

Food webs of two intermittently open estuaries receiving ^{15}N -enriched sewage effluent.

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Running head: Sewage enrichment of $\delta^{15}\text{N}$ signatures

Abstract

Carbon and nitrogen stable isotope signatures were used to assess the response of food webs to sewage effluent discharged into two small intermittently open estuaries in northern New South Wales, Australia. One of these systems, Tallows Creek, has a history of direct sewage inputs, whilst the other, Belongil Creek, receives wastewater via an extensive wetland treatment system. The food webs of both systems were driven by algal sources of carbon, reflecting high autotrophic productivity in response to the nutrients entering the system from sewage effluent. All aquatic biota collected from Tallows Creek had significantly enriched $\delta^{15}\text{N}$ signatures relative to their conspecifics from Belongil Creek, indicating that sewage nitrogen had been assimilated and transferred throughout the Tallows Creek food web. These $\delta^{15}\text{N}$ values were higher than those reported from studies in permanently open estuaries receiving sewage effluent. We suggest that these enriched signatures and the transfer of nitrogen throughout the entire food web reflect differences in hydrology and associated nitrogen cycling processes between permanently open and intermittently open estuaries. Although all organisms in Tallows Creek were generally ^{15}N -enriched, isotopically light (less ^{15}N -enriched) individuals of estuary perchlet (*Ambassis marianus*) and sea mullet (*Mugil cephalus*) were also collected. These individuals were most likely recent immigrants into Tallows Creek, as this system had only recently been opened to the ocean. This isotopic discrimination between resident (enriched) and immigrant (significantly less enriched) individuals can provide information on fish movement patterns and the role of heavily polluted intermittently open estuaries in supporting commercially and recreationally valuable estuarine species.

Keywords: stable isotopes, carbon, nitrogen, ^{15}N -enriched, trophic transfer, estuarine ecology, Australia, New South Wales, Byron Bay

1. Introduction

Eutrophication is one of the greatest threats to aquatic ecosystem health and integrity worldwide (Balls et al. 1995, Braga et al. 2000, Davis and Koop 2006). Whilst urban stormwater, industrial and agricultural inputs can represent substantial sources and quantities of nutrients to receiving waters (Vitousek et al. 1997, Anderson et al. 2002, Nedwell et al. 2002), sewage effluent discharges are often the most significant point source of nutrients and contaminants to waterways, particularly in heavily populated regions (Cromeey et al. 1998, Alonso-Rodriguez et al. 2000, Riera et al. 2000). The impacts of sewage effluent on aquatic fauna and flora have received particular attention in open estuaries and bays (Hall et al. 1997, Lake et al. 2001, Seguel et al. 2001, Costanzo et al. 2003, Davis and Koop 2006). However, eutrophication and its effects on aquatic processes and organisms are poorly understood in intermittently open and closed estuaries. Given their prevalence along low energy coastlines worldwide (Roy et al. 2001, Raz-Guzman and Huidobro 2002, Newton and Mudge 2005, Vorwerk *et al.* 2003) and their distinctive biophysical characteristics and ecological, recreational and commercial values (Pollard 1994a, Griffiths 1999, Gray and Kennelly 2003), it is timely that an investigation of the effects of sewage effluent inputs be conducted in representatives from this special class of estuaries (Roy et al. 2001).

In eastern Australia, intermittently open estuaries form in response to the combined influences of seasonally low riverine flow conditions and a consistent north westerly migration of sand along the coast (Griffiths 1999, Roy et al. 2001). When sand bars or berms form and close estuary entrances, these systems become hydrologically isolated from the ocean (Pollard 1994a, Griffiths 1999, Griffiths 2001a). This loss of connectance may last from days up to years (Griffiths 2001b, Young and Potter 2002) and as a result, the prevailing conditions within intermittently open estuaries range from near freshwater to hypersaline depending on local rainfall, tidal and freshwater flows, surface area, catchment size and catchment characteristics including land use. Depending on the relative importance of these factors, intermittently open estuaries are physico-chemically, biologically and ecologically distinct from the large permanently open estuaries that drain mainland Australia (Pollard 1994b, Young et al. 1997, Roy et al. 2001, Davis and Koop 2006). Importantly, in systems that infrequently open to the ocean, reduced flushing frequency and high water residence times are likely to facilitate their role as sinks for nutrients and contaminants (Rasmussen and Josefson 2002, Davis and Koop 2006). Many of these intermittently open estuaries, particularly those along the eastern Australian coastline, are becoming eutrophic yet there is limited information on the ecological effects of ongoing nutrient loading (Davis and Koop 2006).

Many studies examining the impacts of sewage effluent have assessed nitrogen stable isotope signatures ($\delta^{15}\text{N}$), as effluent typically has an enriched $\delta^{15}\text{N}$ signature relative to that of the receiving waters and can therefore be used to map and trace the uptake of nitrogen (Costanzo et al. 2001, Waldron et al. 2001, Parnell 2003, Gaston et al. 2004).

Previous studies have shown sewage effluent to be a major contributor to the nitrogen isotope signatures of algae (Costanzo et al. 2000), macroinvertebrates (Risk and Erdmann 2000, deBruyn and Rasmussen 2002), individual fish species (Gaston et al. 2004) and taxonomic groups (e.g., the fish assemblage - Schlacher et al. 2005). However, few studies have investigated the uptake and assimilation of ^{15}N -enriched sewage effluent through the entire food web (but see Hansson et al. 1997).

In systems that infrequently open to the ocean, reduced flushing frequency and high water residence times are likely to facilitate their role as sinks for nutrients and contaminants (Rasmussen and Josefson 2002, Davis and Koop 2006). Ongoing sewage effluent discharge into an intermittently open estuary is therefore likely to lead to a comparatively greater $\delta^{15}\text{N}$ enrichment, relative to open estuaries and bays, as flushing and tidal action will not have the opportunity to dilute and distribute the effluent (Rasmussen and Josefson 2002). Furthermore, high organic loads like those from sewage treatment plants coupled with extended periods of low dissolved oxygen concentrations may lead to further increases in $\delta^{15}\text{N}$ signatures via the process of denitrification, whereby the lighter (^{14}N) isotope may be preferentially removed from the system (Heggie et al. 1999, Davis and Koop 2006). Examination of $\delta^{15}\text{N}$ signatures in sediments and biota may therefore provide particular insights into the eutrophication process in these distinctive estuarine systems.

Stable isotope signatures have also been used to assess movements of biota within and between aquatic ecosystems (Hansson et al. 1997, Hobson 1999, Herzka 2005). Whilst

most studies have used $\delta^{13}\text{C}$ signatures to examine patterns of movement of consumers between two or more habitats with different source $\delta^{13}\text{C}$ signatures (Hobson 1999), Hansson et al. (1997) showed that enriched $\delta^{15}\text{N}$ signatures from sewage effluent can also provide useful information relating to residency and movement of fish between sites. In intermittently open and closed estuaries, opening events provide opportunities for mass migration and recruitment (Neira and Potter 1992, Griffiths 1999, Bell et al. 2001).

In this study, we used stable isotopes to examine patterns of nitrogen and carbon flow through the food webs of two intermittently open estuaries in northern New South Wales, Australia. Specifically, we aimed to identify a) the principal sources of carbon supporting higher consumers in these systems, b) the effects of sewage effluent in terms of the degree to which resident biota throughout the food web displayed elevated $\delta^{15}\text{N}$ signatures, c) the degree to which $\delta^{15}\text{N}$ signatures of biota in intermittently open estuaries receiving sewage effluent were comparable to values published from studies in macrotidal estuaries and bays. We predicted that the biota in Tallows Creek, the system receiving direct inputs of treated sewage, would have significantly higher $\delta^{15}\text{N}$ signatures than the neighbouring, less enriched system (Belongil Creek) and other estuaries. Finally, we also had the opportunity to investigate variations in $\delta^{15}\text{N}$ signatures between Tallows and Belongil Creeks as a means to identify recruitment patterns of highly mobile fish species.

2. Materials and Methods

2.1. Study sites

This study was conducted in Tallows and Belongil Creeks, two small intermittently open estuaries in northern New South Wales, Australia (Figure 1). Tallows Creek has a catchment area of 4.5 km² and a waterway surface area of 0.125 km² and Belongil Creek has a catchment area of 30 km² and a waterway surface area of 0.3 km² (NSW DNR 2005). Catchments of both systems have a diverse range of land uses including extensive agricultural and urban developments. Both creeks have received large loads of nutrients over the past 50 years, principally from sewage treatment plants (STPs) that discharge into their waters (McAlister et al. 2000). However, Belongil Creek receives wastewater that flows through an extensive wetland treatment system before it enters the waters of the creek. The success of this treatment process is reflected in the nutrient concentrations in this system, with total phosphorus, total nitrogen and chlorophyll a concentrations of 0.05-0.2 mg l⁻¹, 0.5-1.5 mg l⁻¹ and 2-10 mg l⁻¹, respectively (McAlister et al. 2000). These nutrient concentrations fall within the Australia and New Zealand Environment and Conservation Council (ANZECC 2001) recommended water quality guidelines during wet weather conditions, but may partially exceed the guidelines during dry periods (McAlister et al. 2000). In contrast, Tallows Creek receives effluent directly from a STP that uses comparatively old treatment technologies. McAlister et al. (2000) found that nutrient and chlorophyll a concentrations in Tallows Creek consistently exceeded the ANZECC water quality guidelines (ANZECC 2001), with total nitrogen ranging from 1.5 – 3 mg l⁻¹, total phosphorus from 0.4 – 1.6 mg l⁻¹ and chlorophyll a from 5 – 80 mg l⁻¹.

[INSERT FIGURE 1 HERE]

2.2. Site hydrology

Samples for stable isotope analyses of food web structure and function were collected in both systems in early May 2003. Sampling in Tallows Creek occurred during a period of time (May 5-12) when the entrance status changed from closed to open (personal observation). In contrast, Belongil Creek had been open and subject to tidal influences for the two months preceding sampling.

2.3. Sampling methods

Given the relatively small areal extent of Tallows and Belongil Creeks, replicate samples were collected from sites located less than 500 m from the entrance of both systems. The reported mean (\pm S.E.) stable isotope signatures consequently represent the variability in source and consumer isotopic signatures in the proximity of the mouth of both systems.

In each system we collected the dominant primary sources of carbon, namely riparian vegetation, mangroves (*Avicennia marina*), benthic fine particulate organic matter (FPOM), benthic coarse particulate organic matter (CPOM), epilithon and filamentous algae and seston (suspended particulate organic matter). Samples of riparian vegetation and mangroves were collected by hand, while FPOM and CPOM samples were collected by sifting benthic sediments through a series of graded sieves (250 μ m - 500 μ m - 1 cm). FPOM samples were obtained from the 250 μ m sieve and CPOM samples were collected from the 500 μ m sieve. Epilithon was carefully removed from sediment surfaces using forceps and a scalpel. Similarly, we used a scalpel and fine toothed brush to scrape filamentous algae from all available surfaces, including mangrove pneumatophores, rocks

and woody debris. Bulk seston samples were collected and concentrated from surface waters using a plankton tow net (65 μm mesh size) hauled along a 20 transect at each site. Seston samples were usually comprised of suspended organic matter, phytoplankton and zooplankton. There were insufficient quantities of these components to facilitate separate isotopic analyses for each, so bulk seston samples were run as a composite of all epilimnetic sources of organic matter.

Aquatic macroinvertebrates and small fish were collected from littoral habitats using a dip net (mesh size 250 μm) and a small purse seine net (length 2m, mesh size 1mm). Larger fish and macroinvertebrates were collected using a 20m seine net (6mm mesh) trawled over predominantly sandy substrates in close proximity to littoral vegetation and submerged structures and debris. Sediment-dwelling organisms, including bivalve molluscs and polychaete worms, were collected using a yabby pump. Small mobile macroinvertebrates were collected opportunistically by hand.

Upon collection, all samples were immediately placed in individually labelled zip-lock bags and stored on ice. This approach allows sufficient time for small organisms to void their guts, to ensure that isotope signatures reflect consumer tissues only and are not influenced by gut contents (Hadwen and Bunn 2004, Hadwen and Bunn 2005). Samples were then frozen for transportation back to the laboratory.

2.4. Laboratory sample processing

In the laboratory, samples of riparian vegetation, FPOM, CPOM, attached algae and

mangroves were rinsed with distilled water to wash away dirt and debris. All samples were dried in an oven at 60°C for at least 48 hours. Dried samples were pulverised in a puck and ring grinding mill for approximately 3 minutes, or until the sample had been reduced to a fine powder. Ground samples were subsequently stored in 5 ml vials and frozen prior to stable isotope analysis.

All aquatic macroinvertebrates were rinsed and dried before being ground using a mortar and pestle. Individuals were ground whole, but ground individuals of the same taxa were often subsequently pooled to ensure that sample mass was sufficient to enable isotopic analyses. The exoskeletons of all crustaceans were removed using forceps to ensure that accumulated calcium carbonate did not influence carbon isotopic values (*sensu* Bunn et al. 1995, Leggett *et al.* 1999).

2.5. Analytical methods

Stable isotope samples were analysed using a continuous flow-isotope ratio mass spectrometer (Micromass Isoprime EuroVector EA300, Manchester, UK) at Griffith University. Isotope ratios are expressed as either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ and relate to the ratio of ^{13}C : ^{12}C and ^{14}N : ^{15}N , respectively. Values were calculated according to the equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$$

where R_{sample} is the isotopic ratio for the sample and R_{standard} is the isotopic ratio of the standard (PeeDee belemnite carbonate for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$).

Nitrogen and carbon stable isotope signatures of food web components collected from both creeks were compared statistically using a t-test (Sokal and Rohlf 1981).

Food web analyses were conducted using the IsoSource mixing model software developed by Phillips and Gregg (2003). This model calculates feasible combinations (in 1% increments) of autotroph isotope signatures that explain observed consumer isotope signatures. In our analyses, combinations of end member signatures that added to within 0.01‰ of the consumer signature were considered feasible. Trophic fractionations of carbon are generally low (less than 1‰) and we used no correction in these analyses in light of values reported in the literature (Peterson and Fry 1987, McCutchan *et al.* 2003, Hadwen and Bunn 2004). Nitrogen isotope signatures were not included in these analyses due to unknown levels of fractionation in the study organisms (*sensu* Connolly *et al.* 2005), particularly in the sewage-enriched Tallows Creek ecosystem.

The end members we used in the IsoSource mixing model were epilithon, filamentous algae, mangroves (*Avicennia marina*) and riparian vegetation. Seston, CPOM and FPOM were excluded on the basis that each of these sources is a mixture of other end members. Preliminary analyses revealed that CPOM was largely derived from riparian vegetation and FPOM and seston were combinations of autochthonous (algal) and allochthonous (mangroves and riparian vegetation) carbon sources (data not presented).

3. Results

3.1. Comparison of isotope signatures of biota in Tallows Creek and Belongil Creek

At all trophic levels, the Tallows Creek food web was ^{15}N -enriched relative to the Belongil Creek food web (Table 1, Figure 2, Figure 3). This enrichment was substantial, with a mean difference in $\delta^{15}\text{N}$ signatures of 9.1‰ for primary sources and 11.6‰ for consumers. With the exception of riparian vegetation, which did not differ significantly between the two sites, all other components sampled in both sites were significantly ^{15}N -enriched in Tallows Creek, relative to Belongil Creek (Table 1, t-test, $p < 0.05$, Figure 2).

[INSERT TABLE 1 HERE]

Despite the significant differences in nitrogen isotope signatures, there were few statistically significant differences in the $\delta^{13}\text{C}$ signatures of biota sampled from Tallows and Belongil Creeks (Table 1, Figure 2). Sewage effluent did not significantly alter the carbon isotope signatures of primary source materials in Tallows Creek (Figure 2).

Whilst some consumers were significantly less ^{13}C -depleted in Tallows Creek, reflecting a slightly greater dependence on algal carbon as a food resource relative to conspecifics from Belongil Creek, there was surprisingly little variability in carbon isotope signatures for most taxa collected from both systems.

[INSERT FIGURE 2 HERE]

3.2. Food web analyses

The primary producers in Tallows and Belongil Creeks had $\delta^{13}\text{C}$ signatures that spanned a wide range of values (Figure 2, Figure 3), facilitating analyses of the percent contribution of the major food resources to consumer diets. Sources of riparian vegetation had depleted $\delta^{13}\text{C}$ signatures relative to all other primary sources, with values around -29‰ (Figure 3). In both systems, benthic algae were consistently ^{13}C -enriched relative to other primary food sources (Figure 3). However, benthic algal $\delta^{13}\text{C}$ signatures ranged from -17 to -26 ‰, reflecting both the spatial variability of algal sources as well as the variety of algal types sampled. For example, epilithon collected from the sediment had $\delta^{13}\text{C}$ values ranging from -22‰ to -27‰, whereas filamentous algae collected from wood and/or cobbles ranged from -16‰ to -20‰ (Figure 3).

[INSERT FIGURE 3 HERE]

In both Tallows and Belongil Creeks most consumers spanned the range of $\delta^{13}\text{C}$ signatures of algal resources (filamentous green algae and epilithon) (Figure 3). However, the carbon isotope signatures of some individuals collected from Belongil Creek fell outside the range of end members. As a result, we were not able to calculate source contributions for surf clams (*Donax deltoids*) sand whiting (*Sillago ciliata*) and sea mullet #3 (*Mugil cephalus*) from Belongil Creek (Appendix A). For the remaining individuals, IsoSource analyses of carbon stable isotope data revealed that consumers in both systems derived, on average, more than 60% of their carbon from algal sources (Appendix A).

3.3. Intraspecific variation in $\delta^{13}C$ signatures

Despite comparable algal contributions to the food webs of Tallows and Belongil Creeks, we calculated substantial intrapopulation variability in source contributions for some fish species. For example, *Mugil cephalus* individuals from both systems had highly variable carbon isotope signatures (range in Tallows Creek from -17.78‰ to 25.90‰ and in Belongil Creek from -17.01‰ to -29.19‰ , Figure 4) indicative of high variability in the contribution of food sources to individual diets. This carbon isotope variability resulted in highly variable output from the IsoSource analyses (Appendix A), with some individuals deriving most of their carbon from algal sources (especially individuals 1, 2 and 6 from Belongil Creek and individuals 1, 2 and 5 from Tallows Creek) and others relying strongly on riparian contributions of carbon (especially individuals 4 and 5 from Belongil Creek and individual 3 from Tallows Creek).

3.4. Intraspecific variation in $\delta^{15}N$ signatures

Despite mean $\delta^{15}N$ signatures in Tallows Creek being significantly enriched relative to Belongil Creek for all consumer taxa (Table 1, Figure 2), some individuals of *Ambassis marianus* and *Mugil cephalus* had $\delta^{15}N$ signatures that seemed out of step with their local food web and were less ^{15}N -enriched than expected for long-term residents of Tallows Creek (this is reflected in the substantial y axis error bars for these species in Figure 3). Examination of individual nitrogen isotope signatures revealed that for both of these species, at least one individual collected from Tallows Creek had a $\delta^{15}N$ signature more similar to those displayed in Belongil Creek than that measured for their conspecifics in Tallows Creek (Figure 4). These fish may have been new recruits that had recently

entered Tallows Creek, following the opening event that occurred prior to the completion of our sampling.

[INSERT FIGURE 4 HERE]

Further support for our view that the ^{15}N -enriched individuals were residents in Tallows Creek is the absence of any consistent patterns in ^{15}N -enrichment with resource use and size class (Figure 4). For example, the observed $\delta^{15}\text{N}$ signatures of *Mugil cephalus* and *Ambassis marianus* individuals were clearly not related to ontogenetic (size class) shifts in nitrogen assimilation or fractionation (Figure 4). Furthermore, the absence of trends in $\delta^{13}\text{C}$ signatures with size class suggests that the observed $\delta^{15}\text{N}$ signatures were not being driven by feeding preferences or shifts in resource use by individual fish (Figure 4).

Our analyses also revealed an *M. cephalus* individual in Belongil Creek with a $\delta^{15}\text{N}$ signature of 15 ‰ (Figure 4). This signature is considerably higher than those of its conspecifics in Belongil Creek and might, therefore, represent a recent movement into this system (from one with an enriched signature) by this individual.

5. Discussion

5.1. Sewage effluent and $\delta^{15}\text{N}$ signatures of biota in Tallows Creek

In this study, we found that aquatic biota collected from Tallows Creek (with a history of direct inputs of treated sewage) tended to have significantly enriched $\delta^{15}\text{N}$ signatures relative to their conspecifics from Belongil Creek (receiving wetland-treated wastewater).

Our data suggest that sewage-derived nitrogen had been widely assimilated by producers in Tallows Creek and had been transferred through all trophic levels of the food web. The comparatively low $\delta^{15}\text{N}$ signatures of biota in Belongil Creek presumably reflect both the high efficiency of assimilation/denitrification in the artificial and natural wetland systems and the degree to which additional nitrogen sources from other land uses dilute the influence of sewage-derived nitrogen loads in this much larger catchment. Despite the abundance of studies that have used $\delta^{15}\text{N}$ as a tracer of sewage effluent (Costanzo et al. 2000, Gaston et al. 2004, Schlacher et al. 2005), our findings in Tallows Creek stand out as being some of the first to show the degree to which ^{15}N -enriched sewage effluent has been assimilated and distributed through an entire food web.

At each trophic level in Tallows Creek, the $\delta^{15}\text{N}$ values we measured tended to be higher than those reported in other aquatic systems receiving sewage effluent including rivers (deBruyn and Rasmussen 2002, deBruyn et al. 2003), permanently open estuaries (Costanzo et al. 2003, Schlacher et al. 2005, Barnard et al. 2006), bays (Hansson et al. 1997, Waldron et al. 2001) and coral reefs (Heikoop et al. 2000, Gaston et al. 2004). However Jones et al. (2001) recorded algal $\delta^{15}\text{N}$ values as high as 19.6‰ in a permanently open estuary in southeast Queensland immediately downstream from a sewage treatment plant. Although the highest $\delta^{15}\text{N}$ signatures of algal sources (epilithon and filamentous green algae and red algae) in our study were slightly less ^{15}N -enriched than those from Jones et al. (2001), the range of algal values (8.9 – 17.4‰) fell well within theirs (6.4 – 19.6‰). Jones et al. (2001) did not measure $\delta^{15}\text{N}$ enrichment of higher trophic levels and measuring sewage impacts in aquatic ecosystems only at the

primary producer level (e.g. algae and seagrass) does not provide any evidence of the implications of nutrient enrichment for the higher organisms within the system (Schlacher et al. 2005). In contrast, our results present strong evidence of the assimilation and trophic transfer of effluent nitrogen up through the entire food web to the highest consumers.

The $\delta^{15}\text{N}$ signatures of the highest consumers - fish - measured in this study were also higher than those reported in the estuarine literature. Although direct comparisons of species isotope signatures are not always possible, the recent study by Schlacher et al. (2005) provides a useful data set for comparisons with ours for two reasons. First, their study was geographically close in proximity (less than 300 km to the north of Byron Bay), and second, many of the species they assessed were present in our collections. In our study, *Sillago ciliata* individuals from Tallows Creek had a mean $\delta^{15}\text{N}$ value of 24.64‰ (SE \pm 0.6) (Figure 2). In a comparable system, albeit a permanently open estuary, also receiving sewage effluent discharges, Schlacher et al. (2005) reported mean $\delta^{15}\text{N}$ signatures for *S. ciliata* only as high as 15.11‰ (Table 2). With the exception of *Rhabdosargus sarba*, the $\delta^{15}\text{N}$ signatures of specimens from Tallows Creek were consistently enriched (with a mean difference of + 6.56‰) relative to the $\delta^{15}\text{N}$ signatures presented in Schlacher et al. (2005) (Table 2). We propose that this substantial degree of enrichment (above that reported by Schlacher et al. 2005) highlights the significance of differences in hydrology (tidal influence) and nitrogen cycling (denitrification and sediment residence times) between permanently open macrotidal estuaries and intermittently open estuaries.

[INSERT TABLE 2 HERE]

5.2. Food web structure and function in Tallows and Belongil Creeks

Despite the heavily enriched nitrogen isotope signatures and high ambient nutrient concentrations (McAlister et al. 2000) in Tallows Creek relative to those in Belongil Creek, there was surprisingly little difference in the structure of the food webs of these two intermittently open estuaries (Appendix A). On average, consumers derived between 62% and 69% of their nutrition from the abundant algal (epilithon and filamentous) resources sampled in Belongil and Tallows Creeks, respectively. These results are consistent with the general observation that within-system algal production is primarily responsible for supplying the nutrition required by invertebrates (Bouillon et al. 2002, Page and Lastra 2003, Martineau et al. 2004) and fish (Kitting et al. 1984, Miller et al. 1996, Vizzini and Mazzola 2003) in estuarine environments.

Whilst algae were clearly the most important sources of consumer nutrition in both Tallows and Belongil Creeks, we calculated that mangrove and riparian vegetation sources of carbon contributed up to a one third of the nutrition of consumers in both systems. In contrast, many researchers have found surprisingly little evidence of contributions of mangrove and riparian vegetation carbon to consumers (Boon et al. 1997, Loneragan et al. 1997, Page and Lastra 2003). Bouillon et al. (2002) even suggested that benthic invertebrates showed a strong preference for local algal resources and that the export of mangrove and terrestrial detritus from estuaries during periods of

high flow further reduced the likelihood that these carbon sources would be assimilated by benthic fauna. In light of this suggestion, we propose that the increased residence time of terrestrial and mangrove-derived carbon sources in these intermittently open estuaries (relative to permanently open estuaries) might explain this higher per cent contribution to consumers. Future research will investigate the relative contribution of terrestrial and mangrove sources of carbon in a series of closed and open estuaries, to specifically examine the influence of entrance status (and potential for export of terrestrial and mangrove sources of carbon) on carbon flow pathways in estuarine food webs.

5.3. Intraspecific variability in $\delta^{13}C$ signatures for *Mugil cephalus*

We measured a wide range of carbon isotope signatures for *Mugil cephalus* individuals, both within and between the two study systems. We propose that this variability is most likely due to the benthic feeding strategy employed by this highly mobile species (Coleman and Mobley 1984, Wells 1984, Pusey et al. 2004) and that it might reflect the patchiness of resources within these two intermittently open estuaries. *M. cephalus* is a filter-feeding detritivore that typically filters detritus and other organic particles through a series of minute pharyngeal teeth (Pusey et al. 2004). This foraging strategy tends to lead to a diet dominated (up to 80%) by algal and detritus food sources, although several researchers have also reported high proportions of zooplankton and macroinvertebrates in the guts of some *M. cephalus* individuals (Coleman and Mobley 1984, Pusey et al. 2004). Similarly, Gu et al. (1997) reported a broad range of carbon isotope signatures within a population of blue tilapia (*Oreochromis aureus*) in a lake in Florida. This cichlid also consumes a wide variety of dietary items and Gu et al. (1997) concluded that for species

like blue tilapia and others that feed at low trophic levels, attention should be given to the potential for wide ranges in carbon isotope signatures owing to the feeding plasticity of these organisms. When present, this variability has consequences for assessments of carbon flow and diets in aquatic food webs, particularly when small numbers of individuals are used to generate general conclusions regarding food web structure and function (Gu et al. 1997).

5.4. Patterns of fish movement inferred from nitrogen isotope signatures

Several researchers have used stable isotopes to assess movements of biota within and between aquatic ecosystems (Hansson et al. 1997, Hobson 1999, Herzka 2005). Whilst most studies have used $\delta^{13}\text{C}$ signatures to examine patterns of movement of consumers between two or more habitats with different source $\delta^{13}\text{C}$ signatures (Hobson 1999), Hansson et al. (1997) showed that enriched $\delta^{15}\text{N}$ signatures from sewage effluent can also provide useful information relating to residency and movement of fish between sites. On the basis of our findings, we propose that the greatly enriched nitrogen isotope signatures of biota in Tallows Creek can provide useful information on the movement of commercially important fish species such as *M. cephalus* into and out of this system during the intermittent periods of connectance with the ocean (i.e. when the entrance is artificially or naturally opened).

In large estuaries, the applicability of consumer isotope signatures as indicators of trophic transfer of sewage effluent is likely to be determined by the degree of site fidelity shown by target species. For example, if fish move between sites with varying source isotope

signatures, only moderate levels of ^{15}N -enrichment are likely to be measured in their tissues. Schlacher et al. (2005) proposed the use of fish $\delta^{15}\text{N}$ signatures as an indicator of sewage pollution in estuarine environments receiving wastewater and provided evidence of high site fidelity for a range of estuarine fish species. In our study, resident taxa did have elevated $\delta^{15}\text{N}$ signatures in Tallows Creek, but the presence of comparatively ^{15}N -depleted individuals in Tallows Creek suggests that nitrogen isotope signatures may be used to discriminate between residents and recent recruits. These considerable differences in isotope signatures enable us to gain an insight into patterns of fish movement between intermittently open estuaries and nearby coastal waters with variable sewage effluent inputs.

The use of $\delta^{15}\text{N}$ signatures to examine patterns of fish movement is particularly important given the scant knowledge for most species, including those of commercial and recreational value. For example, for sea mullet (*Mugil cephalus*), there is conflicting evidence for the site fidelity proposed by Schlacher et al. (2005). While Field (1987) reported that juvenile mullet (including *M. cephalus*) show a high level of site fidelity and tend not to mix with neighbouring groups or move between neighbouring estuaries, Pusey et al. (2004) report that at various points in their life cycle, substantial movement within and between estuaries is a common trait of this species, particularly on the east coast of Australia. Specifically, individuals have been reported to re-enter the marine environment (from estuaries) during periods of high rainfall (due to washing out of their detrital food resources). When these mass movements occur, *M. cephalus* individuals tend not to feed (Pusey et al. 2004). This has important implications for the persistence of

elevated nitrogen isotope signatures in individuals from Tallows Creek (due to slower tissue turnover during non-feeding movements – *sensu* Gaye-Siessegger et al. 2004) and their use as an indicator of patterns of movement for this species.

Pusey et al. (2004) indicated that following these *M. cephalus* ‘wash out’ runs, individuals tend to re-enter the original estuary, or move to a neighbouring estuary (typically to the north). Therefore, monitoring stable isotope signatures of immigrants in neighbouring estuaries (like Belongil Creek in this study) might aid in assessing patterns of directional movement in *M. cephalus*. Furthermore, the persistence of enriched isotope signatures in muscle tissues may aid in the determination of contributions of sewage effluent to fish nutrition and fisheries productivity across broader spatial scales.

5.5. Conclusions

We have shown that sewage effluent can be assimilated and subsequently enrich all components of the food web in a small intermittently open estuary. We suggest that given the degree to which biota were ^{15}N -enriched in Tallows Creek (relative to comparable data from permanently open estuaries) in addition to the long residence time in intermittently open estuaries, that these systems act as significant sinks for nutrients. Abundant nutrient sources fuel considerable autochthonous (algal) production in these systems and we determined that the food web is largely based on algal food resources. Finally, we found that the enriched $\delta^{15}\text{N}$ signatures of certain resident biota can be used to assess patterns of movement by mobile fish species (cf. Hansson et al. 1997, Herzka 2005). We propose that heavily enriched systems like Tallows Creek provide excellent

opportunities for understanding ecosystem processes (like nutrient cycling) and assessing patterns of fish recruitment without the inherent costs associated with adding large quantities of ^{15}N -enriched nutrient sources to an otherwise undisturbed site (Peterson 1999, Bedard-Haughn et al. 2003). Studies of this nature are likely to be particularly relevant to coastal resource and fisheries managers, as findings will assist in evaluation of the ecological responses to anthropogenic sources of nutrients and the role that intermittently open estuaries play in sustaining commercially and recreationally valuable fish populations (Griffiths 1999, Watts and Johnson 2004).

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Table 1. Results of statistical comparisons of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope signatures of primary sources and consumers collected from both Tallows and Belongil Creeks in May 2003. Actual $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data (Mean \pm S.E.) is presented in Figure 2. Filamentous algal signatures were not compared given that they were comprised of different algal groups (Belongil – green algae, Tallows – red algae). Consumer species are identified as polychaetes (p), crustaceans (c) or fish (f).

Samples	Tallows Creek	Belongil Creek	P values for comparisons of $\delta^{15}\text{N}$ signatures from Tallows and Belongil Creeks	P values for comparisons of $\delta^{13}\text{C}$ signatures from Tallows and Belongil Creeks
Source Materials				
Riparian Vegetation	2	2	0.670	0.454
Fine Particulate Organic Matter (FPOM)	2	2	0.024*	0.095
Coarse Particulate Organic Matter (CPOM)	2	2	0.010*	0.976
Epilithon	4	3	<0.001*	0.810
Seston	2	2	0.026*	0.907
<i>Avicennia marina</i> (Grey Mangrove)	2	3	0.008*	0.688
Consumers				
<i>Marphysa</i> spp. (p)	4	3	<0.001*	0.006*
Bivalves	3	4	<0.001*	<0.001*
<i>Metapeneus bennettiae</i> ^a (c)	1	6	<0.001*	0.951
<i>Gerres subfasciatus</i> (f)	3	3	<0.001*	0.023*
<i>Ambassis marianus</i> (f)	3	6	0.025*	0.393
<i>Rhabdosargus sarba</i> ^a (f)	1	3	0.011*	0.669
<i>Sillago ciliata</i> (f)	6	3	<0.001*	0.010*
<i>Mugil cephalus</i> (f)	12	7	0.038*	0.700

^a Single sample processed (numerous individuals were pooled to obtain a sample) – no standard error could be calculated.

Table 2. Comparison of mean (\pm S.E.) $\delta^{15}\text{N}$ signatures of species sampled in Tallows Creek (data from this study) and the Maroochy River (data from Schlacher et al. 2005).

	Tallows Creek	Maroochy River	Difference in mean $\delta^{15}\text{N}$ values
Species	$\delta^{15}\text{N}$ ‰	$\delta^{15}\text{N}$ ‰	$\delta^{15}\text{N}$ ‰
<i>Metapenaeus bennettiae</i>	23.23 (^a)	13.07 (0.56)	10.16
<i>Gerres subfasciatus</i>	24.25 (0.12)	14.89 (0.30)	9.36
<i>Ambassis marianus</i>	15.88 (3.46)	15.07 (0.24)	0.81
<i>Acanthopagrus australis</i>	27.44 (1.24)	15.18 (0.52)	12.26
<i>Rhabdosargus sarba</i>	17.01 (^a)	17.52 (^b)	-0.51
<i>Sillago ciliata</i>	24.64 (0.60)	15.11 (0.36)	9.53
<i>Platycephalus fuscus</i>	22.82 (2.25)	14.65 (14.86)	8.17
<i>Mugil cephalus</i>	15.20 (2.03)	12.45 (1.11)	2.75
Mean difference (\pm S.E.)			6.56 (1.70)

^a Composite sample processed (numerous individuals were pooled to obtain a sample) – no standard error could be calculated.

^b no standard error term reported

<i>Mugil cephalus</i> 5	0.04	0.03	0.00	0.10	0.01	0.01	0.00	0.03	0.13	0.09	0.00	0.33	0.83	0.08	0.67	0.96
<i>Mugil cephalus</i> 6	0.06	0.05	0.00	0.17	0.84	0.01	0.81	0.86	0.06	0.04	0.01	0.14	0.04	0.04	0.00	0.11
<i>Mugil cephalus</i> 7	0.26	0.19	0.00	0.76	0.38	0.06	0.23	0.48	0.19	0.14	0.00	0.57	0.17	0.13	0.00	0.51
Averages	0.14	0.10	0.00	0.40	0.55	0.03	0.48	0.61	0.13	0.09	0.00	0.37	0.18	0.08	0.05	0.38
Tallows Creek	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
<i>Marphysa</i> spp.	0.17	0.12	0.00	0.48	0.60	0.04	0.51	0.67	0.12	0.09	0.00	0.35	0.11	0.08	0.00	0.33
Corixids	0.21	0.14	0.00	0.50	0.13	0.07	0.00	0.28	0.34	0.21	0.00	0.77	0.32	0.18	0.00	0.72
Bivalves	0.23	0.15	0.00	0.54	0.14	0.08	0.00	0.30	0.32	0.20	0.00	0.76	0.31	0.18	0.00	0.70
<i>Metapenaeus bennettiae</i>	0.29	0.21	0.00	0.83	0.45	0.11	0.16	0.62	0.14	0.10	0.00	0.38	0.13	0.09	0.00	0.38
<i>Ocypode</i> sp.	0.13	0.10	0.00	0.39	0.75	0.06	0.61	0.82	0.06	0.05	0.00	0.18	0.06	0.04	0.00	0.17
<i>Hallicarcinus</i> sp.	0.25	0.16	0.00	0.56	0.15	0.08	0.00	0.31	0.32	0.19	0.00	0.74	0.29	0.17	0.00	0.66
<i>Anguilla</i> sp.	0.28	0.21	0.00	0.80	0.46	0.11	0.19	0.63	0.13	0.10	0.00	0.40	0.12	0.09	0.00	0.34
<i>Gobiomorphus</i> sp.	0.34	0.23	0.00	0.81	0.25	0.12	0.00	0.45	0.22	0.14	0.00	0.57	0.20	0.13	0.00	0.53
<i>Centropogon australis</i>	0.08	0.06	0.00	0.22	0.04	0.03	0.00	0.12	0.39	0.26	0.00	0.93	0.49	0.23	0.00	0.87
<i>Pelates sexlineatus</i>	0.29	0.21	0.00	0.82	0.43	0.11	0.15	0.60	0.15	0.11	0.00	0.43	0.13	0.10	0.00	0.39
<i>Gerres subfasciatus</i>	0.30	0.22	0.00	0.90	0.42	0.12	0.10	0.60	0.14	0.11	0.00	0.43	0.14	0.10	0.00	0.40
<i>Rhabdosargus sarba</i>	0.33	0.24	0.00	0.97	0.37	0.13	0.03	0.56	0.16	0.11	0.00	0.46	0.14	0.10	0.00	0.41
<i>Sillago ciliata</i>	0.29	0.21	0.00	0.87	0.44	0.11	0.13	0.61	0.14	0.10	0.00	0.41	0.13	0.09	0.00	0.37
<i>Achylopa nigra</i>	0.22	0.16	0.00	0.62	0.58	0.09	0.37	0.71	0.10	0.08	0.00	0.30	0.09	0.07	0.00	0.26

<i>Ambassis marianus 1</i>	0.32	0.21	0.00	0.76	0.22	0.11	0.00	0.42	0.24	0.15	0.00	0.60	0.22	0.14	0.00	0.57
<i>Ambassis marianus 2</i>	0.27	0.17	0.00	0.61	0.17	0.09	0.00	0.34	0.29	0.18	0.00	0.71	0.27	0.16	0.00	0.65
<i>Ambassis marianus 3</i>	0.24	0.15	0.00	0.56	0.15	0.08	0.00	0.31	0.31	0.20	0.00	0.75	0.30	0.17	0.00	0.68
<i>Acanthopagrus australis 10</i>	0.34	0.23	0.00	0.82	0.25	0.12	0.00	0.45	0.21	0.14	0.00	0.57	0.20	0.13	0.00	0.52
<i>Acanthopagrus australis 20</i>	0.16	0.11	0.00	0.42	0.69	0.06	0.55	0.78	0.09	0.06	0.00	0.24	0.07	0.05	0.00	0.20
<i>Acanthopagrus australis 30</i>	0.24	0.18	0.00	0.69	0.54	0.10	0.30	0.68	0.12	0.09	0.00	0.35	0.11	0.08	0.00	0.30
<i>Platycephalus fuscus 1</i>	0.18	0.13	0.00	0.49	0.65	0.07	0.49	0.76	0.09	0.06	0.00	0.24	0.08	0.06	0.00	0.23
<i>Platycephalus fuscus 2</i>	0.15	0.11	0.00	0.40	0.71	0.06	0.58	0.80	0.07	0.05	0.00	0.19	0.07	0.05	0.00	0.19
<i>Mugil cephalus 1</i>	0.20	0.14	0.00	0.52	0.63	0.07	0.46	0.74	0.09	0.07	0.00	0.25	0.09	0.06	0.00	0.23
<i>Mugil cephalus 2</i>	0.23	0.16	0.00	0.67	0.57	0.09	0.33	0.70	0.11	0.08	0.00	0.29	0.10	0.07	0.00	0.28
<i>Mugil cephalus 3</i>	0.18	0.11	0.00	0.41	0.10	0.06	0.00	0.23	0.36	0.23	0.00	0.82	0.36	0.20	0.00	0.76
<i>Mugil cephalus 4</i>	0.24	0.15	0.00	0.55	0.14	0.08	0.00	0.30	0.32	0.20	0.00	0.74	0.30	0.18	0.00	0.68
<i>Mugil cephalus 5</i>	0.06	0.05	0.00	0.15	0.89	0.03	0.84	0.92	0.03	0.03	0.00	0.08	0.03	0.02	0.00	0.06
<i>Mugil cephalus 6</i>	0.24	0.17	0.00	0.67	0.53	0.09	0.30	0.67	0.12	0.09	0.00	0.34	0.11	0.08	0.00	0.32
<i>Mugil cephalus 7</i>	0.32	0.22	0.00	0.77	0.23	0.12	0.00	0.42	0.24	0.15	0.00	0.63	0.21	0.14	0.00	0.55
<i>Mugil cephalus 8</i>	0.27	0.17	0.00	0.61	0.17	0.09	0.00	0.34	0.29	0.18	0.00	0.71	0.27	0.16	0.00	0.65
<i>Mugil cephalus 9</i>	0.32	0.23	0.00	0.90	0.39	0.14	0.08	0.58	0.16	0.11	0.00	0.43	0.14	0.10	0.00	0.42
<i>Mugil cephalus 10</i>	0.28	0.18	0.00	0.64	0.18	0.10	0.00	0.35	0.28	0.18	0.00	0.71	0.26	0.16	0.00	0.64
<i>Mugil cephalus 11</i>	0.33	0.22	0.00	0.79	0.24	0.12	0.00	0.44	0.23	0.15	0.00	0.61	0.21	0.14	0.00	0.55
<i>Mugil cephalus 12</i>	0.34	0.25	0.00	0.98	0.34	0.13	0.00	0.54	0.16	0.12	0.00	0.49	0.15	0.11	0.00	0.42

Averages	0.24	0.17	0.00	0.64	0.38	0.09	0.18	0.53	0.19	0.13	0.00	0.50	0.18	0.12	0.00	0.45
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† In Belongil Creek, filamentous algae was a green alga. In Tallows Creek, this component was a red alga.

Figure 1. Map of Australia, showing location of Byron Bay, New South Wales (NSW).

Inset shows location of Belongil and Tallows Creeks.

Figure 2. Mean (\pm SE) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures of all food web components collected from Tallows (solid symbols) and Belongil (open symbols) Creeks in May 2003.

Statistical comparisons of signatures from Tallows and Belongil Creeks are presented in Table 1.

Figure 3. Biplots of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures of all food web components collected from A) Tallows Creek and B) Belongil Creek in May 2003. Boxes represent mean (\pm SE) values from replicate samples of all components. Numerical codes represent taxa as follows: 1 = *Centropogon australis*, 2 = Bivalve molluscs, 3 = *Ambassis marianus*, 4 = Corixids (Corixidae, Insecta), 5 = *Marphysa* spp., 6 = *Halicarcinus* sp., 7 = *Gobiomorphus* sp., 8 = *Mugil cephalus*, 9 = *Anguilla* sp, 10 = *Rhabdosargus sarba*, 11 = *Pelates sexlineatus*, 12 = *Metapenaeus bennettiae*, 13 = *Gerres subfasciatus*, 14 = *Sillago ciliata*, 15 = *Acanthopagrus australis*, 16 = *Achylopa nigra*, 17 = *Platycephalus fuscus*, 18 = *Ocypode* sp. and 19 = *Philypnodon grandiceps*.

Figure 4. Variability in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope signatures for individuals of *Mugil cephalus* and *Ambassis marianus* collected from Tallows (solid symbols) and Belongil (open symbols) Creeks in May 2003. Relationships between $\delta^{13}\text{C}$ signatures and fish total length (cm) are presented in Figures 4A (*Mugil cephalus*) and 4B (*Ambassis marianus*)

and relationships between $\delta^{15}\text{N}$ signatures and fish total length (cm) are presented in Figures 4C (*Mugil cephalus*) and 4D (*Ambassis marianus*).







