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Gut content- and stable isotope-derived diets of four commercially and recreationally important fish species in two intermittently open estuaries.

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Running Head – Fish diets in intermittently open estuaries

Abstract

20 Despite remaining closed for variable periods, intermittently open estuaries provide habitat for estuarine and marine fish species of commercial and recreational value. To better understand how these systems trophically support their fish assemblages, the diets of four valued fish species, namely *Acanthopagrus australis*, *Platycephalus fuscus*, *Sillago ciliata* and *Mugil cephalus*, were examined in two intermittently open estuaries in New South Wales,
25 Australia. Fish diets were determined using both gut contents and stable isotope analyses, because the different temporal resolutions afforded by these methods can provide insight into the flexibility of fish diets. Stable isotope signatures of prey and fish proved to be particularly useful in analyses of the diets of *M. cephalus* and *P. fuscus* individuals, as these species

consume large quantities of unidentifiable organic matter and have high incidences of empty guts, respectively. Diet reconstructions across methods were generally consistent for *A. australis*, but differed substantially for *S. ciliata*, with fewer prey taxa identified in the guts than expected. This result suggests that *S. ciliata* individuals switch between local resources
5 on the basis of their fluctuating temporal availability. Trophic flexibility, coupled with broad physicochemical tolerances, enables these species to flourish in the challenging environment of intermittently open estuaries.

Introduction

10 Aquatic ecosystems that maintain only an intermittent connection with the ocean represent a diverse and numerous, yet understudied, subset of estuaries (Roy *et al.* 2001, Haines *et al.* 2006). Often referred to as coastal lagoons, intermittently closed and open lakes and lagoons (ICOLLs) or intermittently open estuaries (as in this paper), these systems are found throughout the world, particularly in regions with mediterranean climates like southern
15 Australia (Kench 1999, Roy *et al.* 2001), South Africa (Strydom *et al.* 2003), Mexico (Raz-Guzman and Huidobro 2002) and Portugal and Spain (Perez-Ruzafa *et al.* 2000, Newton and Mudge 2003, Tancioni *et al.* 2003, Vizzini *et al.* 2005).

As their name suggests, intermittently open estuaries differ from macrotidal or permanently
20 open estuaries by virtue of their variable connectivity with the ocean (Bell *et al.* 2001, Roy *et al.* 2001). In eastern Australia, seasonally low riverine flows and a constant northerly migration of sand up the coast (longshore drift) foster the formation and maintenance of sand bars above the high tide level in the mouth of many estuaries (Roy *et al.* 2001, Haines *et al.* 2006). Under these conditions, up to 92% of the estuaries in New South Wales, Australia, do
25 not maintain a permanent connection with the ocean (Williams *et al.* 1998). Whilst these closed entrance conditions may persist for months to years (Haines *et al.* 2006, Harrison and Whitfield 2006), seasonal flows and storm events can breach the bar and restore tidal flows and connectivity with the marine environment (Jones and West 2005). Once open,

intermittently open estuaries can remain open for any length of time, from just a few days to many years (Griffiths 1999, Roy *et al.* 2001).

Several researchers have investigated differences in fish community composition in geographically proximate estuaries with different opening regimes and entrance behaviours (Pollard 1994a, Pollard 1994b, Raz-Guzman and Huidobro 2002, Vorwerk *et al.* 2003). Typically, fish assemblages in permanently open estuaries have been found to be more diverse than those in intermittently open estuaries and several studies have shown that estuary type (permanently open versus intermittently open) explains a significant amount of variation in the composition of fish communities (Strydom *et al.* 2003, Jones and West 2005, Harrison and Whitfield 2006). Griffiths (2001a) suggested that these compositional differences were likely to be driven by salinity stability, as this factor influences the capacity of an estuary to support both marine and estuarine fish species. Despite harbouring fewer fish species, Pollard (1994b) found that intermittently open estuaries can be much more productive than neighbouring permanently open estuaries. Indeed, some intermittently open estuaries represent disproportionately important nursery grounds for certain species, particularly those that can tolerate variability in physicochemical conditions (Pollard 1994b).

In addition to comparisons between permanently and intermittently open estuaries, within-system studies have shown that temporal and spatial variations in salinity, resulting from extended periods of closure, rainfall events and/or bar breaching, can cause a shift in ichthyofaunal composition and abundance (Young *et al.* 1997, Young and Potter, 2002). As might be expected, macroinvertebrate communities respond similarly to changes in salinity within intermittently open estuaries (Williams and Williams, 1998, Dye and Barros 2005, Gladstone *et al.* 2006). Given the strong influence of entrance status on overall biotic composition, we would expect food resource diversity, temporal availability to consumers and food web structure to be highly variable in intermittently open estuaries (Hadwen and Arthington 2007). As a result, the foraging strategies of fish species inhabiting these systems

would be expected to accommodate this variability in ways that maximize survival and reproduction.

Despite the importance of intermittently open estuaries to coastal and nearshore fisheries
5 (Pollard 1994b, Watts and Johnson 2004), there have been surprisingly few dietary studies in
these systems. One notable exception is the work of Sarre *et al.* (2000), who found that the
dietary composition of black bream (*Acanthopagrus butcheri*) individuals differed
significantly between a series of intermittently and permanently open estuaries in Western
Australia. These findings support the hypothesis that intermittently open estuaries may only
10 support species that are opportunists and/or generalists in their feeding habits, enabling them
to shift readily among prey items based on the dynamic nature of their availability over time
(Mariani *et al.* 2002, Poizat *et al.* 2004, Dye and Barros 2005).

In this paper, we examine the diets of four commercially and recreationally important fish
15 species in two intermittently open estuaries in northern New South Wales, Australia. We used
gut content analysis to document instantaneous diet composition and stable isotope techniques
to reconstruct fish diets over longer periods (weeks to months). To gain further insight into
how intermittently open estuaries trophically support estuarine and marine species, we
compared the diets of each target species within our study systems with dietary information
20 from the literature that has principally been collected from permanently open estuaries and
marine environments. Furthermore, given differences in the scope, accuracy and temporal
resolution of diets using gut content and stable isotope analyses (Peterson 1999, Cocheret de
la Moriniere *et al.* 2003, Melville and Connolly 2003), we sought to compare and contrast the
results generated using these two methods.

25

Materials and Methods

Study Area

Samples were collected from two small intermittently open estuaries located in northern New

South Wales, Australia (Figure 1). Belongil Creek has a catchment area of 30 km² and a waterway surface area of 0.3 km² and Tallows Creek has a catchment area of 4.5 km² and a waterway surface area of 0.125 km² (NSW DNR 2005). Both systems have histories of receiving large quantities of nutrients over the past 50 years, principally from sewage treatment plants (STPs) that discharge treated effluent into their waters (McAlister *et al.* 2000).

[INSERT FIGURE 1 HERE]

10 Samples were collected between November 27 and December 18 2003. One study site located at the mouth of each system was selected in an attempt to reduce the influence of within-system variability on comparisons between systems. Previous research (Hadwen and Arthington 2007, WLH unpublished data) has shown that within this region, the target fish species are most often encountered in these near-entrance areas of intermittently open
15 estuaries.

At the time of sampling, Belongil Creek had been open to the ocean for several months, whilst Tallows Creek had been closed for more than 6 months (WLH, personal observation). As a result, the physico-chemical characteristics of the two systems were quite different, with
20 the tidally active Belongil Creek displaying variable salinity levels, depending on the tidal cycle during which specimens were collected. For example, salinity was observed to drop from 29.0 to 26.9 over a falling tide on December 12, 2003. In contrast, the closed Tallows Creek had consistently low salinities (< 6.0) over the course of the study. Other physicochemical variables measured were generally not different between systems, with pH
25 and dissolved oxygen concentrations consistently around 8 and 6 ppm, respectively.

Study Species

The four fish species studied were yellowfin bream (*Acanthopagrus australis*), dusky flathead

(*Platycephalus fuscus*), sand whiting (*Sillago ciliata*) and sea mullet (*Mugil cephalus*). They were selected on the basis of their known presence in intermittently open estuaries (Pollard 1994b, Griffiths 2001b) and their importance to both recreational and commercial fisheries (West and Gordon 1994, Butcher *et al.* 2003, Gray and Kennelly 2003).

5

Comparing stable isotope and gut contents analyses

Combined stable isotope and gut content analyses are quite rare in estuarine fish diet studies (Cocheret de la Moriniere *et al.* 2003), yet both methods offer interesting and different insights into trophic relations. The major point of difference between the methods is that gut contents can provide information on recently (within hours) consumed food items, whereas stable isotope signatures of muscle tissues tell us more about which food items have been consumed and assimilated over recent weeks to months (Cocheret de la Moriniere *et al.* 2003, Melville and Connolly 2003). Furthermore, since the application of gut contents analyses is limited for some herbivorous and benthic feeding species (like *M. cephalus*) due to problems associated with the identification of the food items that have been consumed and some ambush predators that tend to have a high frequency of empty guts (like *P. fuscus*), our capacity to examine diets of these species in a quantitative way is likely to be best served using stable isotope techniques.

20 *Fish Collection*

Individuals of each of the four target species were collected in trawls of a 20m seine net (with a 1.5 m bag and 6 mm mesh size). Seine net trawls were conducted between 9:00 am and 2:00 pm on each sampling occasion. Captured fish were removed from the net after each trawl and placed immediately into an ice-slurry in accordance with our approved animal ethics procedures (Permit #AES/11/02/AEC).

25

Fish Processing and Gut Contents Analyses

The total length (TL) of each fish was measured and recorded in the laboratory to the nearest

5 mm. The guts of each fish were then removed and preserved in 4% formalin. A small portion of muscle was removed from each gutted fish for stable isotope analysis (see below). Two days prior to microscopic investigation of gut contents the preserved guts were transferred to a solution of 70% ethanol.

5

Before ingested items were removed, the gut cavity contents were dried with blotting paper and the excess ethanol was allowed to evaporate before being weighed in g to two decimal places. The entire contents of the digestive tract were removed by dissection and placed in a petri dish with 70% ethanol and sorted under a dissecting microscope (Wild M3Z, 6.5-40X).

10 Gut contents were taxonomically classified into 9 broad dietary categories adapted from existing literature and personal observation of potential prey items found in the food web sampling.

Analyses of fish diets were based on three parameters proposed by Hyslop (1980), namely the
15 percent frequency of occurrence of each dietary item in the stomachs of all fish (%F) and the relative contributions of each dietary item to the number (%N) and volume (%V) in the stomach of each fish. As these methods have been widely adopted for the analysis of fish diets in estuarine environments (Edgar and Shaw, 1995, Hyndes et al. 1997, Sarre et al. 2000), their use also facilitated comparisons between this study and others. Volumes of dietary
20 categories were calculated indirectly, whereby the items within each diet category were pooled and their relative percent contribution estimated against a standard 1 mm grid, with the number of filled grids being divided by the total number of grids examined. This method takes into account the stomach fullness of each individual analysed (Hyslop 1980). Gut content analyses were restricted to those individuals whose stomachs contained dietary items
25 at the time of capture.

Since volumetric data best represent the relative importance of each dietary category, especially when a combination of both large and small prey items are eaten that may over or

under-emphasise their importance (Hyslop 1980), subsequent analyses were performed using only volumetric data for each dietary category. The use of the volumetric parameter also enables the inclusion of amorphous or difficult to identify items (such as algae and detritus) to be included in analyses (Hyslop 1980). Furthermore, comparison of diet reconstructions using gut contents and stable isotope methods is facilitated using this parameter (Jones and Waldron 2003).

Food Source Collection

Stable isotope analyses of diet composition rely on the matching (including isotopic fractionation where necessary) of source (prey) material signatures with those in the tissues of the target species (Peterson and Fry 1987, Peterson 1999). To this end, we collected triplicate samples of primary sources of organic matter and all potential prey items from Tallows and Belongil Creeks. At most sites primary carbon sources consisted of riparian vegetation, grey mangroves (*Avicennia marina*), benthic fine particulate organic matter (FPOM), benthic coarse particulate organic matter (CPOM), attached and free-floating algae and seston (suspended particulate organic matter). Samples of riparian vegetation and mangroves were collected by hand and FPOM and CPOM samples were collected by sifting benthic sediments through a series of graded sieves (250 μm - 500 μm - 1 cm). FPOM samples were obtained from the 250 μm sieve and CPOM samples were collected from the 500 μm sieve. Using a scalpel blade and brush, attached algae were carefully scraped 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 m 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 (*sensu* Hadwen and Arthington 2007), so bulk seston samples were run as a composite of all epilimnetic sources of organic matter.

Aquatic insects, crustaceans 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 20 m seine net (with a 1.5m bag and 6 mm mesh size), trawled over sandy substrates, often in close proximity to littoral vegetation and submerged substrates. Sediment-dwelling organisms, including bivalve molluscs and polychaete worms, were collected using a hand pump. In addition, some small mobile macroinvertebrates (especially crabs, isopods and amphipods) were collected opportunistically by hand, generally off sand and wood substrates.

Upon collection, all samples were immediately placed in individually labelled zip-lock bags and stored on ice. For small organisms, this approach has been shown to allow sufficient time for them to void their guts to ensure that isotope signatures reflect consumer tissues only and is not influenced by gut contents (*sensu* Hadwen and Bunn 2005). Samples were subsequently frozen for transportation and storage in the laboratory.

15

Stable Isotope Sample Processing and Analyses

In the laboratory, samples of riparian vegetation, FPOM, CPOM, 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 aquatic crustaceans were removed (prior to drying) to ensure that accumulated calcium carbonate did not influence carbon isotopic values (*sensu* Hadwen and Bunn 2004).

Fish muscle samples (collected at the time of gut removal) were dried in an oven at 60°C for 48 hours, before being ground into a fine powder in a mortar and pestle. Carbon: nitrogen ratios revealed that the vast majority of tissues (87 of 92 samples) had values below 3.2 (data not presented), which is considered to be the threshold beyond which lipid extraction prior to stable isotope processing is suggested (Kiljunen *et al.* 2006, Sweeting *et al.* 2006). To this end, we did not subject fish muscle tissues to lipid extraction prior to stable isotope analyses.

Ground and dried samples were analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a continuous flow-isotope ratio mass spectrometer (Micromass Isoprime EuroVector EA300, Manchester, UK) at Griffith University. Standards against which materials were assessed were ANU sucrose for $\delta^{13}\text{C}$ and atmospheric N for $\delta^{15}\text{N}$. Isotope ratios are expressed as either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ and relate to the ratio of $^{13}\text{C}:^{12}\text{C}$ and $^{14}\text{N}:^{15}\text{N}$, respectively (Hadwen and Bunn 2005).

Fish diets were reconstructed using the IsoSource mixing model software (Phillips and Gregg 2003), which calculates feasible combinations (in 1% increments) of prey item stable isotope signatures that explain 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 difficulties associated with determining fractionation increments in the study organisms (*sensu* Connolly *et al.* 2005), particularly in the sewage-enriched Tallows Creek ecosystem (Hadwen and Arthington 2007).

Whilst most studies using stable isotopes of carbon and nitrogen aim to assess the relative importance of primary food sources (autochthonous and allochthonous) to the diets of target consumers (Connolly 2003, Melville and Connolly 2003, Hindell 2006, Hadwen and

Arthington 2007), we adopted a modified approach to facilitate comparisons between our gut contents and stable isotope analyses. Specifically, we selected end members for use in the IsoSource mixing model on the basis of a) the results from our gut contents analyses, and b) published data on the diets of each species. Given that we did not use $\delta^{15}\text{N}$ isotope signatures in our mixing models, this approach also avoided the need to account for significant trophic fractionation between prey items and their consumers and represents a more appropriate analysis of diets of carnivorous and omnivorous species. As our specific focus in this study was to examine the diets of four key fish species, we felt that this trophically relevant approach provided greater insights to contributions of known prey items to the diets of each species. Furthermore, the contribution of primary carbon sources to consumer organisms in Tallows and Belongil Creeks has recently been evaluated by Hadwen and Arthington (2007). Sampling of all components of the food web also enabled us to appreciate prey item abundance and variability within and between the two estuaries. The end members chosen for *A. australis*, *S. ciliata* and *P. fuscus*, were epilithic algae, polychaete worms, bivalve molluscs, decapod crustaceans and small fish (predominantly flathead gudgeons (*Philypnodon* spp) and estuary perchlet (*Ambassis marianus*)). For *M. cephalus*, epilithic algae, seston (suspended particulate matter) and riparian vegetation were the end members used in mixing model analyses, as this species is known to consume large quantities of benthic and suspended particulates (Thomson 1954, ICLARM 2006). CPOM and FPOM were excluded from all mixing model analyses on the basis that these sources represent mixtures of other end members (*sensu* Hadwen and Bunn 2004, Hadwen and Arthington 2007). Preliminary analyses revealed that CPOM was largely derived from riparian vegetation and FPOM represented a combination of autochthonous (algal) and allochthonous (riparian vegetation) carbon sources (data not presented).

25

As Hadwen and Arthington (2007) found significant differences between stable isotope signatures in Tallows and Belongil Creeks (and the Maroochy River), we also undertook a statistical comparison (t-tests) of stable carbon and nitrogen isotope signatures of the four

target fish species. Specifically, we sought to examine the variability and differences in isotope signatures between Tallows and Belongil Creeks.

Results

5 *Catch Data*

We collected a total of 93 fish (across the four target species) from Belongil and Tallows Creeks (Table 1). Catches differed considerably between the two systems, with two-thirds of all individuals collected from Belongil Creek. Catches of *S. ciliata* and *A. australis* showed the greatest differences between systems, with much larger catches of both species from
10 Belongil Creek (Table 1). Comparable numbers of the other two species (*M. cephalus* and *P. fuscus*) were recorded from each system. Overall, *S. ciliata* was the most commonly caught species, comprising 41.9 % of all of the individuals captured. There was little variation in the size classes of each species collected in each system (Table 1), therefore eliminating the difficulties associated with accounting for ontogenetic variation in diet composition. Within
15 species, the occurrence of empty stomachs was similar across both systems. However, the proportion of individuals with an empty digestive tract varied greatly between species: no individuals of *M. cephalus* were found to have an empty stomach whereas 47 % of *P. fuscus* individuals had empty stomachs (Table 1).

20 [INSERT TABLE 1 HERE]

Gut Content Analyses

Gut content analyses revealed that yellowfin bream (*A. australis*) consumed the most diverse range of prey items of the four species examined (Table 2). In Belongil Creek, bivalve
25 molluscs dominated the diet of *A. australis* with a mean volumetric contribution of 22.17 % and a frequency of occurrence of 66.6 %. In contrast, the gut of the single bream individual collected from Tallows Creek contained no molluscs and had a greater proportion of decapod crustaceans and polychaetes (Table 2). Although algae were the most commonly ingested

items in the diet of *A. australis* from Belongil Creek, being found in 83.33 % of individuals, algae contributed only 15% to the average diet by volume.

5 The diet of dusky flathead, *P. fuscus*, from Tallows Creek was consistently less diverse than those of the other three species (Table 2). Contents of guts from *P. fuscus* were comprised predominately of decapods of the genus *Metapenaeus* (62.5 %V) and small unidentifiable fish (31.67 %V) (Table 2). In Belongil Creek, 40 % of the fish collected had empty guts, but those individuals with food in the gut had recently consumed small fish (Table 2).

10 [INSERT TABLE 2 HERE]

Reconstruction of *M. cephalus* diets on the basis of gut contents was difficult given their benthic feeding habits and tendency to consume large quantities of unidentifiable material. All individuals had relatively full stomachs, with large quantities of sediment, algae and
15 unidentifiable particulate matter (Table 2). In addition, one large individual collected from Tallows Creek had consumed some crustaceans and aquatic insects (Table 2). For that individual, about 10 % of the stomach volume was taken up by these aquatic macroinvertebrates, with the remainder represented by contributions from algae, sediments and particulate organic matter.

20

Polychaete worms were the most commonly consumed prey item in the diet of *S. ciliata*, being present in all guts (with contents) in both systems (Table 2). Volumetrically, polychaetes contributed 84.26 % and 55.00 % of the diets of *S. ciliata* in Belongil and Tallows Creeks, respectively (Table 2). Bivalve molluscs were the second most important
25 item by volume in each system and were the dominant item ingested by number in Belongil Creek (59.72 % of the total abundance of food items). Crustaceans, namely decapods and amphipods, occurred in 50 % of the fish from Tallows Creek, but none were recorded from the guts of any of the 27 individuals examined from Belongil Creek.

Stable Isotope Analyses

Stable carbon and nitrogen isotope signatures for the four target fish species were significantly different between systems, with fish from Tallows Creek consistently ¹³C-depleted and ¹⁵N-enriched relative to their conspecifics from Belongil Creek (Table 3, Figure 5 Figure 2). These differences were also observed for all mixing model end members (Algae, Seston, Riparian Vegetation, Polychaetes, Molluscs, Decapods and Small Fish) suggesting that patterns of carbon and nitrogen assimilation, fractionation and trophic transfer were different in Tallows and Belongil Creeks at the time of sampling (Figure 2). Specifically, the 10 comparatively enriched signatures of $\delta^{13}\text{C}$ in Belongil Creek reflect the marine influence at the time of sampling (entrance was open to the ocean), whereas the depleted $\delta^{13}\text{C}$ values in Tallows Creek represents a food web more like those likely to encountered in freshwater systems, with algal isotope signatures around -22‰.

15 [INSERT TABLE 3 HERE]

The stable isotope data suggest that the food webs of both Tallows and Belongil Creeks are primarily fuelled by autochthonous sources of carbon (Figure 2). Consumer $\delta^{13}\text{C}$ signatures in Belongil Creek fall between those of algae and seston, with a significant component of carbon and nitrogen likely to be derived from these two sources. For the four focal fish species in this 20 study, algae appears to be a major primary carbon source, while seston clearly contributes to the signatures of polychaetes and decapods (Figure 2). The ¹⁵N-enriched Tallows Creek food web is also largely driven by seston and algae, although seston appears to play a more important role in supporting *M. cephalus* and *A. australis*. In contrast, *S. ciliata* and decapod 25 crustaceans appear to derive their carbon predominantly from algal sources (Figure 2). There is also some evidence of riparian vegetation contributions of carbon to consumers in Tallows Creek by virtue of the $\delta^{13}\text{C}$ depleted signatures of small fish, polychaetes and, to a lesser degree, molluscs (Figure 2).

[INSERT FIGURE 2 HERE]

Whilst an appreciable number of the collected fish had empty guts (except *M. cephalus*, see
5 Table 1), the diets of all individuals could be assessed using stable isotope data from muscle
tissues. However, for those individuals with gut contents, the use of both methods allowed
comparison of instantaneous diet composition (gut contents) with that based on the range of
food items consumed over the antecedent months (stable isotopes). Unfortunately, extremely
low *A. australis* and *S. ciliata* catches in Tallows Creek (Table 1) meant that comparisons of
10 results obtained using these two methods were only possible for the Belongil Creek
individuals. Similarly, there were too few individuals (with and without gut contents) to
facilitate quantitative comparisons between gut contents and stable isotope dietary
reconstructions for *P. fuscus* (Table 1).

15 *Composition of diets – comparison between systems within species*

On the basis of our catch characteristics, the only species for which comparison of diet
between the two intermittently open estuaries was possible was *M. cephalus*. Mixing model
results for stable isotope signatures of muscle tissue indicated that algae contributed almost 90
% of the carbon and nitrogen to individuals from Belongil Creek (Figure 3). In contrast, about
20 50% of the dietary carbon and nitrogen of *M. cephalus* individuals collected from Tallows
Creek was derived from seston (particulate organic matter), with 40% coming from algal
sources and the remainder derived from riparian vegetation (Figure 2, Figure 3).

[INSERT FIGURE 3 HERE]

25

Composition of diets – comparison between techniques within systems

For *A. australis* individuals collected from Belongil Creek, there was reasonable agreement
between gut content and stable isotope analyses of the contributions of prey items to

individual diets (Figure 4A). However, there was a significant quantity of miscellaneous/algal items identified in *A. australis* guts and this resulted in an apparent underestimation of decapod crustacean and polychaete worm contributions on the basis of the stable isotope mixing model output (Figure 4A). These data highlight the difficulties associated with diet
5 reconstructions from gut contents when the identification of some prey items is particularly difficult. This is especially the case for omnivorous fish, like *A. australis*, that consume a wide range of prey across multiple trophic levels. These identification difficulties can make direct comparisons of gut content- and stable isotope-derived diets difficult to undertake.

10 [INSERT FIGURE 4 HERE]

In contrast to the findings for *A. australis*, there was considerable disagreement in the diet reconstructions based on stable isotope mixing model output and gut contents for *S. ciliata* individuals collected from Belongil Creek (Figure 4B). Stable isotope data suggested that *S.*
15 *ciliata* individuals had previously fed on a broader range of prey taxa than that observed in their gut contents at the time of capture (Figure 4B). Specifically, while the guts of many individuals were full of polychaete worms (Table 2), isotope data indicate that these prey items had contributed a relatively small proportion (< 20%) of the dietary carbon and nitrogen to muscle tissues at the time of capture (Figure 4B). Furthermore, the stable isotope data
20 revealed that small fish, decapod crustaceans and algae had all made substantial contributions to *S. ciliata* diets in Belongil Creek in the preceding weeks and months.

Discussion

Like other intermittently open estuaries (Pollard 1994b, Dye and Barros 2005, Mariani 2001,
25 Hadwen and Arthington 2007), Belongil and Tallows Creek support diverse biological assemblages, with freshwater, estuarine and marine species present in our collections. The representation of these groups across systems was largely dependent on the prevailing status of the entrance of each estuary, with the tidally influenced Belongil Creek dominated by

estuarine taxa and the closed Tallows Creek containing a diverse range of freshwater insect taxa, particularly those belonging to the Corixidae, Chironomidae and Odonata groups. Although all four target fish species were also collected from Tallows Creek, this system was more like a freshwater-dominated coastal lake or lagoon than an estuary at the time of
5 sampling. Having been closed for more than 6 months (WLH, personal observation), low catches of *A. australis* and *S. ciliata* (relative to those from Belongil Creek) reflected the comparatively freshwater conditions that prevailed in Tallows Creek over the period of our study.

10 Given the presence of freshwater macroinvertebrates in both Tallows and Belongil Creeks during the sample period, it came as no surprise that we found some freshwater macroinvertebrates in the guts of fish from both systems. However, the incidences and numbers of freshwater macroinvertebrates in guts were generally low. For example, in Tallows Creek, corixids were identified in the gut of just one very large *M. cephalus*
15 individual (total length - 49 cm). This general tendency for low numbers of freshwater insects in the diet of this species is consistent with a handful of other studies from northern New South Wales and southern Queensland (Pusey *et al.* 2004). In addition to this *M. cephalus* record, we identified chironomid larvae in the guts of both *S. ciliata* individuals from Tallows Creek with gut contents. Whilst there are no records of freshwater macroinvertebrates in the
20 diet of *S. ciliata* in the estuarine literature, it is likely that they will be consumed when encountered, as this species is generally considered to be a generalist benthic carnivore (Kailola *et al.* 1993). Finally, we also found some *A. australis* individuals from the tidally-active Belongil Creek that had recently consumed aquatic beetles (Coleoptera), suggesting that freshwater macroinvertebrate taxa can also contribute to the diets of this generalist
25 omnivore. This finding supports those of Mazumder *et al.* (2006), who reported freshwater macroinvertebrates in the guts of some *A. australis* individuals collected on ongoing tides near saltmarshes fringing Botany Bay (NSW). As stated by Kailola *et al.* (1993), *A. australis* is an opportunistic omnivore that is likely to consume a wide range of plant and animal

species. In highly variable intermittently open estuaries like those examined in this study, this dietary breadth and flexibility clearly favours species like *A. australis* over others with narrower limits of resource and habitat use (Pollard 1994b).

5 Despite their abundance at the time of sampling, the low frequency and numbers of freshwater macroinvertebrates identified in the guts of fish across species and systems suggested that these taxa do not substantially support the productivity of any of the focal fish species on a regular basis. This conclusion is further supported by the discrepancies between stable isotope signatures of freshwater macroinvertebrates and the four focal fish species. In
10 general, the stable isotope signatures of each fish species were much more strongly aligned with the signatures of their more typical or preferred prey types. For example, the extremely high abundance of polychaetes observed in Tallows Creek at the time of sampling was mirrored by their high frequency of occurrence in fish guts and their proportional importance to diets on the basis of their stable isotope signatures. Given that polychaetes represent highly
15 nutritious prey items that are relatively easy to catch and are often represented in the diets of all four focal fish species (Coleman and Mobley 1984, Burchmore *et al.* 1988, Kailola *et al.* 1993, Pusey *et al.* 2004, Mazumder *et al.* 2006), it is likely that the resident fish selectively fed on this food resource to the exclusion of the aquatic insects present, at least at the time of sampling (*sensu* Levin *et al.* 1997).

20 The high proportion of empty guts recorded in this study, particularly for *P. fuscus* and *S. ciliata*, suggest that despite being productive ecosystems (Pollard 1994b), intermittently open estuaries can be hard systems to live in. *P. fuscus*, a cryptic ambush predator (Humphries *et al.* 1992, Kailola *et al.* 1993, Edgar and Shaw 1995, Butcher *et al.* 2003), had the highest
25 incidence of empty stomachs in both systems (40% in Belongil Creek and 50% in Tallows Creek). The sporadic nature of this feeding strategy, which requires movement of prey items within an individual's striking range, lends itself to extended periods when no prey items are ingested and similar proportions of empty stomachs have been observed in dietary analyses

for other species within this genus (Humphries *et al.* 1992, Edgar and Shaw 1995). For *S. ciliata*, we found higher incidences of empty guts than reported by Burchmore *et al.* (1988). In that study, conducted in Botany Bay, New South Wales, around 15% of the collected *S. ciliata* individuals were found to have empty guts (Burchmore *et al.* 1988). In contrast, we
5 found between 25% (Belongil Creek) and 33% (Tallows Creek) of the individuals collected in this study had empty guts. This higher than previously reported occurrence of empty guts for this species presumably reflects the greater variability in food resource availability in intermittently open estuaries, particularly when contrasted with the availability of resources in permanently open estuaries and large marine bays.

10

As noted by Griffiths (2001b) and Connolly (2003), there is limited quantitative information on *A. australis* diets in the scientific literature, despite the fact that this species is a highly valued fisheries species (Kailola *et al.* 1993, Gray and Kennelly 2003). Nevertheless, *A. australis* can feed on a wide range of prey items and is assumed to have a broad diet (Kailola
15 *et al.* 1993, Connolly 2003). Both our stable isotope mixing model and gut contents data from Belongil Creek supported this suggestion of considerable dietary breadth for *A. australis*. Similar findings have been reported from a study of diet variability and flexibility across a range of estuary types in Western Australia, for a related species, *A. butcheri* (Sarre *et al.* 2000), suggesting that individuals from this genus can feed on a broad range of taxa across
20 daily, weekly and monthly time scales. Sarre *et al.* (2000) concluded that coupled with their broad environmental tolerances (Partridge and Jenkins 2002, Doupe *et al.* 2005), this dietary breadth and flexibility ensures that acanthopagrids are well suited to the variable conditions encountered in intermittently open estuaries (Sarre *et al.* 2000).

25 Gut contents analyses of *M. cephalus* individuals pose considerable analytical problems given the large quantities of miscellaneous and/or unidentifiable matter encountered. Indeed, even for the handful of quantitative studies of *M. cephalus* diets that have been published, at least 20% of the gut contents tend to remain unidentified (Pusey *et al.* 2004). This figure falls

between our values for Belongil (15%) and Tallows (35%) Creeks and highlights the difficulties associated with gut contents analyses for examining the diets of filter-feeding detritivores. Despite these limitations, we found a greater abundance of sediment in the guts of *M. cephalus* from Belongil Creek than in their conspecifics from Tallows Creek. On the basis of our stable isotope analyses, this abundance of sediment in the guts of *M. cephalus* collected from Belongil Creek is likely to relate to their high reliance (around 90% of their dietary carbon) on epilithic algae as a primary food source. This result mirrors those of other studies suggesting that benthic algae contribute heavily to *M. cephalus* diets in estuaries and rivers (Kailola *et al.* 1993, Pusey *et al.* 2004). In contrast, the stable isotope data from Tallows Creek suggested that there was a much higher contribution of seston to *M. cephalus* diets, reflecting an increased reliance of water column sources of organic matter relative to their conspecifics in Belongil Creek. We suggest that the relative importance of seston to the diets of *M. cephalus* in Tallows Creek may be enhanced by the closed entrance status at the time of sampling, as sewage effluent is retained within the system and is not flushed or diluted by riverine and tidal flows (Hadwen and Arthington 2007). Furthermore, high water column algal production, driven by this high nutrient loading, may encourage *M. cephalus* within Tallows Creek to feed on algae (phytoplankton) and particulate organic matter suspended in the water column. Indeed, many *M. cephalus* individuals were observed feeding directly on seston at the STP outflow pipe in Tallows Creek at the time of this study (WLH, personal observation), indicating that this is palatable and selectively consumed resource in this system. We suggest that stable isotope evaluations of diet for this species, like those presented in this study, serve to get around the problem of high proportions of unidentified items and can provide important insights into the relative importance of different sources of nutrition (allochthonous vs. autochthonous, benthic vs. pelagic etc) for individuals in different habitats.

Despite low catch numbers, our limited dietary data for *P. fuscus* from Belongil and Tallows Creeks support the literature evidence that this species is a cryptic ambush predator that feeds

predominantly on decapod crustaceans and small fish (Kailola *et al.* 1993, Butcher *et al.* 2003). We suggest that future dietary analyses of *P. fuscus* should use stable isotope analyses to overcome the difficulties associated with the high incidence of empty guts commonly encountered within the sample population.

5

For *S. ciliata*, there was considerable disagreement in diet reconstructions using stable isotope mixing model and gut content analyses, with fewer prey items accounted for in guts than in the mixing model analyses. These discrepancies suggest that while individuals can focus their foraging on particularly abundant prey items on a day-to-day basis, they feed on a wider range
10 of taxa over longer periods (from weeks to months). Significantly, our gut contents analyses indicated that decapod crustaceans had not been recently consumed by any of the sampled individuals. This finding is in stark contrast to the reported dietary compositions identified for sillaginid species in other coastal waters, where decapods have routinely been found in the guts of between 52% and 100% of all individuals examined (Burchmore *et al.* 1988, Hyndes
15 *et al.* 1997). Although we collected penaeid prawns (*Metapenaeus bennettiae*) and small crabs from both estuaries over the course of the study, the absence of crustaceans from the diet of the 27 individuals from Belongil Creek suggests that *S. ciliata* may preferentially feed on (and switch between) other locally abundant prey items. As polychaetes were extremely abundant at the time of sampling (WLH, personal observation), it may be that *S. ciliata* individuals
20 focus on this high energy and comparatively easily captured resource. Previous studies in permanently open estuaries have also found that polychaetes can be a significant food source for this species (Kailola *et al.* 1993, Hyndes *et al.* 1997).

Whilst polychaetes had clearly been an important food resource supporting *S. ciliata* in
25 Tallows and Belongil Creeks in the days before and during our study, as evidenced by their dominance in the gut contents, the stable isotope data suggest that decapod crustaceans had contributed significantly to the nutrition of this species in the preceding weeks and months. This finding suggests that temporal variability in prey abundance might be detected when

using paired stable isotope and gut content techniques, owing to their different temporal resolutions (Creach *et al.* 1997, Davenport and Bax 2002). Our stable isotope diet reconstruction is in general agreement with published diet records for *S. ciliata* (Burchmore *et al.* 1988, Hyndes *et al.* 1997), indicating that over longer periods, the sampled individuals had
5 consumed a more typical range of prey items than indicated by their gut contents, which were generally dominated by the locally abundant polychaetes.

Stable Isotope Signatures of fish in Tallows and Belongil Creeks

The stable carbon isotope signatures of fish and food resources reported in this study fall
10 within the range of values reported in the literature (Connolly 2003, Melville and Connolly 2003, Hadwen and Arthington 2007). The data from the tidally-active Belongil Creek look like those anticipated from a typical estuarine ecosystem, with algal $\delta^{13}\text{C}$ signatures spanning the range from -12‰ to -18‰. In contrast, the stable carbon isotope data from the Tallows Creek food web are in the range of those more likely to be encountered in a study of a
15 freshwater lake, with algal $\delta^{13}\text{C}$ signatures from -19‰ to -24‰ (*sensu* Hadwen and Bunn 2004). Future studies are required to examine the temporal shifts in algal communities (and their stable isotope signatures) to further examine the degree to which intermittently open estuaries shift between these apparent 'estuarine' and 'freshwater lake' states.

20 Whilst the stable nitrogen isotope signatures from Belongil Creek are typical of estuarine ecosystems (Melville and Connolly 2003, Connolly 2003), the significantly enriched $\delta^{15}\text{N}$ signatures of samples collected from Tallows Creek are greater than those reported elsewhere (*sensu* Hadwen and Arthington 2007). These greatly enriched $\delta^{15}\text{N}$ signatures are presumably due to the low flushing frequency and high water residence times in intermittently open
25 estuaries and the associated within-system nitrogen recycling and continued loss of the light isotope fraction (Hadwen and Arthington 2007).

Direct comparisons of our diet reconstructions with those from previous studies are not

possible, as we adopted a paired (gut contents versus stable isotopes) consumptive approach to reconstruct diets that used known food items as end members in the mixing model analyses. Other studies have tended to use a basal resource (primary producers and particulate organic matter) approach to examine the autochthonous and allochthonous contributions to the trophic ecology of target species. Nevertheless, our data and the findings of Hadwen and Arthington (2007) suggest that both Tallows and Belongil Creeks are predominantly fueled by algal sources of carbon, which is consistent with the findings of stable isotope studies in estuarine and marine ecosystems (Kitting *et al.* 1984, Miller *et al.* 1996, Connolly *et al.* 2005).

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Conclusions

Paired gut contents and stable isotope analyses can provide useful insights into the trophic ecology of fish in intermittently open estuaries. Indeed, and as demonstrated in a number of studies, discrepancies between gut contents and stable isotope diet reconstructions can provide insights that neither method can deliver in isolation (Creach *et al.* 1997, Davenport and Bax 2002). In our study, comparisons of *S. ciliata* diets using both methods highlighted considerable temporal variability in their diets, with decapod crustaceans absent from the guts at the time of sampling, but important over longer periods on the basis of the stable isotope mixing model analyses. The stable isotope evidence supports existing diet data for this species, whilst the gut contents data indicates that *S. ciliata* individuals in Belongil Creek can switch between local prey items and, importantly, target locally abundant resources over relatively short time frames. For commercially valuable species like those examined in this study, information regarding this short-term diet flexibility could aid in fisheries management, by providing managers with a better understanding of the role that intermittently open estuaries play as nursery grounds for species of interest.

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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) stable carbon and nitrogen isotope signatures of potential prey items and target fish species (*Acanthopagrus australis*, *Mugil cephalus*, *Platycephalus fuscus* and *Sillago ciliata*) collected from Tallows (solid symbols) and Belongil (open symbols) Creeks.

Figure 3. Mixing model analyses of average percent contributions of dominant carbon sources to *M. cephalus* diets from individuals collected from Tallows (n=12) and Belongil (n=13) Creeks.

Figure 4. Comparison of average percent contribution of prey types to the diet of A) *Acanthopagrus australis* and B) *Sillago ciliata* on the basis of gut contents and stable isotope analyses of dietary composition for individuals collected in Belongil Creek.

Table 1. Catch data and size class information for *A. australis*, *P. fuscus*, *S. ciliata* and *M. cephalus* individuals caught in Belongil and Tallows Creeks in November-December 2003.

Site	Belongil Creek		Tallows Creek	
Species	Sample Size	Mean TL (SE)	Sample Size	Mean TL (SE)
<i>A. australis</i>	13	180.8 (1.12)	1	90.0 (-)
# Empty guts	1 (7.69%)	170.0 (-)	0 (0%)	-
0-100 mm	-	-	1	90.0 (-)
101-150 mm	4	140 (0.58)	-	-
151-200 mm	6	186.6 (0.61)	-	-
201-300 mm	2	245.0 (0.5)	-	-
301-400 mm	-	-	-	-
400+ mm	-	-	-	-
<i>P. fuscus</i>	5	235.0 (3.27)	12	335.0 (1.43)
# Empty guts	2 (40%)	320.0 (0)	6 (50%)	298.3 (3.49)
0-100 mm	-	-	-	-
101-150 mm	-	-	-	-
151-200 mm	1	170.0 (-)	-	-
201-300 mm	2	267.5 (0.75)	1	300.0 (-)
301-400 mm	-	-	5	342.0 (1.53)
400+ mm	-	-	-	-
<i>M. cephalus</i>	10	212.5 (3.16)	13	180.8 (2.75)
# Empty guts	0 (0%)	-	0 (0%)	-
0-100 mm	-	-	-	-
101-150 mm	5	120	10	143.0 (0.15)
151-200 mm	-	-	1	160.0 (-)
201-300 mm	3	286.6 (0.88)	1	270.0 (-)
301-400 mm	2	332.5 (1.25)	-	-
400+ mm	-	-	1	490.0 (-)
<i>S. ciliata</i>	36	181.1 (0.28)	3	275.0 (1.5)
# Empty guts	9 (25%)	168.3 (0.12)	1 (33.3%)	165.0 (-)
0-100 mm	-	-	-	-
101-150 mm	4	137.5 (0.44)	-	-
151-200 mm	22	171.4 (0.19)	-	-
201-300 mm	1	210.0 (-)	2	275.0 (4.5)
301-400 mm	-	-	-	-
400+ mm	-	-	-	-

Mollusca	44.4	59.7	13.5	50.0	5.0	10.0	-	-	-	-	-	-
Bivalvia	44.4	59.7	13.5	50.0	5.0	10.0	-	-	-	-	-	-
Gastropoda	-	-	-	-	-	-	-	-	-	-	-	-
Teleosts	-	-	-	-	-	-	-	-	-	-	-	-
FW Inverts *	-	-	-	100.0	8.4	7.5	-	-	-	7.7	1.8	0.4
Sediment	-	-	-	-	-	-	50.0	***	45.0	-	-	-
Algae	3.7	***	1.8	-	-	-	40.0	***	40.0	92.3	***	57.3
Plant Material	-	-	-	50.0	***	2.5	-	-	-	30.7	***	2.3
Other	3.7	***	0.4	50.0	***	17.5	20.0	***	15.0	61.5	***	35.4

* FW Inverts were collapsed into one category due to the occurrence of specific taxa in the stomachs of each fish species. (Chironomids – *S. ciliata*,

Coleopterans – *A. australis* and Hemipterans – *M. cephalus*).

*** indicates indiscrete dietary items that are not able to be numerically classified.

Table 3. Mean (\pm S.E.) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures of IsoSource end members (Algae, Seston, Riparian Vegetation, Polychaetes, Molluscs, Decapods and Small Fish) and *Acanthopagrus australis*, *Sillago ciliata*, *Platycephalus fuscus* and *Mugil cephalus* collected from Tallows and Belongil Creeks in November-December 2003.

[Polychaetes were *Marphysa* sp.; Molluscs were bivalves; Decapods included small crabs and prawns (*Metapeneaus bennettiae*) and Small fish included gudgeons (*Philypnodon* sp.) and estuary perchlet (*Ambassis marianus*)].

	Tallows Creek		Belongil Creek	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Algae	-21.16 (2.45)	22.38 (0.93)	-15.32 (2.83)	3.76 (2.09)
Seston	-22.85 (0.69)	18.38 (0.27)	-22.70 (-)	3.09 (-)
Riparian Vegetation	-29.03 (0.66)	5.24 (1.17)	-28.68 (0.77)	-2.47 (0.53)
Polychaetes	-24.45 (0.83)	22.19 (0.96)	-21.61 (-)	7.62 (-)
Molluscs	-23.89 (0.56)	20.39 (0.69)	-11.85 (-)	8.67 (-)
Decapods	-18.82 (0.80)	22.60 (1.20)	-20.52 (1.06)	6.91 (0.84)
Small Fish	-26.81 (1.33)	24.82 (0.82)	-18.60 (0.63)	10.23 (0.48)
<i>Acanthopagrus australis</i>	-23.18 (2.10)*	24.85 (1.12)*	-19.35 (0.34)	11.33 (0.65)
<i>Sillago ciliata</i>	-18.69 (0.62)*	23.30 (2.50)*	-17.84 (0.10)	11.22 (0.11)
<i>Platycephalus fuscus</i>	-19.87 (0.37)*	19.85 (0.93)*	-18.38 (0.37)	11.68 (0.16)
<i>Mugil cephalus</i>	-23.00 (0.68)*	22.63 (0.62)*	-16.43 (0.78)	9.47 (0.54)

- denotes instances where only 1 sample was collected - standard errors could not be calculated.

* denotes statistically significant differences in fish isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) signatures between Tallows and Belongil Creeks ($p < 0.05$).







