CSIRO PUBLISHING

www.publish.csiro.au/journals/mfr *Marine and Freshwater Research*, 2004, **55**, 689–699

The ^{13}C, ^{15}N and ^{34}S signatures of a rocky reef planktivorous fish indicate different coastal discharges of sewage

Troy F. Gaston[A,B,C], *Antionette Kostoglidis*[A] *and Iain M. Suthers*[A]

[A]School of Biological, Earth and Environmental Sciences, Faculty of Science, University of New South Wales, Sydney, NSW 2052, Australia.

[B]Present address: Faculty of Science, University of the Sunshine Coast, Maroochydore DC, Qld 4558, Australia.

[C]Corresponding author. Email: tgaston@usc.edu.au

Abstract. We assessed the effects of primary, secondary and tertiary treated sewage on the stable isotope composition of the viscera and muscle of the zooplanktivorous eastern hula fish (Plesiopidae: *Trachinops taeniatus*). Fish were collected from three regions during three consecutive summer months for 2 years. In comparison to fish from control sites, the muscle $\delta^{15}N$ of fish at outfall sites was significantly enriched by secondary and tertiary treated effluent. We estimate that 30–50% of nitrogen in hula fish near outfalls may be sewage-derived. The $\delta^{34}S$ content of muscle was similar at all regions and sites (20–21‰), although it was significantly depleted by 1–2‰ at the tertiary treated outfall site. Detection of a comparatively minor volume of effluent (<6 ML day^{-1}) in fish muscle may be due to its slower tissue turnover rate and the continuous discharge of effluent at outfall sites, compared to major yet sporadic rainfall or oceanographic events. The isotopic composition of hula fish from near a large primary treated sewage outfall off the coast of Sydney was not significantly different from one of the control sites, indicating a regional effect of four outfalls discharging >1000 ML day^{-1}. With increasing upgrades to sewage treatment, stable isotopes may become useful tracers of anthropogenic nutrients in an oligotrophic environment.

Extra keywords: food chain dynamics, planktivorous fish, sewage, stable isotope analysis.

Introduction

Sewage nutrients can be distinguished from marine, freshwater and terrestrial nutrient sources on the basis of naturally occurring stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$; Burnett and Schaeffer 1980; Sweeney and Kaplan 1980; Sweeney *et al*. 1980; Spies *et al*. 1989; Hansson *et al*. 1997; Gaston and Suthers 2004). The secondary and tertiary treatment of sewage can enrich the concentration of the heavier isotope (Spies *et al*. 1989; Hansson *et al*. 1997; Jones *et al*. 2001), providing the potential to differentiate particular sewage discharges. The stable isotope of sulfur, ^{34}S may be a better source indicator because the isotopic separation between terrestrial and marine organic matter is much larger than it is for carbon and nitrogen. The $\delta^{34}S$ values for terrestrial vegetation is $\sim+4$‰, whereas marine plant material is $\sim+19$‰ (Peterson and Fry 1987). In sediments, however, sulfate reduction results in a large range of values (Kaplan *et al*. 1963). Sewage particulate organic matter (SPOM) typically has sulfur values in the range -1 to $+3$‰ (Sweeney *et al*. 1980; Tucker *et al*. 1999), whereas marine POM (phytoplankton) has more enriched sulfur values of 17–21‰ (Peterson and Fry 1987; Tucker *et al*. 1999), thus making it possible to quantify the relative contribution of sewage particulates in bivalve diets (Tucker *et al*. 1999).

The interpretation of stable isotope ratios relies on the assumption that the isotopic composition of the animal reflects its diet (Gannes *et al*. 1997). Trophic enrichment (or fractionation) is the unequal partitioning of light and heavy stable isotopes between diet and consumer. Trophic enrichment factors have been well-established for carbon and nitrogen, and are typically within the range of 0–2‰ for $\delta^{13}C$ and 2–4‰ for $\delta^{15}N$ (De Niro and Epstein 1978, 1981; Rau *et al*. 1983; Minagawa and Wada 1984; Peterson and Fry 1987). Sulfur is not useful as a trophic level indicator because there is little fractionation of sulfur from prey to predator (Peterson *et al*. 1985). The isotopic composition of animal tissues can differ from that of the diet when the diet is isotopically homogeneous; hence enrichment factors are not only species-specific but also possibly tissue-specific (Macko *et al*. 1982; Tieszen *et al*. 1983). Without knowledge of the trophic enrichment factors for an organism and its tissues, nutrient source identification for the food chain is not possible (Peterson and Fry 1987). Stable isotope ratios of different tissue types can provide both short and long-term nutrient information, as the period of isotope integration is related to tissue type. Tissues with a fast turnover, such as liver, provide a short-term integrator of nutrient source (i.e. weeks), whereas tissues with a slow turnover, such as

 10.1071/MF03142 1323-1650/04/070689

muscle, provide a long-term indicator of nutrient source (Tieszen *et al*. 1983; Hesslein *et al*. 1993). Analysis of the isotopic composition of muscle tissue can identify fish supported by different trophic pathways (Sholto-Douglas *et al*. 1991; Rau *et al*. 1992; Thomas and Cahoon 1993). Some authors have used other tissues, most notably fish livers (Hesslein *et al*. 1993), bone collagen (Schoeninger and De Niro 1984; Sholto-Douglas *et al*. 1991), and fish otoliths (Radtke *et al*. 1996), representing different time scales of nutrient integration.

The ecological effects of sewage discharge have been detected at the community or trophic level (Grigg 1994), and at the species level (Smith *et al*. 1999). In contrast, other studies from the relatively oligotrophic waters off eastern Australia report no significant impact on marine biota (Smith 1994; Scanes *et al*. 1995), presumably due to dilution and temporal variability along the open coast. We examined this contradiction from a planktotrophic perspective, in view of the continuous flow of discharge off the coast of Sydney. The assimilation of anthropogenic nutrients into the marine pelagic food chain (compared to the benthic food chain) of any vertebrate, such as a zooplanktivore, is largely unknown.

First, this study aimed to test if the stable isotope signatures of comparatively small volumes of sewage discharge were detectable in the coastal planktonic food chain at three different discharge sites along the NSW coast, using the eastern hula fish (Plesiopidae: *Trachinops taeniatus*). If sewage-derived nutrients do not enter the food chain, the muscle and viscera of hula fish from within the outfall sites should have similar carbon, nitrogen and sulfur isotope compositions as those of hula fish from control sites. Second, the interpretation of stable isotope data requires comparisons between field data and laboratory experiments (Gannes *et al*. 1997). A laboratory feeding study was carried out to quantify trophic enrichment, to estimate the nutritive contribution of sewage to the pelagic vertebrate food chain.

Materials and methods

Study area and collections

Three regions along the NSW coast were sampled – Port Stephens (PS), Sydney (S) and Jervis Bay (JB; Fig. 1). There were three sites within each region – two controls and one in the vicinity of sewage discharge. Each outfall site had either primary, secondary or tertiary levels of treatment and had different volumes of effluent discharge (Table 1). The effluent is discharged ~600 m from the shore at Boulder Bay (PS), but from the shoreline at Potter Point (S) and Plantation Point (JB). All sites within each region consisted of rocky reef environment with patches of kelp (*Ecklonia radiata*).

The eastern hula fish is an abundant rocky reef fish endemic to eastern Australian waters from southern Queensland to Victoria. It is a zooplanktivorous species that attains a maximum length of ~10 cm (Hutchins and Swainston 1986), forming large schools that appear to be geographically persistent. The hula fish is also quite sensitive to the effects of sewage discharge. It is one of just three species of fish, from a suite of >25 species, that show a significant decline in abundance around sewage outfalls, particularly near primary treated sewage outfalls (Smith and Suthers 1999; Smith *et al*. 1999).

Hula fish were collected from each site by a scuba diver using two small (30 cm diameter) hand-held nets, over three consecutive months (February, March and April) during two replicate years (1999 and 2000). Fish were placed on ice in the field and stored in a laboratory freezer. In the laboratory, fish were thawed and wet weight (ww, ± 0.01 g) and standard length (SL, ± 0.1 mm) were measured (mean $\pm$ s.e.m.; 1.06 ± 0.07 g (ww) and 42.4 ± 1.1 mm (SL)). We found no significant relationship between size and the stable isotope composition.

In the laboratory, adult hula fish were reared on a diet of newly hatched *Artemia* spp. with a constant stable carbon and nitrogen isotope composition of -20.7 ± 0.2‰ and 9.8 ± 0.5‰, respectively. We used *Artemia* rather than wild zooplankton because we could not ensure that wild zooplankton would be isotopically consistent over the 9 months. For this reason the *Artemia* were not fed. We assumed that the effects of fractionation would be similar for *Artemia* to wild zooplankton as they have a comparable size range (Frazer *et al*. 1997). Fish ($n = 12$) were reared on this diet for 9 months in 40 L tanks in a recirculating seawater system of constant temperature (21.5 ± 0.2°C) and salinity (34.8 ± 0.5, mean $\pm$ s.e.m.). Fish had an initial ww of 0.64 ± 0.06 g and a SL of 39.6 ± 1.2 mm (there was no significant growth during the experimental period as the fish were adult or near adult size, and thus representative of our field samples). At the end of this period fish were removed from their tanks (before their daily feed), killed in ice water and frozen until analysed.

For stable isotope analysis, fish were thawed and a piece of white dorsal muscle and the visceral mass were excised from three fish at each site. Lipids were not extracted as the procedure often results in greater variance and poorer resolution of dietary relationships (Pinnegar and Polunin 1999). The liver in hula fish was too small to remove accurately for stable isotope analysis (<0.001 g), so the entire visceral mass (all soft parts of the body cavity) was used as the tissue, reflecting higher metabolic activity. Tissue samples were freeze-dried, ground to a fine powder and 1.2–1.5 mg of each sample was sealed into a tin capsule for carbon and nitrogen stable isotope analysis. Each capsule was analysed with an automated nitrogen and carbon analysis (ANCA) mass spectrometer (20–20, Europa Scientific, SerCon, Cheshire, UK) system. 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 $^{13}C:{}^{12}C$ and $^{15}N:{}^{14}N$. Isotope values are expressed in delta (δ) notation, $\delta^{13}C$ and $\delta^{15}N$, relative to international standards (Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). Sulfur isotope analysis was conducted in a single batch by the Isotope Science Laboratory, University of Calgary, Canada. Due to insufficient visceral mass, muscle tissue only was freeze-dried and 1–5 mg sealed into a tin capsule. Sulfur isotope ratios were determined using a Carlo Erba NA 1500 elemental analyser interfaced with continuous flow – isotope ratio mass spectrometry (CF-IRMS; Thermo Electron Corporation, Milan, Italy). Capsules were combusted at 1020°C and the reaction products separated by GC to give a pulse of pure SO_2 for analysis of total S and $^{34}S:{}^{32}S$, relative to an international standard (NBS 127 $BaSO_4$). Delta values were determined as follows:

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

where $X = {}^{13}C$, ^{15}N or ^{34}S and $R = {}^{13}C:{}^{12}C$, $^{15}N:{}^{14}N$ or $^{34}S:{}^{32}S$. Error bars are expressed as the standard error of the three replicates, or the machine precision of analyses (whichever was greater), which were ± 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, and ± 0.7‰ for $\delta^{34}S$.

Data were tested for homogeneity of variance, transformed if variances were heterogeneous, and analysed using analysis of variance (ANOVA). A four-way ANOVA was used to compare the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ value of each tissue (only muscle for $\delta^{34}S$) among years ($n = 2$),

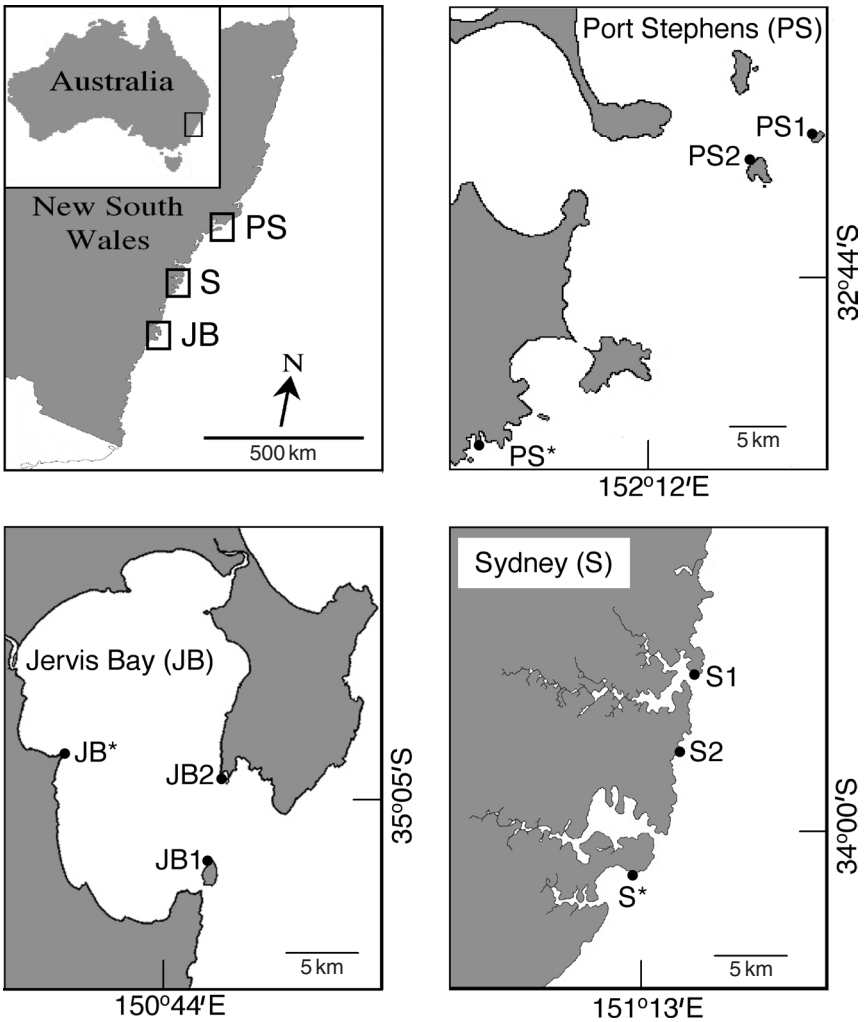

Fig. 1. Map showing locations of sampling sites within the three regions of Port Stephens (PS); Sydney (S); and Jervis Bay (JB). *Indicates the location of a sewage outfall.

Table 1. Location of sites within each of the three regions, including the treatment level and discharge volume of effluent sewage treatment plants (STP)

Region	Site	Site code	Treatment level	ADWF (ML day^{-1})	Depth (m)
PS	Boulder Bay	PS*	Secondary	4.3	7–12
	Little Island	PS1			7–10
	Boondelbah Island	PS2			5–10
S	Potter Point	S*	Primary	46	7–12
	North Head	S1			7–12
	Gordons Bay	S2			4–12
JB	Plantation Point	JB*	Tertiary	5.8	5–10
	Bowen Island	JB1			5–7
	Dart Point	JB2			5–7

ADWF, average dry weather flow; adapted from (MHL 1997); PS, Port Stephens; S, Sydney; JB, Jervis Bay. *Indicates the location of a sewage outfall.

months ($n = 3$), regions ($n = 3$) and sites ($n = 3$). Means were compared using Student–Newman–Keuls (SNK) tests for all ANOVAs using GMAV5 for Windows (Underwood *et al*. 1998).

Results

Stable isotope analysis – muscle

The muscle tissue of fish from the outfall sites in Jervis Bay and Port Stephens were enriched in ^{15}N relative to the control sites during both 1999 (Fig. 2*a*,*e*) and 2000 (Fig. 2*b*,*f*). In 1999, muscle tissue from the site of the secondary treated outfall in Port Stephens (PS*) was significantly enriched (10.7 ± 0.2‰, $P < 0.01$; Table 2) relative to the control sites (PS1 and PS2; 10.2 ± 0.2‰; Fig. 2*a*). In 2000, muscle tissue from PS* was significantly enriched ($P < 0.01$) relative to the control sites, however, muscle tissue from PS2 (9.9 ± 0.2‰; $P < 0.05$) was significantly enriched relative to PS1 (9.3 ± 0.2‰; Fig. 2*b*). In 1999, muscle tissue from

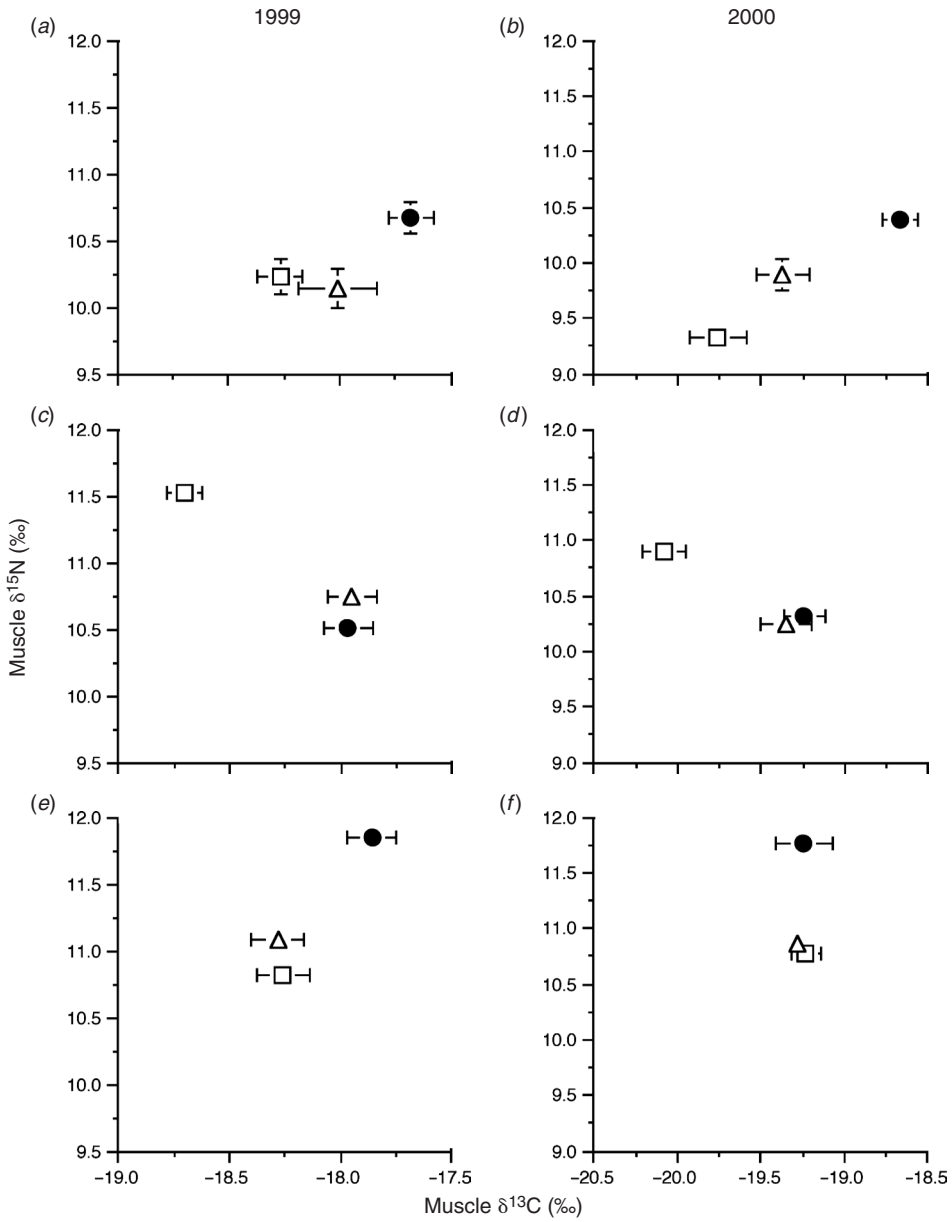

Fig. 2. Scatterplot of yearly averaged stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratio of hula fish (*Trachinops taeniatus*) muscle from (*a*,*b*) Port Stephens (secondary treated discharge at PS*); (*c*,*d*) Sydney (primary treated discharge at S*); and (*e*, *f*) Jervis Bay (tertiary treated discharge at JB*) during (*a*,*c*,*e*) 1999 and (*b*,*d*, *f*) 2000. Note that the axes are different between years (more depleted in 2000 than 1999). The outfall site is indicated by the closed circle, control site 1 is indicated by the open square and control site 2 is indicated by the open diamond, in each region. Values are mean ± s.e.m. ($n = 9$).

the site of a tertiary treated outfall in Jervis Bay (JB*), was significantly enriched in ^{15}N (11.8 ± 0.2‰; $P < 0.01$; Table 2) relative to the controls, JB1 (10.8 ± 0.2‰) and JB2 (11.1 ± 0.2‰; Fig. 2*e*). Similarly, in 2000 muscle tissue from JB* was enriched (11.8 ± 0.2‰; $P < 0.01$; Table 2) relative to JB1 (10.8 ± 0.2‰) and JB2 (10.9 ± 0.2‰; Fig. 2*f*).

The $\delta^{15}N$ and $\delta^{13}C$ composition of fish from S2 (control site off Sydney) were not significantly different to those from S* (site of a primary treated outfall) for both sampling years (Fig. 2*c*,*d*). However, compared to S* and S2, fish from S1 (control site) were significantly enriched in ^{15}N ($P < 0.01$; Table 2) and significantly depleted in ^{13}C ($P < 0.01$; Table 2) during 1999 and 2000 (Fig. 2*c*,*d*).

As with ^{15}N, fish from the outfall site in Port Stephens were enriched in ^{13}C relative to the control sites within that region during both 1999 and 2000 (Fig. 2). Fish from PS* were significantly enriched in ^{13}C (-17.7 ± 0.2‰; $P < 0.05$; Table 2) relative to PS1 (-18.3 ± 0.2‰) and PS2 (-18.0 ± 0.2‰)

Table 2. Summary of analysis of variance of isotope composition of hula fish (*Trachinops taeniatus*) sampled during 1999 and 2000 with $n = 3$ fish per cell

Source	d.f.	Muscle $\delta^{15}N$				Muscle $\delta^{13}C$				Viscera $\delta^{15}N$				Viscera $\delta^{13}C$				Muscle $\delta^{34}S$			
		SS	MS	*F*	*P*	SS	MS	*F*	*P*	SS	MS	*F*	*P*	SS	MS	*F*	*P*	SS	MS	*F*	*P*
Year ($n = 2$)	1	4.1	4.1	62.0	**	61.6	61.6	655.2	**	1.4	1.4	8.4	**	22.3	22.3	40.6	**	<0.1	<0.1	<0.1	ns
Month ($n = 3$)	2	0.1	0.1	0.9	ns	2.0	1.0	10.4	**	2.8	1.4	8.8	**	2.3	1.2	2.1	ns	94.4	46.2	4.6	*
Region ($n = 3$)	2	29.0	14.5	218.6	**	2.3	1.1	12.0	**	13.3	6.6	41.0	**	15.2	7.6	13.8	**	43.2	21.6	2.1	ns
Site ($n = 2$)	2	5.6	2.8	42.0	**	7.6	3.8	40.6	**	7.0	3.5	21.7	**	8.6	4.3	7.8	**	78.1	39.0	3.9	*
Y × M	2	1.4	0.7	10.2	**	1.0	0.5	5.3	**	3.8	1.9	11.7	**	3.9	1.9	3.5	*	28.3	14.1	1.4	ns
Y × R	2	0.9	0.5	6.9	**	0.4	0.2	1.9	ns	3.0	1.5	9.1	**	2.4	1.2	2.2	ns	143.6	71.6	7.1	**
Y × S	2	0.7	0.4	5.5	**	0.1	<0.1	0.3	ns	2.1	1.1	6.5	**	0.3	0.1	0.2	ns	67.3	33.6	3.3	*
M × R	4	1.6	0.4	5.9	**	2.6	0.7	7.0	**	2.5	0.6	3.8	**	3.7	0.9	1.7	ns	43.2	10.8	1.1	ns
M × S	4	0.4	0.1	1.6	ns	0.7	0.2	1.9	ns	0.4	0.1	0.6	ns	6.1	1.5	2.7	*	140.2	35.1	3.5	*
R × S	4	15.9	4.0	59.9	**	3.9	1.0	10.3	**	10.7	2.7	16.6	**	8.1	2.0	3.7	**	64.8	16.2	1.6	ns
Y × M × R	4	0.7	0.2	2.7	*	0.3	0.1	0.7	ns	2.2	0.6	3.4	*	4.4	1.1	2.0	ns	159.3	39.8	3.9	**
Y × M × S	4	1.1	0.3	4.3	**	0.7	0.2	1.8	ns	2.4	0.6	3.8	**	4.1	1.0	1.9	ns	56.0	14.0	1.4	ns
Y × R × S	4	0.2	0.2	0.8	ns	1.5	0.4	4.0	**	1.1	0.3	1.7	ns	2.4	0.6	1.1	ns	113.5	28.4	2.8	*
M × R × S	8	1.2	0.2	2.2	*	2.6	0.3	3.5	**	6.0	0.8	4.7	**	10.3	1.3	2.3	*	344.4	43.0	4.3	**
Y × M × R × S	8	0.7	0.1	1.3	ns	0.9	0.1	1.3	ns	3.3	0.4	2.5	*	3.6	0.5	0.8	ns	410.9	51.4	5.1	**
Residual	108	7.2	0.1			10.2	0.1			17.5	0.2			59.4	0.6			1093.3	10.1		

ns, not significant $P > 0.05$; *$P < 0.05$; **$P < 0.01$. M, month; R, region; S, site; Y, year.

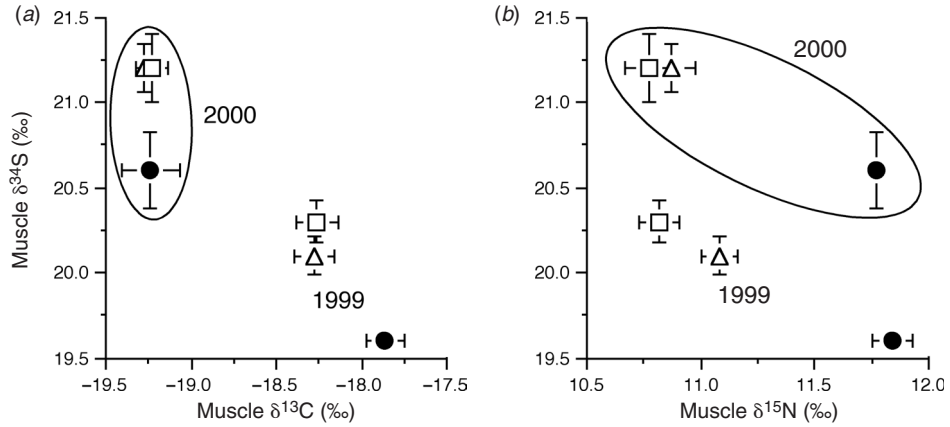

Fig. 3. Scatterplot of yearly averaged stable isotope composition of hula fish (*Trachinops taeniatus*) muscle from Jervis Bay (tertiary treated discharge at JB*, solid circle) for (*a*) sulfur ($\delta^{34}S$) on carbon ($\delta^{13}C$); and (*b*) sulfur ($\delta^{34}S$) on nitrogen ($\delta^{15}N$). Circled values are yearly averaged for 2000 and those outside the circles are yearly averaged for 1999. Values are mean ± s.e.m. ($n = 9$).

in 1999. Similarly in 2000, fish from PS* were enriched (-18.7 ± 0.2‰; $P > 0.01$) relative to PS1 (-19.8 ± 0.2‰) and PS2 (-19.4 ± 0.2‰). In 1999, fish from JB* were significantly enriched (-17.9 ± 0.2‰; $P < 0.05$; Table 2) relative to JB1 and JB2 (-18.3 ± 0.2‰; Fig. 2*e*), however, all sites were similar in 2000 (Fig. 2*f*).

The sulfur isotopic composition of hula fish muscle was remarkably uniform across all sites in all regions and showed little significant variation (Fig. 3). The one exception was JB*, which was significantly depleted by >1‰ in ^{34}S relative to the control sites in Jervis Bay during both 1999 and 2000 ($P < 0.05$; Fig. 3*a*,*b*; Table 2). There were no significant differences among sites within the Port Stephens and Sydney regions for either 1999 or 2000 ($P > 0.05$).

Stable isotope analysis – viscera

The fish from all sites in Port Stephens had similar $\delta^{15}N$ values for both 1999 and 2000 (Fig. 4*a*). The viscera of fish from JB* (9.9 ± 0.2‰) were significantly enriched in ^{15}N ($P < 0.01$; Table 2) relative to JB1 (8.8 ± 0.2‰) and JB2 (9.0 ± 0.2‰) in 2000, but similar to JB2 in 1999 (Fig. 4*c*).

The $\delta^{15}N$ and $\delta^{13}C$ composition of viscera in fish from S2 (control site off Sydney) were not significantly different to those from S* (site of a primary treated outfall) for both sampling years (Fig. 4*b*). However, fish from S1 (control site) were significantly enriched in ^{15}N ($P < 0.01$, Table 2) and depleted in ^{13}C ($P < 0.01$, Table 2) during 1999, yet similar to the other sites in Sydney in 2000 (Fig. 4*b*). There was no significant difference among sites in the $\delta^{13}C$ composition of viscera in fish from Port Stephens and Jervis Bay during both 1999 and 2000 (Fig. 4).

There was no significant correlation between $\delta^{15}N$ and $\delta^{13}C$ for either tissue or either year. Muscle $\delta^{34}S$ showed a weak but significant correlation to muscle carbon and nitrogen stable isotope ratios, but the sign of the correlation changed from negative to positive between 1999 and 2000.

Inter-annual variability and diet–viscera–muscle fractionation

There was a significant shift in $\delta^{13}C$ of muscle between 1999 and 2000. Fish sampled in 1999 were significantly enriched in ^{13}C relative to those sampled in 2000 (Table 2; Fig. 2), across all sites and regions. A similar increase in $\delta^{13}C$ of the viscera occurred between sampling years but this shift was not as pronounced (Fig. 4).

In the laboratory, hula fish reared on a diet of constant stable carbon (-20.7 ± 0.2‰) and nitrogen (9.8 ± 0.5‰) isotope composition had a different trophic enrichment factor for muscle and viscera. Muscle was enriched by the diet (1.9‰ for ^{13}C and 2.3‰ for ^{15}N), whereas in the viscera, ^{13}C was depleted (by 0.5‰) and ^{15}N was enriched (by 0.9‰; Fig. 5) by the diet.

Discussion

Effects of secondary and tertiary treatment

The $\delta^{13}C$ and $\delta^{15}N$ isotope composition of hula fish muscle was significantly different among sites and regions. Fish from the outfall sites in Port Stephens and Jervis Bay were enriched in ^{15}N relative to the control sites within each region. The Port Stephens outfall (Boulder Bay, PS*) is the only large source of freshwater in the local area, and is the main continuous source of new N for local hula fish. Jervis Bay also receives little freshwater input other than from the Plantation Point (JB*) outfall (Holloway 1996). Secondary (Boulder Bay, PS*) and especially tertiary (Plantation Point, JB*) treated effluent is enriched in ^{15}N due to the preferential removal of ^{14}N via ammonia volatilisation during the treatment process (Heaton 1986; Compton 2000; Lancaster and Waldron 2001).

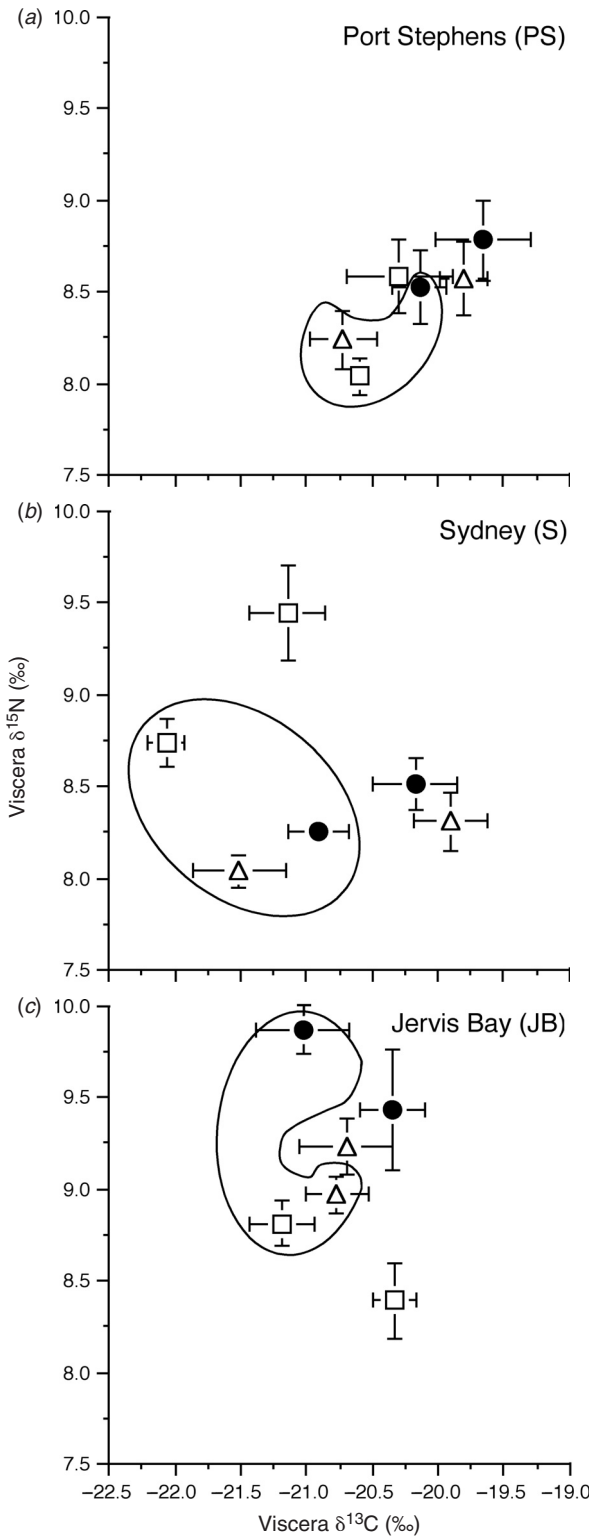

Fig. 4. Scatterplot of yearly averaged stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratio of hula fish (*Trachinops taeniatus*) viscera from (*a*) Port Stephens (secondary treated discharge at PS*, solid circle); (*b*) Sydney (primary treated discharge at S*, solid circle); and (*c*) Jervis Bay (tertiary treated discharge at JB*, solid circle) in 1999 and 2000 (circled). Values are mean ± s.e.m. ($n = 9$).

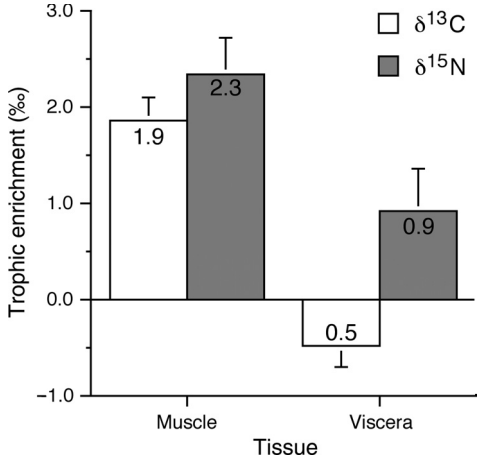

Fig. 5. Trophic enrichment (‰) for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition of muscle and viscera of eastern hula fish (*Trachinops taeniatus*) reared on a single diet for 9 months. Values are mean ± s.e.m. ($n = 12$).

The enrichment of muscle tissue in the vicinity of outfalls is consistent with sewage effluent as the dominant nutrient source in these areas. Elevated $\delta^{15}N$ signatures of marine organisms were observed at the site of secondary treated sewage discharge, however, the amount of effluent discharged is relatively small (2.3 ML day^{-1}; Jones *et al*. 2001). This indicates that municipal discharges can have a significant impact on receiving waters. Muscle has a comparatively slow turnover, making it a long-term indicator of dietary sources of nutrients (Hesslein *et al*. 1993). The relatively depleted $\delta^{15}N$ composition of muscle tissue in fish from control sites around Port Stephens and Jervis Bay reflects organic-matter nitrogen of a marine origin (Rau *et al*. 1991; Riera 1998).

The muscle tissue of fish near outfall sites in both Port Stephens and Jervis Bay was also enriched in ^{13}C relative to the control sites. Tertiary treated sewage effluent of Jervis Bay is enriched in ^{13}C (Compton 2000) compared to terrestrial organic matter (−30 to −27‰; Richard *et al*. 1997) and compared to the overall average of marine particulate organic matter (−21‰; Gaston and Suthers 2004). Thus, the enrichment of ^{13}C in muscle tissue in the vicinity of these outfalls is consistent with sewage effluent being the dominant nutrient source in these areas. The signatures of secondary and tertiary treated sewage in hula fish are possibly regionally specific and only indicative of general stable isotope characteristics, as we did not have replicate secondary or tertiary treatment in each region.

The results from the viscera were less clear, showing greater variance among replicate fish and between years (residual in Table 2). The viscera C–N signature showed less inter-annual difference at the outfall compared to those fish from control sites (inspection of Fig. 4), consistent with our finding that sewage provides a constant source of nutrients. We attempted to relate known upwelling events with the

viscera stable isotope composition but found no consistent pattern.

A proportion of the viscera consisted of the gut containing prey items, and hence the viscera's isotope ratio would be influenced by the food source and recent feeding history. The visceral mass also included the liver, which in another zooplanktivorous fish (*Atypichthys strigatus*), can significantly change isotope composition in 3 days for ^{13}C and in 12 days for ^{15}N (Gaston 2003). Therefore, the isotope composition of the visceral mass would be a combination of the most recent diet (gut contents) and that of only a few days old (due to the rapid turnover rate of liver tissue). This accounts for the larger error bars for viscera compared to muscle.

A striking feature of the $\delta^{34}S$ values of hula fish muscle from this study is the relatively uniform, marine nature. Only the tertiary treated outfall site in Jervis Bay (JB*) was significantly depleted in ^{34}S relative to the control sites in both years. The $\delta^{34}S$ values of sewage POM found in this study were enriched (10‰; Gaston 2003) compared to the reported values of Sweeney *et al*. (1980) but remain significantly different from that of marine POM (Peterson and Fry 1987; Tucker *et al*. 1999). Hence, the depleted $\delta^{34}S$ value of hula fish muscle at the JB outfall site probably reflects the incorporation of sewage-derived sulfite into the food chain. It is unclear why similar depletions were not observed at Port Stephens, other than a difference in secondary treatment. Our findings may represent a unique characteristic for the Jervis Bay sewage treatment plant. These results provide a testable hypothesis for future studies of replicated secondary or tertiary treated discharges.

Sulfur isotopes have been used successfully to trace sewage in estuarine or benthic habitats, but not in pelagic food webs in coastal areas (Peterson *et al*. 1985; Moore *et al*. 1996). The use of nitrogen and sulfur stable isotopes revealed widespread evidence of sewage-derived particulate and dissolved materials in Boston Harbour and Massachusetts Bay (Tucker *et al*. 1999). The sulfur stable isotope provided an unambiguous sewage signal, and the researchers were able to identify that blue mussels were consuming a food source with a significant sewage component. $\delta^{15}N$ and $\delta^{34}S$ isotopic analyses of winter flounder (*Pleuronectes sanguinolenta*) have also been used to indicate the influence of sewage dumping (Moore *et al*. 1996). Therefore, failure of the sulfur isotope to show an unambiguous sewage signature in this study is possibly due to the mixing and dynamics of the open coast or to some characteristics of the pelagic habitat. This study suggests that there could be a treatment process peculiar to a particular treatment plant, thus providing a location-specific signature.

Effects from primary treatment

The Sydney coast has three deepwater ocean outfalls as well as the coastal outfall site of this study (Potter Point, S*; MHL 1997), which together discharge over 1000 ML day^{-1} of primary treated effluent. Primary treated effluent (SPOM) is depleted in ^{15}N (∼0.4‰) relative to estuarine (∼7‰) and marine particulate organic nitrogen (∼8‰; Gaston and Suthers 2004). The primary treated sewage is enriched in ^{13}C (−25‰) relative to estuarine-derived organic matter (∼−28‰; Richard *et al.* 1997; Riera 1998). Consequently it was surprising that the coastal outfall site near Sydney (Potters Point, S*) was not significantly different to one of the control sites (Gordons Bay, S2) in either ^{13}C, or ^{15}N, for muscle or viscera. The hula fish signature from these two sites may be due to a large-scale combined effect of the Potters Point coastal outfall and the three major deep ocean outfalls (Gaston and Suthers 2004), highlighting the difficulty of finding genuine control areas near cities.

Another, co-occurring zooplanktivorous fish, *Atypichthys strigatus* (the mado), was found to be significantly depleted in ^{15}N in the Sydney region regardless of the proximity to Sydney's outfalls, consistent with a general 'Sydney impact' (Gaston and Suthers 2004). The $\delta^{15}N$ and $\delta^{13}C$ of muscle at the other control site (North Head, S1), near the mouth of Sydney Harbour, was enriched in ^{15}N and depleted in ^{13}C relative to the outfall site (Potters Point, S*) and the coastal control site (Gordons Bay, S2). Particulate organic matter along the Sydney coast is depleted in ^{15}N, indicating an influence of primary treated effluent in the coastal zone, relative to estuarine-derived material (Gaston 2003). Compared to marine sources and primary treated effluent, estuarine material is depleted in ^{13}C by 2–6‰ (Cloern *et al*. 2002; Gaston 2003; Darnaude *et al*. 2004), consistent with the hula's $\delta^{13}C$ value at this site. Hence, the hula fish also reveal a 'Sydney impact' that is only overridden by fish residing within estuary mouths.

Inter-annual changes

Changes between years and among months in the C, N and S isotope signatures of hula fish were evident, reflecting temporal changes of nutrient inputs. Our analysis of tissues with differing metabolic rates was essential for determining the short and long-term nutritive changes in the food chain. There was a 1‰ decrease in $\delta^{13}C$ stable isotope composition of muscle tissue from 1999 to 2000 among all sites and across all regions. Such a shift may be indicative of a change in species composition of phytoplankton communities along the NSW coast. Laboratory cultures of phytoplankton communities have found flagellates to be more depleted in ^{13}C than diatoms (Wong and Sackett 1978). This finding has also been observed in field studies. Enriched ^{13}C content of phytoplankton on central Georges Bank was consistent with a diatom-dominated community (Fry 1988). Diatoms in Narragansett Bay were on average 2‰ enriched in ^{13}C relative to nanoplankton (Gearing *et al*. 1984). While the $\delta^{13}C$ of phytoplankton were not measured in this study, a shift in algal dominance from diatoms to dinoflagellates would account for the shift in $\delta^{13}C$ composition of muscle and viscera to values

that are more negative. Consistent with this speculation, is the fact that hula fish had significantly better body condition (weight/length^3) in 1999 than in 2000 (Gaston 2003). The decrease in body condition of hula fish may account for the doubling in ^{15}N viscera-to-muscle fractionation from 1999 to 2000 (from 1.2 to 2.2‰). Starvation is typically associated with increased δ^{15}N (Hesslein *et al*. 1993). Therefore, the potential for species-, tissue- and even annually specific fractionation is probably large. Furthermore, sulfur values were more depleted in 1999 than 2000 across all regions.

Contribution of sewage nutrients

When sewage enters the marine environment it mixes with marine nutrients and terrestrially-derived organic matter from river runoff, causing the sewage signature to be masked (Thornton and McManus 1994). With the use of mixing models, the origins of organic matter can be delineated to give proportions of marine versus terrestrially-derived POM (Fry and Sherr 1984; Faganeli *et al*. 1988). Field studies have shown that changes in δ^{15}N correlate better to food-web structure than do changes in δ^{13}C, where differences in trophic fractionation are smaller and often less consistent (Fry 1988; Hansson *et al*. 1997; Gaston and Suthers 2004).

Our laboratory study provided the enrichment values for such an approach. The enrichment values for muscle, +1.9‰ and +2.3‰ for δ^{13}C and δ^{15}N, respectively, fall within the range reported in the literature (De Niro and Epstein 1978, 1981; Rau *et al*. 1983; Minagawa and Wada 1984). Also, the isotopic composition of muscle is enriched in both ^{13}C and ^{15}N relative to viscera. This difference between tissues is attributed to the relative biochemical fractions present within the tissues and is enhanced in this study because a proportion of the viscera consisted of gut contents (Tieszen *et al*. 1983; Hesslein *et al*. 1993; Pinnegar and Polunin 1999). The viscera had a positive enrichment for δ^{15}N (+0.9‰), yet a negative enrichment for δ^{13}C (−0.5‰) over diet (Fig. 5), which could be due to the lipid content of the liver present in the visceral mass. Tissues that contain large amounts of lipids, such as fish liver, tend to be more depleted in ^{13}C than other tissues (De Niro and Epstein 1977; Tieszen *et al*. 1983; Hesslein *et al*. 1993).

Using the stable isotope signature of sewage, marine POM and our trophic enrichment value for hula fish muscle over diet (+2.3‰), an idealised model of the food chain was constructed for nitrogen in Jervis Bay (Fig. 6). In order to determine the per cent (%) contribution of sewage effluent to the Jervis Bay coastal food chain (Y) a mixing equation was used:

$$(\mathrm{Y}) = \frac{(\delta^{15}\mathrm{N}_m - \delta^{15}\mathrm{N}_h)}{(\delta^{15}\mathrm{N}_m - \delta^{15}\mathrm{N}_s)} \times 100$$

For Jervis Bay nitrogen, $\delta^{15}\mathrm{N}_m$ is the actual average isotope composition of hula fish muscle supported entirely by marine-derived POM (10.9‰; JB1 and JB2, both years),

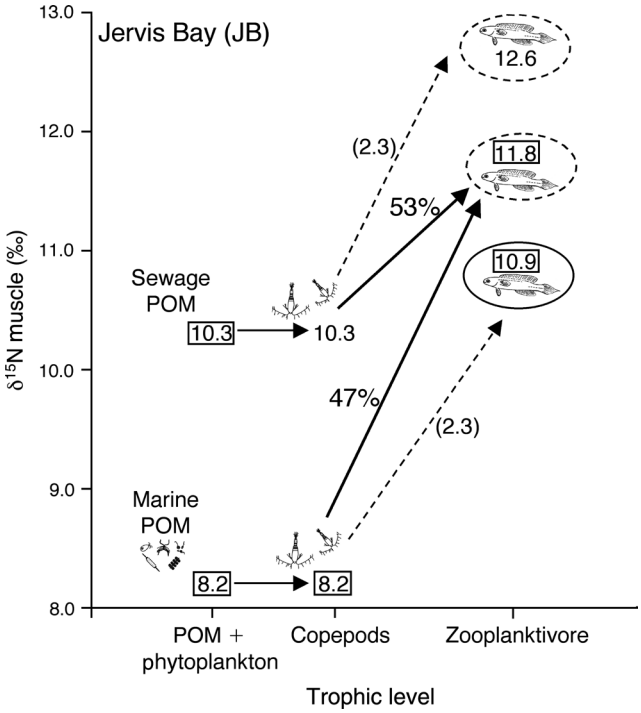

Fig. 6. Model of the Jervis Bay coastal planktonic food chain for δ^{15}N. Values are based on *in situ* measurements of marine and sewage particulate organic matter (POM), zooplankton in Jervis Bay (the copepod *Temora turbinata*), average δ^{15}N for hula fish (*T. taeniatus*) muscle tissue at the control sites and the outfall site, and the trophic enrichment factor (2.3‰) was determined in the laboratory for hula fish (see Table 3 for source of values). Actual measurements are in rectangles. Calculated values (see text) are in normal font. Fish in an open circle represent a food chain based entirely on marine POM, and fish in a dashed circle represent a food chain with some contribution from sewage POM. Arrows in **bold** represent the relative contribution from marine and sewage-based food chains to fish off Plantation Point. Approximately 53% of nitrogen in the hula fish food chain off Plantation Point (Jervis Bay) could be derived from tertiary treated sewage effluent.

Table 3. Summary of sources of C and N for the Jervis Bay mixing model

Trophic level	Value (‰)	Source
Marine particulate organic matter	8.2	Gaston (2003)
Sewage particulate organic matter	10.3	Compton (2000)
Marine zooplankton	8.2	Compton & Suthers (unpublished data)
Sewage-supported zooplankton	10.3	Calculated
Fractionation	2.3	This study
Marine hula fish	10.9	This study
Sewage-supported hula fish	11.8	This study
Sewage-supported hula fish (100%)	12.6	Calculated

which is similar to 10.3‰, calculated by adding marine zooplankton (8‰) to our fractionation value for hula fish muscle. Marine zooplankton δ^{15}N was determined from samples of the abundant copepod *Temora turbinata* made in

Jervis Bay during the mid-summer of 1999, and exhibited no enrichment from the marine POM (T. Compton and I. M. Suthers, unpublished data). $\delta^{15}N_h$ is the actual average isotope composition of hula fish muscle measured at JB* (11.8‰). $\delta^{15}N_s$ is the calculated isotope composition of hula fish muscle if supported entirely by sewage-derived POM (12.6‰; Compton 2000; Gaston 2003; Fig. 6). Using our mixing model, 50% of the nitrogen of the hula fish food chain around the Jervis Bay outfall may be derived from tertiary treated sewage effluent. Similarly, using the mixing model of Phillips and Gregg (2001), 53% of the nitrogen was derived from the Jervis Bay outfall (39% lower and 67% upper, 95% confidence interval). Off Port Stephens, $\delta^{15}N_m$ is 9.6‰, $\delta^{15}N_h$ is 10.6‰ and $\delta^{15}N_s$ is similarly calculated as 12.6‰ (using bulk zooplankton as 7‰, and sewage POM 10.3‰). As a result, ~33% of the nitrogen in the hula fish food chain around the Port Stephens outfall may be derived from secondary treated sewage effluent. Our estimate of 30–50% is remarkable considering the potential for dilution in the coastal ocean, and highlights the nurturing that is provided by a continuous discharge of sewage effluent, regardless of its volume. With ongoing upgrades to sewage treatments from primary to tertiary, ^{15}N and ^{34}S may become useful tracers of anthropogenic nutrients in an oligotrophic environment. The shift in stable isotope values of hula fish between years highlights the importance of a sampling design incorporating multiple temporal scales.

Acknowledgments

We acknowledge the financial support of the Australian Research Council and the Sea World Research and Rescue Foundation Inc. Thanks to Richard Piola who assisted with many of the analyses, and to Sharon Longford, Steven Rutten and Jocelyn Dela-Cruz for assistance with fieldwork.

References

Burnett, W. C., and Schaeffer, O. A. (1980). Effect of ocean dumping on $^{13}C/^{12}C$ ratios in marine sediments from the New York Bight. *Estuarine, Coastal and Shelf Science* **11**, 605–611.

Cloern, J. E., Canuel, E. A., and Harris, D. (2002). Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology and Oceanography* **47**, 713–729.

Compton, T. (2000). 'Tracing Marine Nutrients Using Novel ^{15}N Stable Isotope Techniques.' Honours Thesis. (University of New South Wales: Sydney, Australia.)

Darnaude, A. M., Salen-Picard, C., Polunin, N. V. C., and Harmelin-Vivien, M. L. (2004). Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Oecologia* **138**, 325–332. doi:10.1007/S00442-003-1457-3

De Niro, M. J., and Epstein, S. (1977). Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197**, 261–263.

De Niro, M. J., and Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506. doi:10.1016/0016-7037(78)90199-0

De Niro, M. J., and Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* **45**, 341–351. doi:10.1016/0016-7037(81)90244-1

Faganeli, J., Malej, A., Pezdic, J., and Malacic, V. (1988). C:N:P ratios and stable C isotopic ratios as indicators of sources of organic matter in the Gulf of Trieste (Northern Adriatic). *Oceanologica Acta* **11**, 377–382.

Frazer, T. K., Ross, R. M., Quetin, L. B., and Montoya, P. (1997). Turnover of carbon and nitrogen during growth of larval krill, *Euphuasia superba* Dana: a stable isotope approach. *Journal of Experimental Marine Biology and Ecology* **212**, 259–275. doi:10.1016/S0022-0981(96)02740-2

Fry, B. (1988). Food web structure on Georges Bank from stable C, N and S isotopic compositions. *Limnology and Oceanography* **35**, 1182–1190.

Fry, B., and Sherr, E. B. (1984). $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* **27**, 13–47.

Gannes, L. Z., O'Brien, D. M., and Martínez del Rio, C. (1997). Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* **78**, 1271–1276.

Gaston, T. F. (2003). 'Nutrient Sources of Urban Coastal Food Chains.' PhD Thesis. (University of New South Wales: Sydney, Australia.)

Gaston, T. F., and Suthers, I. M. (2004). 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. *Journal of Experimental Marine Biology and Ecology* **304**, 17–33. doi:10.1016/J.JEMBE.2003.11.022

Gearing, J. N., Gearing, P. J., Rudnick, D. T., Requejo, A. G., and Hutchings, M. J. (1984). Isotopic variability of organic carbon in a phytoplankton-based temperature estuary. *Geochimica et Cosmochimica Acta* **48**, 1089–1098. doi:10.1016/0016-7037(84)90199-6

Grigg, R. W. (1994). Effect of sewage discharge, fishing pressure and habitat complexity on coral ecosystems and reef fishes in Hawaii. *Marine Ecology Progress Series* **103**, 25–34.

Hansson, S., Hobbie, J. E., Elmgren, R., Larsson, U., Fry, B., and Johansson, S. (1997). The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* **78**, 2249–2257.

Heaton, T. H. E. (1986). Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology* **59**, 87–102. doi:10.1016/0009-2541(86)90046-X

Hesslein, R. H., Hallard, K. A., and Ramlal, P. (1993). Replacement of sulphur, carbon, and nitrogen tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 2071–2076.

Holloway, P. E. (1996). A field investigation of water exchange between a small coastal embayment and an adjacent shelf. In 'Mixing in Estuaries and Coastal Seas'. (Ed. C. Pattiaratchi.) pp. 145–158. (American Geophysical Union: Washington DC, USA.)

Hutchins, B., Swainston, R. (1986). 'Sea fishes of southern Australia.' (Swainston Publishing: Perth, Australia.)

Jones, A. B., O'Donohue, M. J., Udy, J., and Dennison, W. C. (2001). Assessing ecological impacts of shrimp and sewage effluent, biological indicators with standard water quality analyses. *Estuarine, Coastal and Shelf Science* **52**, 91–109. doi:10.1006/ECSS.2000.0729

Kaplan, I. R., Emery, K. O., and Rittenberg, S. C. (1963). The distribution and isotopic abundance of sulfur in recent marine sediments off southern California. *Geochimica et Cosmochimica Acta* **27**, 297–331. doi:10.1016/0016-7037(63)90074-7

Lancaster, J., and Waldron, S. (2001). Stable isotope values of lotic invertebrates: Sources of variation, experimental design and statistical interpretation. *Limnology and Oceanography* **46**, 723–730.

Macko, S. S., Lee, W. Y., and Parker, P. L. (1982). Nitrogen and carbon isotope fractionation by two species of marine amphipods: laboratory and field studies. *Journal of Experimental Marine Biology and Ecology* **63**, 145–149. doi:10.1016/0022-0981(82)90028-4

MHL (1997). 'Review of Sewage Effluent Ocean Outfall Performance in NSW.' Report MHL769. (Manly Hydraulics Laboratory, Department of Public Works and Services: Sydney, Australia.)

Minagawa, M., and Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta* **48**, 1135–1140. doi:10.1016/0016-7037(84)90204-7

Moore, M. J., Shea, D., Hillman, R. E., and Stegeman, J. J. (1996). Trends in hepatic tumours and hydropic vacuolation, fin erosion, organic chemicals and stable isotope ratios in winter flounder from Massachusetts, USA. *Marine Pollution Bulletin* **32**, 458–470. doi:10.1016/0025-326X(96)84962-9

Peterson, B. J., and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**, 293–320. doi:10.1146/ANNUREV.ES.18.110187.001453

Peterson, B. J., Howarth, R. W., and Garritt, R. H. (1985). Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* **227**, 1361–1363.

Phillips, D. L., and Gregg, J. W. (2001). Uncertainty in source partitioning using stable isotopes. *Oecologia* **127**, 171–179. doi:10.1007/S004420000578

Pinnegar, J. K., and Polunin, N. V. C. (1999). Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. *Functional Ecology* **13**, 225–231. doi:10.1046/J.1365-2435.1999.00301.X

Radtke, R. L., Showers, W., Moksness, E., and Lenz, P. (1996). Environmental information stored in otoliths: insights from stable isotopes. *Marine Biology* **127**, 161–171.

Rau, G. H., Ainley, D. G., Bengston, J. L., Torres, J. J., and Hopkins, T. L. (1992). $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell Sea birds, seals and fish: implications for diet and trophic structure. *Marine Ecology Progress Series* **84**, 1–8.

Rau, G. H., Mearns, A. J., Young, D. R., Olson, R. J., Schafer, H. A., and Kaplan, I. R. (1983). Animal $^{13}C/^{12}C$ correlates with trophic level in pelagic food webs. *Ecology* **64**, 1314–1318.

Rau, G. H., Sullivan, C. W., and Gordon, L. I. (1991). $\delta^{13}C$ and $\delta^{15}N$ variations in Weddell Sea particulate organic matter. *Marine Chemistry* **35**, 355–369.

Richard, P., Riera, P., and Galois, R. (1997). Temporal variations in the chemical and carbon isotope compositions of marine and terrestrial organic inputs in the Bay of Marennes-Oléron, France. *Journal of Coastal Research* **13**, 879–889.

Riera, P. (1998). $\delta^{15}N$ of organic matter sources and benthic invertebrates along an estuarine gradient in Marennes-Oléron Bay (France): implications for the study of trophic structure. *Marine Ecology Progress Series* **166**, 143–150.

Scanes, P. R., Scanes, K., and Otway, N. M. (1995). Environmental problems due to disposal of wastes. In 'Coastal marine ecology of temperate Australia'. (Eds A. J. Underwood and M. G. Chapman.) pp. 297–310. (UNSW Press: Sydney, Australia.)

Schoeninger, M. J., and De Niro, M. J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta* **48**, 625–639. doi:10.1016/0016-7037(84)90091-7

Sholto-Douglas, A. D., Field, J. G., James, A. G., and van der Merwe, N. J. (1991). $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios in the Southern Benguela Ecosystem: indicators of food web relationships among different size-classes of plankton and pelagic fish; differences between fish muscle and bone collagen tissues. *Marine Ecology Progress Series* **78**, 23–31.

Smith, A. K., and Suthers, I. M. (1999). Effects of sewage effluent discharge on the abundance, condition and mortality of hulafish, *Trachinops taeniatus* (Plesiopidae). *Environmental Pollution (Barking, Essex: 1987)* **106**, 97–106. doi:10.1016/S0269-7491(99)00054-8

Smith, A. K., Ajani, P. A., and Roberts, D. E. (1999). Spatial and temporal variation in fish assemblages exposed to sewage and implications for management. *Marine Environmental Research* **47**, 241–260. doi:10.1016/S0141-1136(98)00120-2

Smith, S. D. A. (1994). Impact of domestic sewage effluent versus natural background variability: an example from Jervis Bay, New South Wales. *Australian Journal of Marine and Freshwater Research* **45**, 1045–1064.

Spies, R. B., Kruger, H., Ireland, R., and Rice, D. W. Jr (1989). Stable isotope ratios and contaminant concentrations in a sewage-distorted food web. *Marine Ecology Progress Series* **54**, 157–170.

Sweeney, R. E., and Kaplan, I. R. (1980). Natural abundances of ^{15}N as a source indicator for near-shore marine sedimentary and dissolved nitrogen. *Marine Chemistry* **9**, 81–94. doi:10.1016/0304-4203(80)90062-6

Sweeney, R. E., Kalil, E. K., and Kaplan, I. R. (1980). Characterisation of domestic and industrial sewage in Southern California coastal sediments using nitrogen, carbon, sulphur and uranium tracers. *Marine Environmental Research* **3**, 225–243. doi:10.1016/0141-1136(80)90029-X

Thomas, C. J., and Cahoon, L. B. (1993). Stable isotope analyses differentiate between different trophic pathways supporting rocky-reef fishes. *Marine Ecology Progress Series* **95**, 19–24.

Thornton, S. F., and McManus, J. (1994). Application of organic carbon and nitrogen stable isotope and C/N ratios as sources indicators of organic matter provenance in estuarine systems: evidence from the Tray Estuary, Scotland. *Estuarine, Coastal and Shelf Science* **38**, 219–233. doi:10.1006/ECSS.1994.1015

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., and Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia* **57**, 32–37.

Tucker, J., Sheats, N., Giblin, A. E., Hopkinson, C. S., and Montoya, J. P. (1999). Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. *Marine Environmental Research* **48**, 353–375. doi:10.1016/S0141-1136(99)00069-0

Underwood, A. J., Chapman, M. G., Richards, S. A. (1998). 'GMAV5 for Windows.' (Institute of Marine Ecology, University of Sydney: Sydney, Australia.)

Wong, W. W., and Sackett, W. M. (1978). Fractionation of stable carbon isotopes by marine phytoplankton. *Geochimica et Cosmochimica Acta* **42**, 1809–1815. doi:10.1016/0016-7037(78)90236-3

Manuscript received 23 September 2003; revised 19 July 2004; and accepted 19 August 2004.