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Spatial variation in $\delta^{13}C$ and $\delta^{15}N$ of liver, muscle and bone in a rocky reef planktivorous fish: the relative contribution of sewage

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Abstract

Carbon and nitrogen stable isotope ratios in the liver, muscle and bone of a rocky reef planktivorous fish (*Atypichthys strigatus*) were compared amongst sewage impacted and non-impacted regions off eastern Australia. Fish were sampled in summer and autumn over 2 years from replicate sites across the three regions. Liver and muscle ^{15}N ratios, representing short- (weekly) and long-term (yearly) nutrient integration, were depleted within the impacted region (10.0‰ and 12.0‰, respectively) relative to both non-impacted regions (11.4‰ and 13.6‰, respectively), consistent with the lighter ^{15}N composition of primary treated sewage effluent (0.4‰), compared to marine derived particulate organic matter (POM, 8.2‰). Bone with the slowest turnover and longest integration time exhibited the least difference in ^{15}N among regions (generally <1‰). Within fish variation in stable isotope ratios was found to differ among tissue types and between elements. Bone showed the least variation (5%), whereas liver had the greatest variation (29%). Up to 70% of the variance was observed amongst regions in almost all tissue/stable isotope combinations. With adequate replication at several spatial scales, small sample sizes had sufficient power to detect significant ecological variation. Compared to the massive but sporadic upwelling events, we find that the comparatively small yet continuous discharge of sewage off Sydney is an important nutrient source for planktivorous fish.

Keywords: Spatial variability; Stable isotopes; Planktivorous fish; Mado; *Atypichthys strigatus*; Sewage; Food chain dynamics

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1. Introduction

Sewage derived nutrients may be assimilated by marine organisms, as revealed by the naturally occurring stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$, (Hansson et al., 1997; Spies et al., 1989; van Dover et al., 1992). For example, the stable isotopic composition of muscle, established that fish could be supported by different trophic pathways (Rau et al., 1992; Sholto-Douglas et al., 1991; Thomas and Cahoon, 1993). Analysis of different tissues has the advantage of revealing the time scale of assimilating a new nutrient source (Hobson and Clark, 1992). The slower turnover rate of stable isotope ratios in bone collagen (Schoeninger and De Niro, 1984; Sholto-Douglas et al., 1991) and of muscle provides a long-term dietary indicator (Hesslein et al., 1993; Tieszen et al., 1983), compared to fish liver (Hesslein et al., 1993).

Off Sydney, over 1200 ML of effluent is discharged per day via three deepwater ocean outfalls (MHL, 1997). In contrast to the relatively continuous flow of sewage discharge, upwelled sources of nutrients are mainly episodic, occurring only 2–3 times per summer and are comparatively massive (Middleton et al., 1996). The continual flow of ocean outfalls provides the principal source of ammonia to local waters (Pritchard et al., 1997), while the intrusion of nutrient rich slope water is the prime source of nitrates (Rochford, 1984). The relative contribution of sewage and upwelling to the assimilated nutrients of the planktivorous food chain could be revealed by tissue-specific stable isotope analyses.

The interpretation of these ratios relies on the assumption that the isotopic composition of the animal reflects that of its diet (Gannes et al., 1997). Trophic enrichment factors, from diet to tissue, for carbon and nitrogen have been well established and applied in many investigations (De Niro and Epstein, 1978, 1981; Minagawa and Wada, 1984; Rau et al., 1983), but these enrichment factors are species-specific and possibly tissue-specific. Gannes et al. (1997) argue that laboratory determined, organism- and tissue-specific, trophic enrichment factors are necessary to interpret stable isotope data from the field.

Early studies of stable isotope composition of marine organisms were based on relatively few samples with little replication in space or time (Fry and Sherr, 1984; Owens, 1987). Within-species variation was apparent at almost all scales of investigation (Fry and Sherr, 1984; Hemminga and Mateo, 1996), and with low levels of replication, ecologically significant variation was possibly masked. Many inshore reef fish species may switch their trophic position in response to local conditions, thus without spatial replication on a number of different scales it would be difficult to infer the nutrient sources of reef fishes (Jennings et al., 1997). Biological variation and machine error in isotope ratio mass spectrometry (IRMS) are the two main sources of variation of the mean isotope value of a population (Lancaster and Waldron, 2001). Spatial differences in the $\delta^{13}C$ and $\delta^{15}N$ composition of reef fishes on spatial scales of tens of kilometers has been demonstrated (Thomas and Cahoon, 1993), but there has been no attempt to compare variability among individuals on smaller scales (Jennings et al., 1997).

In the present study, carbon and nitrogen stable isotope analysis of the zooplanktivorous mado (*Atypichthys strigatus* Günther) is used to examine the variability at several spatial scales in regions impacted and non-impacted by anthropogenic nutrients.

Juvenile mado are an abundant, non-migratory schooling species occurring on local rocky-reefs, and may be an ecologically important species due to their high abundance (Glasby and Kingsford, 1994). We compared the stable isotope composition of liver, muscle and bone tissue of mado, as short- and long-term integrators of their nutrient source, which was indicated by stable isotope analysis of marine-derived and sewage-derived particulate organic matter (POM). If sewage effluent is a significant contributor to the coastal food chain compared to sporadic upwelling, then we predict that the tissues of fish from impacted regions to have isotope ratios consistent with that of sewage effluent. Trophic enrichment factors were determined for C and N of each tissue by laboratory rearing.

2. Methods

2.1. Study area and sampling design

Mado samples for this study were collected from three regions (Port Stephens, Sydney and Jervis Bay) along the New South Wales (NSW) coast of southeastern Australia (Fig. 1). The East Australian Current (EAC) and local winds influence the vertical stratification and upwelling intensity as well as dispersion of sewage effluent discharged (Lee and Pritchard, 1996). No major upwelling events were documented within 2 weeks prior to our sampling, as determined by inspection of ocean temperature data from the Ocean Reference Station (ORS), which is moored 3 km off the coast of Sydney. Sydney is served by three deepwater ocean outfalls (North Head, Bondi and Malabar; Fig. 1) that discharge approximately 1000 ML day^{-1} of primary treated sewage effluent 3–4 km offshore from multiport diffusers in 60–80 m of water (MHL, 1997; Pritchard et al., 1997; Pritchard et al., 1996). Another local, subtidal primary treated sewage outfall remains at Potter Point (Fig. 1) that discharges 46 ML day^{-1} (MHL, 1997).

We sampled two sites within each of the three regions: Region 1 (non-impacted: Jervis Bay—Plantation Point and Bowen Island), Region 2 (Sydney—Potter Point and Gordons Bay) and Region 3 (non-impacted: Port Stephens—Little Island and Boondelbah Island, Fig. 1). All sites were sampled once in summer and once in autumn of 1997. This was repeated in 1998. Fish were collected with SCUBA, using a multi-prong hand spear, and placed on ice before being stored in a laboratory freezer.

Marine particulate organic matter (POM, mainly phytoplankton particles, 0.2–150 μm) was collected from water samples during the second year of sampling (1998; once in summer and once in autumn). Water samples were collected using 5 l Niskin bottles from 2 m above the bottom, pre-filtered through a 150-μm-mesh sieve, and collected on Whatman GF/F glass fibre filters under moderate vacuum then stored in a laboratory freezer. Final effluent from four major outfalls within the impacted region (Fig. 1) was also collected, prior to discharge, during the second year of sampling at monthly intervals. Sewage particulate organic matter (SPOM) was collected on Whatman GF/F glass fibre filters under moderate vacuum then stored in a laboratory freezer before

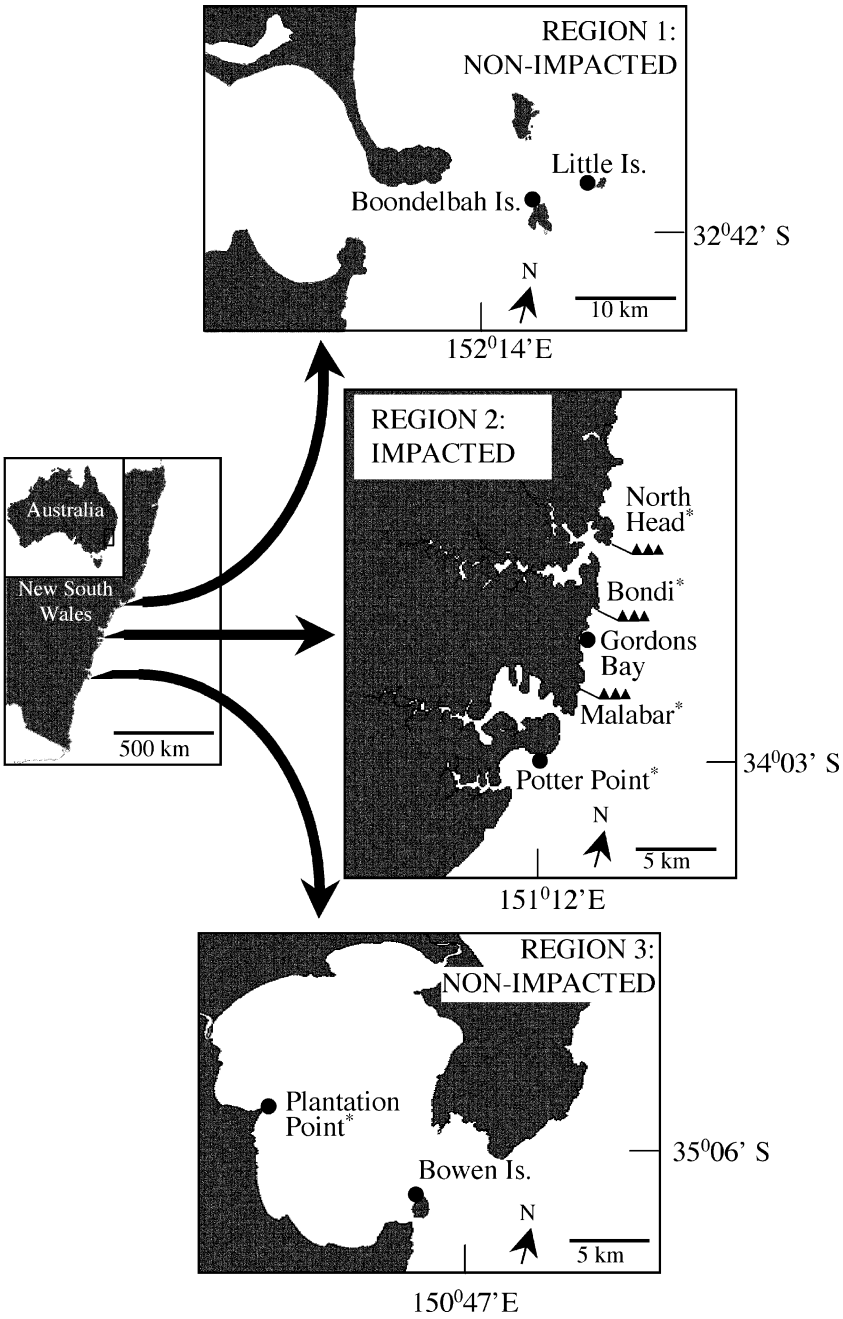

Fig. 1. Map showing locations of sampling sites within the three regions: Region 1: Non-impacted (Port Stephens), Region 2: Impacted (Sydney) and Region 3: Non-impacted (Jervis Bay). * indicates the location of a sewage outfall, and (↘▲▲▲) indicates the location of a deepwater ocean outfall.

Table 1
Mean within site coefficient of variation (CV, %) for $\delta^{13}C$ and $\delta^{15}N$ composition of liver, muscle and bone in mado (*A. strigatus*) from each region and all regions pooled

Region[a]	Liver		Muscle		Bone	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
1	3.1	6.0	1.4	3.9	2.7	6.0
2*	3.2	3.8	2.1	2.6	5.5	4.5
3	2.9	3.0	1.8	2.5	5.4	3.4
Pooled	3.1	4.3	1.8	3.0	4.5	4.6

Region 2* receives >1000 ML day^{-1} of primary treated sewage.
[a] Region 1 = Port Stephens (non-impacted), Region 2 = Sydney (impacted), Region 3 = Jervis bay (non-impacted).

analysis. For SPOM samples we used 200 ml of final effluent. The filtrate was collected (70 ml), representing sewage dissolved organic matter (SDOM), and stored in a laboratory freezer before analysis.

2.2. Laboratory study of trophic enrichment

Mado were caught using handheld nets from one location in the summer of 1997 and reared on a diet of commercial flake food for 12 months. The diet had a stable carbon and nitrogen composition of -24.2 ± 0.1 ‰ and 7.1 ± 0.1 ‰, respectively (values are mean ± S.E. for samples taken at 6 intervals during the experiment, total $n = 18$). Fish were reared at a density of 4 mg l^{-1} in 40 l tanks with a continuous flow of filtered (30 μm) seawater (4 l min^{-1}; 34.9 ± 0.1 ‰ salinity; 20.2 ± 0.3 °C). Fish were fed to satiation three times a day, and their standard length (SL, mm) and wet weight (g) measured at the beginning and end of the experiment. Fish were removed from their tanks, sacrificed in ice water, then frozen until analysis.

2.3. Stable isotope analysis

Fish were thawed and a piece of white dorsal muscle, liver and bone (vertebrae) was excised from three replicate fish at each site. Bone was only sampled in the first year of sampling (1997) and sub-samples of all tissue were analysed only during the first year (1997). Tissue samples were freeze-dried, ground to a fine powder using a mortar and pestle, and 1.2 to 1.5 mg of the sample sealed into a tin capsule. POM and SPOM were scraped from the glass fibre filter, freeze-dried and 1.5–1.7 mg of the particulate material sealed into a tin capsule. SDOM were freeze-dried, ground to a fine powder using a mortar and pestle, and 1.2 to 1.5 mg of the sample sealed into a tin capsule. Stable isotope analysis on each capsule was performed at the CSIRO Land and Water Adelaide Laboratory on an Automated Nitrogen Carbon Analysis (ANCA)-Mass Spectrometer (20-20 Europa Scientific). Capsules were combusted and the reaction products separated by GC (Gas Chromatography) to give pulses of pure CO_2 and N_2 for analysis of total C and N, and $\delta^{13}C$ and $\delta^{15}N$. Isotope values were expressed in del (δ) notation, $\delta^{13}C$ and $\delta^{15}N$, relative to international standards (Pee

Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). Delta values were determined as follows:

$$\delta X(‰) = \left[\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1\right] \times 1000$$

where $X = {}^{13}C$ or ${}^{15}N$ and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, respectively. The repeatability in $\delta^{13}C$ and $\delta^{15}N$ values was $\pm 0.2‰$ for ${}^{13}C$ and ${}^{15}N$, respectively.

2.4. Statistical analysis

Data were tested for homogeneity of variance using Cochran's test, and were successfully transformed to log $(x+1)$ if variances were heterogeneous, before using analysis of variance (ANOVA). Variation in stable isotope ratios at spatial scales, for the three tissue types, was contrasted by calculating the variance components of the mean squared estimates from a four-way nested ANOVA; among date (two sampling times in only 1997 for all three tissues), region (three regions), site (two sites, nested within region) and fish (three fish, nested within site, region and date), with $n = 2$ subsamples. Mean square estimates were determined by construction of analysis from general principles.

A three-way nested ANOVA was used to compare the $\delta^{13}C$ and $\delta^{15}N$ value of each tissue (liver and muscle), among date (four sampling times over both years),

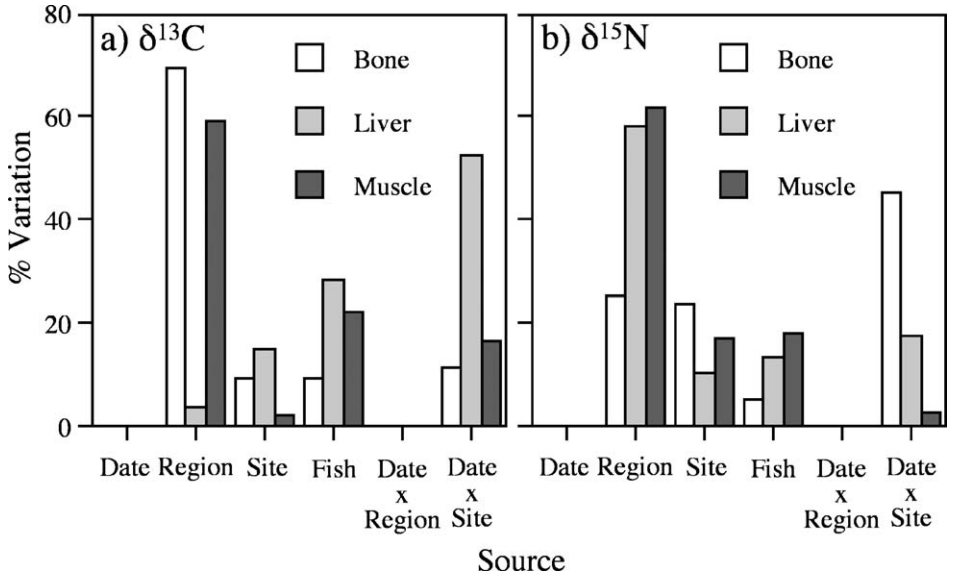

Fig. 2. Variance components for within fish, site, region and date × region interaction from bone, liver and muscle for (a) $\delta^{13}C$ and (b) $\delta^{15}N$. Variance components were determined from the mean square estimates from a four-way nested ANOVA performed on the 1997 data. Replicate samples of each tissue, from each fish, were only collected during 1997.

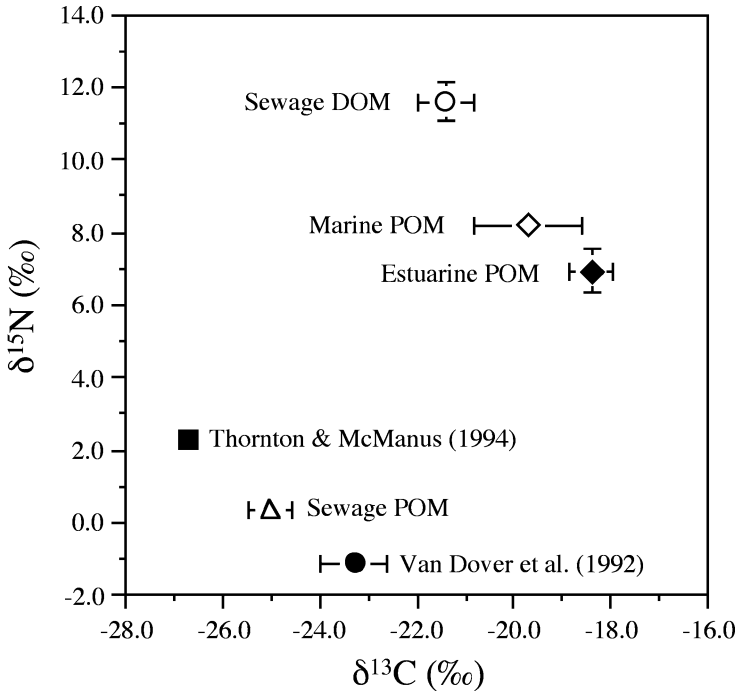

Fig. 3. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope composition of marine and sewage derived particulate organic matter (POM) sampled in 1998. The sewage POM is derived from the final, primary treated effluent of four Sydney outfalls. The marine POM is oceanic samples taken from each region. The solid diamond represents estuarine POM collected from an estuary in Region 1 during 1998 (Gaston, 2003). The solid square and circle represent stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values from primary treated effluent as determined by Thornton and McManus (1994) and van Dover et al. (1992), respectively. Values are mean ± S.E.

region (three regions) and site (two sites, nested within region). A three-way nested ANOVA was used to compare the δ^{13}C and δ^{15}N value of bone among date (two sampling times in 1997), region (three regions) and site (two sites, nested within region), with $n = 3$ fish. Means between dates and means between sites were compared using Student–Newman–Keuls (SNK) tests for all ANOVAs. All ANOVAs were

Table 2
Summary of analysis of variance of (a) δ^{13}C and (b) δ^{15}N isotope composition of SPOM in final effluent collected from Sydney's four (4) major STPs

Source of variation	SS	*df*	MS	*F*	p[a]
(a) $\delta^{13}C$					
STP (S)	10.59	3	3.53	0.62	ns
Residual	866.23	152	5.70		
(b) $\delta^{15}N$					
STP (S)	4.70	3	1.58	0.57	ns
Residual	415.49	152	2.73		

[a] ns, $p>0.05$; *$p<0.05$; **$p<0.01$.

Table 3
The percent (%) carbon and nitrogen composition and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition of sewage particulate organic matter (SPOM) and sewage dissolved organic matter (SDOM) of final effluent from Sydney's four major sewage treatment plants (STPs)

Fraction	Carbon		Nitrogen	
	$\delta^{13}C$ (‰)	%C	$\delta^{15}N$ (‰)	%N
SPOM	−25.0 ± 0.5	71.0 ± 0.4	0.4 ± 0.4	71.0 ± 0.2
SDOM	−21.4 ± 0.6	29.0 ± 1.0	11.6 ± 0.5	29.0 ± 0.2

Values are mean ± S.E.

performed using the GMAV5 for Windows program (Underwood et al., 1998). Power analysis was performed using the GPOWER for MS-DOS program (Faul and Erdfelder, 1992).

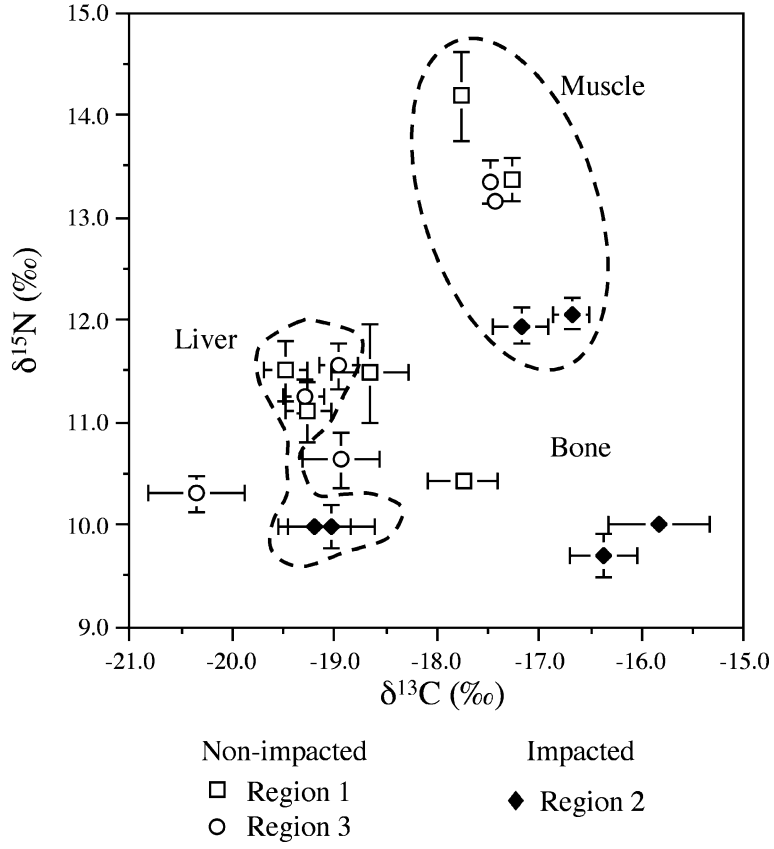

Fig. 4. Average $\delta^{13}C$ versus $\delta^{15}N$ for all sampling occasions of liver, muscle and bone (1997 only) from mado (*A. strigatus*) sampled from impacted (Region 2, closed diamonds) and non-impacted (Region 1 and 3, open squares and circles, respectively) regions. Values are mean ± se ($n = 12$) for liver and muscle and ($n = 6$) for bone.

3. Results

3.1. Relative variability in liver, muscle and bone

The mean coefficient of variation (CV) of stable isotope ratios within sites ($n = 3$ fish) varied among tissues and between C and N (Table 1). CVs of both stable isotopes were lower for muscle than liver or bone. Most CVs were < 5% and for muscle they were < 3%. The percentage variance component at different spatial scales also varied for different tissues and stable isotopes. Bone had the lowest within fish variance for both δ^{13}C (10%) and δ^{15}N (5%, Fig. 2). Liver had the greatest within fish variance for δ^{13}C (29%, Fig. 2a) and muscle tissue had the greatest for δ^{15}N (18%, Fig. 2b). The variance component among regions for δ^{13}C was the greatest for muscle (59%) and bone (70%, Fig. 2a). Among region variance component for δ^{15}N was the greatest for liver (58%) and muscle (62%, Fig. 2b). The within date and date x region interaction components did not contribute to the variance of this model (Fig. 2). This fully nested experimental design had high statistical power (β>0.95) for observing inter-site and inter-region differences in the stable isotope composition of all tissues.

3.2. Stable isotope analysis of particulate organic matter (POM)

The mean δ^{15}N and δ^{13}C of marine POM was 8.2‰ and −19.7‰, respectively, whereas primary treated sewage POM was depleted in both isotope ratios relative to

Table 4
Summary of analysis of variance of (a) δ^{13}C and (b) δ^{15}N isotope composition from replicated subsamples of liver, muscle and bone in mado (*A. strigatus*) from all dates ($n = 4$), regions ($n = 3$) and sites ($n = 2$, nested within region)

Source of variation	Liver					Muscle					Bone				
	SS	*df*	MS	*F*	p[a]	SS	*df*	MS	*F*	p[a]	SS	*df*	MS	*F*	p[a]
(a)$\delta^{13}C$															
Date (D)	12.9	3	4.3	3.52	ns	2.6	3	0.9	2.25	ns	1.2	1	1.2	0.56	ns
Region (R)	1.0	2	0.5	1.26	ns	3.8	2	1.9	2.46	ns	75.8	2	37.9	12.14	*
Site (Region) (S(R))	1.2	3	0.4	0.90	ns	2.3	3	0.8	5.87	**	9.4	3	3.1	3.88	*
D × R	15.3	6	2.6	2.09	ns	8.7	6	1.5	3.81	*	1.9	2	1.0	0.43	ns
D × S(R)	11.0	9	1.2	2.71	**	3.4	9	0.4	2.91	**	6.7	3	2.2	2.78	ns
Residual	21.6	48	0.5			6.3	48	0.1			19.3	24	0.8		
(b)$\delta^{15}N$															
Date (D)	9.2	3	3.1	2.69	ns	21.6	3	7.2	10.33	**	0.1	1	0.1	0.05	ns
Region (R)	30.1	2	15.0	28.98	*	40.7	2	20.4	14.55	*	7.2	2	3.6	2.76	ns
Site (Region) (S(R))	1.6	3	0.5	1.62	ns	4.2	3	1.4	5.64	**	3.9	3	1.3	3.78	*
D × R	6.8	6	1.1	1.00	ns	6.2	6	1.0	1.48	ns	0.7	2	0.3	0.28	ns
D × S(R)	10.2	9	1.1	3.55	**	6.3	9	0.7	2.81	**	3.6	3	1.2	3.56	*
Residual	15.3	48	0.3			11.9	48	0.3			8.1	24	0.3		

[a] ns, p>0.05; *p < 0.05; **p < 0.01.

marine POM (0.4 ‰ and −25.0 ‰, respectively; Fig. 3). The $\delta^{13}C$ and $\delta^{15}N$ of sewage POM did not vary among the four primary treated outfalls over 12 months (ANOVA, $p>0.05$; Table 2). The filtrate (DOM) from the preparation of SPOM had an enriched $\delta^{13}C$ and $\delta^{15}N$ composition of -21.4 ± 0.6 ‰ and 11.6 ± 0.5 ‰, respectively (Table 3, Fig. 3). We found >70% of the total C and N was particulate rather than the filtrate (Table 3). Marine POM varied significantly within regions but not among regions (ANOVA, $p>0.05$; Fig. 3). To examine the possible effect of local Sydney estuaries, we found that estuarine POM overlapped with the stable isotope values for marine POM (Fig. 3; Gaston, 2003).

3.3. Stable isotope analysis of fish tissues

Fish liver from the impacted region (Region 2, 10.0 ± 0.3 ‰) was significantly depleted in ^{15}N (as was primary treated sewage effluent) relative to fish from the non-impacted regions, Region 1 (11.4 ± 0.1 ‰) and Region 3 (11.3 ± 0.2 ‰) across all sampling times (Fig. 4, Table 4; ANOVA $p<0.05$). Muscle tissue of fish from within the impacted region (Region 2, 12.0 ± 0.1 ‰) was significantly depleted in ^{15}N (as was primary treated sewage effluent) relative to fish from Region 1 (13.3 ± 0.1 ‰) and Region 3 (13.8 ± 0.3 ‰) across all sampling times (Fig. 4, Table 4; ANOVA $p<0.05$). The ^{13}C composition of bone was significantly enriched in fish from the impacted region

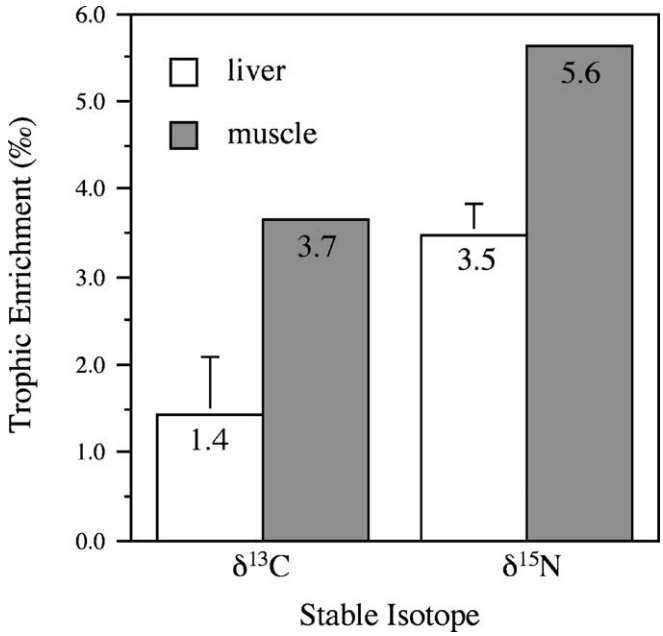

Fig. 5. Trophic enrichment (‰) for carbon and nitrogen stable isotope composition of liver and muscle of mado (*A. strigatus*) reared on a single diet for 12 months. Values are mean ± S.E. Note the increased trophic enrichment in muscle for both carbon and nitrogen isotope composition greater than the reported values of <1 ‰ and 3–4 ‰, respectively.

(Region 2, -16.1 ± 0.3 ‰) relative to one of the non-impacted regions (Region 3: -19.6 ± 0.4 ‰; Fig. 4, Table 4; ANOVA $p < 0.05$).

Trophic enrichment between diet and fish tissue was different for muscle and liver of fish reared on the control diet for 12 months. Between diet and fish there was an increase in the δ^{15}N value of muscle and liver of 5.6 ‰ and 3.5 ‰, respectively (Fig. 5). The δ^{13}C value of muscle and liver increased by 3.7 ‰ and 1.4 ‰, respectively, over diet (Fig. 5).

4. Discussion

Both liver and muscle of fish from within the impacted region were depleted in ^{15}N relative to fish from the non-impacted regions, which was consistent with the lighter isotopic composition of particulate organic matter (POM) in primary treated sewage effluent compared to marine POM. Our finding is surprising considering the dynamic coastal waters off eastern Australia and the episodic intrusion of slope water (Middleton et al., 1996). To make our assessment of sewage nutrients we needed to characterise the stable isotope sources and determine the trophic enrichment from diet to fish tissue. We then paid particular attention to partition the tissue-specific variability in stable isotope ratios in a marine fish over a range of spatial and temporal scales.

4.1. Nutrient sources

Marine POM was significantly enriched in both carbon and nitrogen stable isotopes with respect to sewage POM (SPOM), thus providing a characteristic isotope signature. The δ^{15}N value of marine POM in this study are consistent with reported values of POM found in other marine environments (Mariotti et al., 1984; Rau et al., 1991; Riera, 1998). The δ^{13}C value spans the range typical of that of phytoplankton in temperate seas (Fontugne and Duplessy, 1981; Gearing et al., 1984). Similarly, the δ^{13}C and δ^{15}N value of primary treated SPOM discharged within the impacted region are within the range reported for other studies (Burnett and Schaeffer, 1980; Sweeney et al., 1980; Thornton and McManus, 1994; van Dover et al., 1992). The stable carbon and nitrogen isotope ratios of marine and sewage POM off southern California were found to differ by 2 ‰ and 8 ‰, respectively (Rau et al., 1981). Therefore marine and sewage POM were distinct enough to be conserved higher up the food chain resulting in site differences among invertebrates and fish. The stable carbon and nitrogen isotope ratios of marine and sewage particulate organic matter found in this study (Fig. 3) were also distinct enough to assess their relative influence to the coastal food chain.

We were unable to characterise the dilute marine DOM, but we found the SDOM of primary treated sewage was ^{15}N enriched, similar to that of secondary and tertiary treated sewage (Gartner et al., 2002), due to the relative volatilisation of $^{14}NH_3$:$^{15}NH_3$. In the ocean, DOM is the largest reservoir of organic matter and usually predominates over POM (Sharp, 1973), while POM is predominantly more important in coastal waters where highest productivity is generally encountered (Bodineau et al., 1999). Therefore we assumed that the nutritional importance of SPOM is greater than SDOM, coupled with

the fact that >70% of the C and N in sewage effluent was particulate. We acknowledge that the actual pathway of ^{15}N from SPOM or SDOM, via bacteria, phytoplankton, zooplankton is unknown, yet it would seem from our fish tissue results that SPOM is a major source, consistent with other studies (e.g. Hansson et al., 1997).

Potential contamination of the two nutrient signatures from the Sydney estuaries is unlikely, since estuaries contribute minimal loads of nutrients to the coast (Pritchard et al., 1997). POM sampled from within a Sydney estuary revealed an enriched $\delta^{13}C$ of $-18.6‰$ and $\delta^{15}N$ of 7.3‰ (Gaston, 2003), which is similar to that of marine POM. Hence, the stable carbon and nitrogen isotope ratios of primary treated sewage POM represent a characteristic signature that can be traced in an otherwise marine dominated system.

4.2. Trophic enrichment

Our interpretation of stable isotope ratios relies on the assumption that the isotopic composition of fish tissue reflects that of its diet (Gannes et al., 1997). Trophic enrichment factors for carbon and nitrogen have been well established and applied in many investigations (De Niro and Epstein, 1978, 1981; Minagawa and Wada, 1984; Rau et al., 1983). Our results show that enrichment factors are species-specific and possibly tissue-specific. From our laboratory study we found a greater enrichment of muscle over liver (Fig. 5). The accepted trophic enrichment factor of 3–4‰ for ^{15}N per trophic level (Minagawa and Wada, 1984) holds true for liver, but muscle tissue has an enrichment factor in the order of 5–6‰. The accepted trophic enrichment factor of 1‰ for ^{13}C (De Niro and Epstein, 1978) was also true for liver, but muscle tissue was threefold greater (3.7‰). Should we have applied the assumed enrichment factor for ^{15}N of 3–4‰ we would have incorrectly presumed an additional trophic level in this planktonic food chain, which we outline below.

4.3. Fish tissues

There were significant differences in liver $\delta^{15}N$ values between regions, as fish from within the impacted region were depleted in ^{15}N relative to the non-impacted regions, parallel to the POM comparison. Mado primarily feed upon zooplankton (Glasby and Kingsford, 1994), and zooplankton off Sydney has a $\delta^{15}N$ value of $7.0 \pm 0.2‰$ (Rutten, 1998), which is approximately one trophic level (i.e. 3.5‰, Fig. 5) below the $\delta^{15}N$ value for liver. As marine POM has a $\delta^{15}N$ value that is greater than zooplankton, we conclude that sewage POM is a significant contributor to the Sydney coastal planktonic food chain. Also, sewage discharge represents the major source of nitrogen to coastal waters off Sydney (Pritchard et al., 1997). Hence, we conclude that sewage POM is a significant contributor to the Sydney coastal planktonic food chain.

The diverse and flexible feeding nature of mado (Glasby and Kingsford, 1994) may result in short-term changes in diet and thus the liver. Liver tissue has a faster turnover rate than muscle when the isotope composition of the diet is changed (Tieszen et al., 1983). The turnover rate was found to be the same for muscle and liver for the stable isotopes of carbon, nitrogen and sulfur in broad whitefish (Hesslein et al., 1993), although in fast

growing fish the rate of change would directly reflect the growth rate. For example, we have found in juvenile mado at 20 °C that changes in isotopic composition of diet can be detected in liver tissue 3–4 days (Gaston, 2003). Hence the isotope variations observed in liver tissue could result from short-term dietary differences among individuals, and would account for the spatial and temporal variation between sites within a region. The broader range of $\delta^{13}C$ and $\delta^{15}N$ values in liver tissue at outfall sites probably reflects mixing of marine and sewage POM within these areas. Upwelling could result in differential mixing of POM sources, resulting in short-term changes of the isotope composition of mado diet.

Muscle revealed the same significant differences in $\delta^{15}N$ values between regions as for liver. Muscle is a long-term integrated measure of dietary source due to its slower turnover relative to liver (Tieszen et al., 1983). Muscle from within the impacted region was consistently depleted in ^{15}N relative to the non-impacted regions. Other studies show that fish collected from near a primary treated sewage outfall showed depletion of ^{15}N in their muscle tissue relative to those from a non-outfall region (depletion of 2–4‰, Rau et al., 1981; Spies et al., 1989). Therefore a major and continued influence of ^{15}N depleted primary treated sewage effluent to the coastal food chain resulted in fish being depleted in ^{15}N relative to non-impacted regions.

A small discharge of tertiary treated sewage effluent in the vicinity of site 1 in Region 1 (2–3 ML day^{-1}, Jervis Bay) had little detectable effect on the stable isotope composition of liver, but it did influence muscle. The $\delta^{15}N$ value of tertiary treated effluent was greater than that of marine POM (10.3 ± 0.2‰; Compton, 2000), and fish muscle in the vicinity of the discharge site (site 1, Region 1) was significantly enriched in ^{15}N relative to the other site in Region 1 by ~ 0.9‰ (Fig. 4b). This small example in Jervis Bay is consistent with our overall regional findings.

Analysis of bone revealed no consistent trends in differences of $\delta^{13}C$ or $\delta^{15}N$ isotope composition between impacted and non-impacted regions (Fig. 4c). The stable isotope signature of bone was surprisingly similar to liver, which is difficult to interpret and there are few studies of fish bone with which to compare. The variance component for among fish (within a site) is 9% and 24% for $\delta^{13}C$ and $\delta^{15}N$, respectively, which is comparable to liver and muscle. Within fish variation of stable isotope ratios in bone was less than half that for liver and muscle, possible due to its long turnover time. Since collagen forms the major structural component in vertebrate bone (Gröcke, 1997) and represents most of the weight of fresh bone (Sullivan and Krueger, 1981) it has the greatest influence in the isotopic composition of entire bone. The isotopic composition of collagen may differ from one area of bone to another because of ontogentic diet changes and the accumulation or replacement of collagen (Chisholm, 1989). Different bones in different regions of the body also mature at different times. The turnover rate of bone collagen has not been adequately documented, although (Bumstead, 1985) suggested a period of 2–10 years, possibly accounting for our result.

4.4. Fish tissue: liver versus muscle and bone

We found that muscle was consistently enriched in ^{13}C and ^{15}N relative to liver, while bone was depleted in ^{15}N relative to liver, yet enriched in ^{13}C. This difference has been attributed to the relative lipid fractions present within the tissues (Hesslein et al.,

1993; Pinnegar and Polunin, 1999; Tieszen et al., 1983). Tissues that contain large amounts of lipid, such as fish liver (Hesslein et al., 1993), tend to be more depleted in ^{13}C than other tissues (De Niro and Epstein, 1977; Tieszen et al., 1983). This is ascribed to lipid synthesis discriminating against ^{13}C in favour of the lighter isotope ^{12}C (De Niro and Epstein, 1977). However, removal of lipids from liver tissue only describes a small portion of the difference between liver and muscle (Hesslein et al., 1993). In contrast, clear shifts in $\delta^{13}C$ (and non-significant shifts in terms of $\delta^{15}N$) resulted after extraction of lipids from fry (Pinnegar and Polunin, 1999). They conclude that greater variance and poorer resolution of dietary relationships result from lipid extraction. Therefore, lipid extraction was omitted from our sample preparation. The difference in $\delta^{15}N$ between tissues is less easily understood, and is thought to reflect the relative abundances of different amino acids in tissues (Gaebler et al., 1966). Pinnegar and Polunin (1999) suggest that greater ^{15}N enrichment of muscle relative to other tissues might be a general feature of fish.

4.5. Variability at different spatial scales

Food web studies comparing the stable isotope values of fish from different habitats, must consider variability in stable isotope ratios to avoid potential confounding (France, 1995). Where within-population variance is detectable (i.e. over and above the variance attributable to the measurement technique), additional replication is necessary to quantify that variation (Lancaster and Waldron, 2001). In this study we found that the variance (measured as the CV) among fish within a site was less than that attributable to the measurement technique (0.2 ‰). The mean CV for $n=3$ replicate fish within each site was sufficiently small to characterise the site mean and observe inter-site (and regional) differences with high statistical power ($\beta>0.95$). Our CVs are similar or better than those reported for invertebrates using greater sample sizes (Lancaster and Waldron, 2001). Hansson et al. (1997) found that sample sizes of $n=2$ to 4 fish were sufficient to observe significant changes in the $\delta^{15}N$ values of whole food chains due to discharges from a sewage treatment plant. The level of within tissue variation differs among tissue type (liver, muscle and bone) and between elements. The greatest variation was found for the $\delta^{13}C$ composition of liver. This reflects the fast turnover rate of carbon in liver tissue where changes in isotopic composition of diet can be reflected in the liver within days (Gaston, 2003). Bone had the least within fish variation of all tissues for both ^{13}C and ^{15}N, probably due to its relatively slow turnover rate of bone (2–10 years, Bumstead, 1985), preventing its use as an indicator of dietary source at seasonal or yearly scales, hence bone was not sampled in the second year of this study.

The variance component at the inter-region scale is far greater than that at any other level for almost all tissue/stable isotope combinations. Up to 70% of the variation in stable isotope signatures is explained by the difference among regions. This is clearly observable in the stable isotopic composition of tissues in fish from within the impacted region (Region 2) being depleted in ^{15}N relative to the non-impacted regions (Region 1 and 3). The discharge of ^{15}N-depleted primary treated sewage in Region 2 appears to drive the variability in isotope composition in an otherwise marine dominated system. Therefore, with appropriate replication at several spatial scales, smaller sample sizes have sufficient

power to detect significant ecological variation and providing trophic enrichment factors are known to provide the link to their nutrient sources.

5. Conclusion

Field studies have shown that changes in $\delta^{15}N$ values correlate better with food-web structure than do changes in $\delta^{13}C$ values, where differences in trophic fractionation are smaller and often less consistent (Fry, 1988; Hansson et al., 1997). The variability of $\delta^{13}C$ is often too large and may not corroborate with the $\delta^{15}N$ results (Roelke and Cifuentes, 1997). Discharges from a tertiary treated sewage plant can significantly increase $\delta^{15}N$ values in whole food webs (Hansson et al., 1997) as were observed at one site in Jervis Bay. Conversely, discharges from primary treated sewage outfalls can decrease the $\delta^{15}N$ values in whole food webs. Our results are consistent with previous studies (Rau et al., 1981; Spies et al., 1989) despite Sydney's open and physically dynamic coastal waters. While the annual amount of discharged nutrients is small compared to that from upwellings, its continuous flow acts to nurture a planktonic food chain.

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