (Crosby et al., 1990; Langdon and Newell, 1990).

The ^{15}N-enrichment observed in outfall Site 3 oysters compared to oysters from other sites may be due to the uptake of sewage particulates directly from the effluent. Yet this is unlikely since sewage effluent was observed to contain few particulates during the sample filtration process (pers. obs.). Further, if there were large amounts of sewage particulates available to oysters at the outfall site, the $\delta^{13}C$ and $\delta^{15}N$ values of estuarine POM would reflect this and be more similar to that of SPOM.

Integumental uptake of dissolved organic and inorganic matter is known to occur among soft-bodied animals (Wright and Manahan, 1989). Another mechanism for the consistently elevated $\delta^{15}N$ in oyster tissues at the outfall site is the direct uptake of ^{15}N-enriched dissolved nutrients. While the integumental uptake of nutrients has been reported for invertebrate larvae (Manahan, 1983, 1989; Silva and Wright, 1992) it has been shown to contribute only a minor fraction of the nitrogen and energy demands in adult invertebrates such as giant clams (Ambariyanto and Hoegh-Guldberg, 1999). Therefore, it is an unlikely mechanism for $\delta^{15}N$ enrichment of adult oysters.

The depleted $\delta^{13}C$ and $\delta^{15}N$ values for mangroves in this study indicate that they are within the isotopic range to be contributors of detrital matter to the POM pool, though the high C:N ratio for mangroves (79.0) suggests it would have very little nutritional value for oysters. Seagrass epiphytes are another possible source of detrital carbon (−24.3‰), though the relatively small amount of seagrass beds within the estuary (0.33 km^2, GTCC, 1997) and the presence of seagrass beds at only one sampling site (Site 2) suggest that their contribution would be minor. Seagrass detritus has been shown to be an important source of organic matter in coastal ecosystems (Fry et al., 1987), but the very high $\delta^{13}C$ values for seagrasses sampled in this study (−11.7‰) were quite distinct from other potential food sources and appear too high relative to the $\delta^{13}C$

of oysters from all sites to make any notable contribution to oyster diet, even at Site 2.

4.4. Summary

The dual C–N isotope signature of *Saccostrea glomerata* muscle, ctenidia and viscera were clearly distinguished from one another for each site. While all tissues were able to define the presence of sewage nutrients and an ocean-to-estuarine gradient, ctenidia and viscera were more sensitive than muscle in elucidating this nutrient source over the time-scale of this study. Viscera, however, showed large with-in sample variability due to its multi-tissue composition, making it problematic for this type of study and possibly confounding the results of whole-oyster analyses. Ctenidia and muscle of *S. glomerata* appear useful indicators of sewage effluent over small and large spatio-temporal scales, respectively. This study also highlights the utility of farmed oysters as indicators of both point and non-point source inputs of anthropogenic nitrogen, with their easy deployment to specific locations reducing the common problem associated with the use of biotic indicators of being unable to find suitable organisms in desired study locations.

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