
Available at: https://doi.org/10.3354/meps12498

Accessed from: http://hdl.handle.net/1959.13/1420973
Title: Functional role of the soft coral *Dendronephthya australis* in the benthic food web of temperate estuaries

Authors: Michael Corry¹,³,* , David Harasti², Troy Gaston¹, Debashish Mazumder³, Tom Cresswell³, Natalie Moltschaniwskyj¹,²

¹ School of Life and Environmental Sciences, University of Newcastle, Ourimbah, New South Wales 2258, Australia
² Fisheries Research, New South Wales Department of Primary Industries, Port Stephens, New South Wales 2316, Australia
³ Australian Nuclear Science and Technology Organisation, Locked Bag 2001 Kirrawee DC, New South Wales 2232, Australia

*Corresponding Author: corrym@anso.gov.au
Abstract

The soft coral *Dendronephthya australis*, with its limited distribution along the central New South Wales (NSW) coastline, forms a habitat within the benthic estuarine environment that supports commercially significant and protected marine species. However, the functional role of the soft coral within this system is unknown. Organisms from primary producers through to secondary consumers were sampled from soft coral and sponge habitats inside the Port Stephens estuary NSW, Australia in 2014. A food web model of the benthic habitat, created using stable isotopes of carbon and nitrogen, was used to describe the functional role of the soft coral in comparison to sponges, another important habitat for commercially significant and protected marine species. Primary consumers accessed a range of benthic and pelagic energy sources; however, secondary consumers were almost entirely dependent on pelagic energy sources. Soft coral and sponges accessed different primary sources for their energy requirements. There was no evidence that *D. australis* was used as a direct food source by consumers other than nudibranchs. In contrast, sponges were trophically linked with secondary consumers and are likely to play a direct role in pelagic energy transfer. Amphipods collected from the branches of *D. australis* were identified as major prey components in the diet of protected syngnathids, suggesting that while the soft coral functions as critical habitat it is indirectly linked to higher trophic levels.

Keywords

Stable isotopes; critical habitat; sponge; syngnathidae; estuary; conservation
Introduction

Measures of diversity such as species richness and abundance are closely linked to ecosystem function; the more species present, the greater the range of functional traits and the more dynamic and productive the ecosystem (Lawton 1994, Bu et al. 2014). However, ecosystem function depends not only on the numbers of species present (Stuart-Smith et al. 2013), but also the nature of intra-specific and inter-specific interactions occurring among species. These interactions are a consequence of functional traits of individual species and it is the range and value of these traits in the community (functional diversity) that drives ecosystem processes, such as productivity, nutrient cycling, and energy transfer (Power 1992, Diaz & Cabido 2001). Interactions within a community will determine a species’ contribution to the ecosystem processes and the functional role a species performs within its habitat (Tilman 2001). Therefore, information on a species functional role can assist ecosystem conservation by allowing conservation management to be focussed on the protection of species whose roles are closely linked to ecosystem processes and function (Cadotte 2011).

Food web models link species and map energy flow by describing the sources of energy for organisms in a community, allowing the trophic structure of a community to be described (Pimm et al. 1991). Therefore, food web models can be used to identify the functional role of particular species that have considerable influence over the flow of energy within an ecosystem. Stable isotope analysis allows the production of food web models by comparing the natural abundance of carbon and nitrogen isotopes in the tissue of resident organisms (Gillies et al. 2013). The technique relies on identifying a consistent pattern of isotopic enrichment with increasing trophic level (Peterson & Fry 1987). The nitrogen isotope ratio ($^{15}\text{N}:^{14}\text{N}$) in a consumer is enriched in $^{15}\text{N}$ by 3–4‰ relative to its
diet (DeNiro & Epstein 1981) and represents a species trophic position (Peterson & Fry 1987). The carbon isotope ratio ($^{13}$C:$^{12}$C) is only slightly enriched in $^{13}$C ($\leq 1\%$) amongst trophic levels (DeNiro & Epstein 1981) and traces the flow of energy within an ecosystem by linking carbon sources at the base of a food web with higher order consumers (Sun et al. 2011).

The soft coral *Dendronephthya australis* (family Nephtheidae) occurs along the southern shoreline of the Port Stephens estuary in New South Wales (NSW), with a distribution suspected to be limited to central NSW (Poulos et al. 2015). The estuary is part of the Port Stephens Great Lakes Marine Park; however, the soft coral habitat exists exclusively outside the no-take areas leaving the species at risk from human disturbance.

Whilst soft coral habitat has been linked to high fish and invertebrate biodiversity that include commercially important snapper (Poulos et al. 2013) and members of the protected Syngnathidae family (Harasti et al. 2014), little is known about their functional role in the temperate estuarine community. In tropical environments soft corals access phytoplankton as a major dietary source (Fabricius et al. 1995), while temperate species in the northern hemisphere select zooplankton (Sebens & Koehl 1984). Tropical soft corals have some predators, eg Opisthobranch, Pomacentridae, Chaetodontidae (Fabricius & Alderslade 2001) as do Antarctic species, eg Asteroidea, Pycnogoida (Slattery & McClintock 1995).

Less is known of the trophic links to soft corals in southern temperate estuarine environments (Fabricius & Alderslade 2001) and describing these could provide insights into the functional role of the benthic invertebrate in these marine systems, potentially highlighting the importance of conservation for this habitat.
Within the Port Stephens Great Lakes Marine Park there exists a nearby sponge habitat that also supports diverse fish and invertebrate communities (Van Lier et al. 2017) and unlike the soft coral, is protected inside marine park no-take areas. Also a sessile, benthic macro-invertebrate, sponges may perform a similar functional role as the soft corals and therefore, potentially mitigate the loss of ecosystem services like habitat structure and energy transfer in the event of soft corals disappearing from the estuary (Naeem & Li 1997). Comparing the functional role of soft corals with the sponges will determine if the ecosystem services provided by these habitats are different; such information is also critical in the justification of conservation management for soft corals.

The aim of the study was to develop a food web model of soft coral and sponge habitats using stable isotopes of carbon and nitrogen to trace the flow of energy and determine trophic structure within the temperate estuarine benthic food web. We explored the trophic interactions occurring among primary sources, filter feeders, and higher consumer organisms, focusing specifically on the involvement of soft corals and sponges. Based on the identified trophic links, the functional roles of soft coral and sponges were proposed to provide insight into their significance in the ecosystem.

Materials and methods

Location

Port Stephens is a tide dominated estuary (Roy et al. 2001) located approximately 200 km north east of Sydney in NSW, Australia (32°42'44"S, 152°9'37"E; Figure 1). The eastern section of the estuary contains a diverse range of marine habitats that include soft coral dominated and sponge dominated benthic communities (Davis et al. 2015). Sampling was conducted in both habitat types at two locations; Seahorse Gardens which contained a dense cover of the soft coral *D. australis*, and Pipeline dominated by sponges with adjacent
patches of *D. australis* (Harasti 2016). Seahorse Gardens is located 750 m east of Pipeline and both locations occur within 100 m of the shoreline, at a bottom depth of 5-11 m. Recent mapping suggests that depth, seabed slope, tidal velocity, and distance from the estuary mouth are very similar for both locations (Poulos et al. 2015).

**Fig. 1** Location of Nelson Bay and Port Stephens estuary, NSW including Seahorse Gardens and Pipeline sampling locations.

**Sampling design**

Given the close proximity and abiotic similarity of the two locations, spatial variation of isotopic signatures between locations was unlikely and not considered. Instead, the sampling design treated the two habitat types as part of one community represented by one food web. Samples of marine organisms from primary producers through to tertiary consumers were collected by scuba diving soft coral habitats at Seahorse Gardens and Pipeline, and from sponge habitat at Pipeline, in December 2014. Divers collected vertebrates and invertebrates during each dive with the aim of collecting five of as many
different species as possible, across a range of feeding guilds and trophic levels to allow
the production of a comprehensive food web model. Part time resident species were
included to allow the investigation of many possible links to the soft coral. Zooplankton
and macro invertebrate species not sampled in December 2014 were sampled in August
2015. To investigate the effect of seasonal variability on isotopic signature, multiple
species already collected in December, including soft coral and sponges, were also
collected for analysis in August 2015 and compared to the summer results.

Primary source collection

Sediment organic matter (SOM) was collected using a 10 cm$^2$ plastic scoop to
scrape the top 5 cm of sediment from bare patches adjacent to the soft coral and sponge
structures. Dissolved organic matter (DOM) and particulate organic matter (POM) were
sampled from seawater collected at 1 m above the bottom. Water samples were filtered
through multiple 0.45 µm glass fibre filters with POM remaining on the filters and DOM
collected in the filtrate. Zooplankton samples in surface waters above the soft coral and
sponge habitats were collected using a plankton net (150 µm mesh). It was noted that
although zooplankton is typically considered a primary consumer (Le Loc'h et al. 2008,
Wyatt et al. 2012), in this study zooplankton was referred to as a primary source of carbon
and nitrogen to other consumers in the food web because the $\delta^{15}$N values of all size classes
were similar to the autotroph’s (seagrass and epiphytes) and $\geq 2\%$ lower than the $\delta^{15}$N
value of any other primary consumer. All primary source samples were frozen within six
hours from the time of collection and kept at -20$^\circ$C until preparation and analysis was
conducted.
A knife was used to remove sections (approximately 5 cm³) from three sponge species (*Echinoclathria* sp., *Holopsamma* sp. and *Siphonochalina* sp.) as well as similar size pieces of *D. australis* branches. Intact soft coral colonies were also collected and later sieved to retrieve resident macro-invertebrates. All other invertebrates within the habitats the exception of cephalopods were collected by hand. Cephalopods and smaller, slow moving fish were collected with hand nets. The tip (~3 mm) of each seahorse’s prehensile tail was clipped and collected underwater (Valladares & Planas 2012) to avoid having to take a species listed as protected. All collected samples were held in zip lock bags with sufficient water so to keep the organisms alive and reduce unnecessary stress until the conclusion of each dive. Samples were then taken to the surface and directly euthanized in an ice water slurry (Blessing et al. 2010). Within six hours of collection, all consumer samples were identified, labelled, and frozen at -20°C until preparation and analysis was conducted. Given the time restraints of diver collections and the difficulty of collecting some highly mobile species it was not possible to obtain five replicates of each species. Many mobile species were represented by one individual (Supplement Table 1) and the lack of replication noted as a study limitation.

*Sediment samples were washed with reverse osmosis (RO) water through a series (4 mm, 1 mm, 0.5 mm and 0.25 mm) of sieves and the finest size-fraction (< 0.25 mm) was kept as the SOM (Mazumder et al. 2011). Since stable isotope analysis (SIA) in this study targeted organic carbon, sediment samples were treated with acid to remove the inorganic fraction. Filtrate from water samples was evaporated in trays in an oven at 60°C for up to 96 hours to expose the dissolved fraction (DOM). POM was left on the filters for analysis. Sieves were used to separate zooplankton into three size classes, 150- 250 µm, 250-500
µm, and >500 µm. Zooplankton were rinsed in RO water and dried at 60°C for 48 h.

Depending on the consumer organism, different tissue types were dissected for isotope analysis. A scalpel was used to remove small pieces of epidermal tissue from the branch sections of the soft coral. Care was taken not to include the polyp end of the corals structure since it was typically covered in juvenile brittle stars. Small 3 cm³ sections of sponge tissue were sliced, rinsed with RO water, and gently squeezed to remove foreign material. Epidermal tissue was dissected from echinoderm samples except for *Phyllacanthus parvispinus* where internal soft tissue was dissected. Molluscs and arthropods too small to obtain adequate tissue samples were analysed whole following the removal of stomach and internal organs. Clean white muscle tissue was dissected from large molluscs, large arthropods, and all chordates with the exception of seahorses in which the tail tissue was analysed whole.

All samples (primary sources, invertebrates and fish) were rinsed in RO water and dried to constant weight in an oven at 60°C for up to 72 h. Samples were homogenised by grinding to a fine powder using a mortar and pestle for small amounts of sample, and a ball and mill grinder for larger amounts. Seahorse tail clippings and minute crustacean samples too small to grind were sliced into smaller sections using a scalpel until enough mass for analysis was obtained.

The carbonates in a sample can alter δ¹³C values (Bosley & Wainright 1999) and were removed from samples in which carbonate free tissue extraction was not possible (small invertebrates, soft coral, sponges, plankton and SOM). Half of each powdered sample was saturated in 0.1M hydrochloric acid for one hour, rinsed with RO water and re-dried and re-ground (Mazumder et al. 2011). As the acidification step can lead to an
enrichment of $^{15}$N (Pinnegar & Polunin 1999), the half of each sample that was not acidified was used to analyse $\delta^{15}$N. The presence of lipid in tissue samples can also influence $\delta^{13}$C signatures (Post et al. 2007). Sample preparation for analysis targeted lipid free tissue; however, small amounts of lipids may have still been present. Therefore, to standardise lipid content amongst different tissue types, the normalisation mathematical formula was applied (Post et al. 2007). Since the lipid content of tissue is related to the molar ratio of carbon to nitrogen (C:N) in the tissue, when C:N was > 3.5 (e.g. high lipid content), stable isotope values were normalised using the following equation:

$$\delta^{13}\text{C}:\text{N}_{\text{normalised}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C:N}$$

Powdered samples were stored in sterile 5ml screw cap vials until being weighed to the nearest microgram in tin capsules. Carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) signatures were then analysed on a continuous-flow stable isotope ratio mass spectrometer (CF-IRMS), model Delta V Plus (Thermo Scientific Corporation, U.S.A.), interfaced with an elemental analyser (Thermo Fisher Flash 2000 HT EA, Thermo Electron Corporation, U.S.A.) at the Australian Nuclear Science and Technology Organisation, Sydney. Data were reported relative to International Atomic Energy Agency secondary standards calibrated against global standards of Vienna PeeDee Belemnite for carbon and air for nitrogen. A two point calibration was used to normalise the data, using standards that bracket the samples being analysed. Stable isotope values were reported in delta ($\delta$) units, in parts per thousand ($\%$) relative to the international standard and determined using the equation:

$$\delta X = ((R_{\text{sample}} / R_{\text{standard}}) - 1) \times 1000$$
Where $X$ is carbon or nitrogen and $R$ is the ratio of the heavy isotope over the light isotope.

Two replicate samples were included in each run for quality control and standard deviations of replicate samples ($n = 50$) were 0.3‰ for both $\delta^{13}C$ and $\delta^{15}N$.

**IsoSource mixing model**

The IsoSource model was used to determine the feasible contribution of multiple energy sources (selected possible prey species) to the diets of *D. australis* and sponges. The diet of syngnathids was also investigated given that any links observed between *D. australis* and these protected organisms would give weight to the conservation importance of the soft coral. IsoSource model calculates the feasible combinations of each source (provided there was at least one more sources than elements used) that could explain one consumer’s signature (Phillips & Gregg 2003). The sources were selected for Isosource modelling if they were known prey items for a particular consumer and if their $\delta^{13}C$ signature suggested a dietary link. Four sources were selected for soft coral and sponge diet analysis and five sources were selected for the analysis of syngnathids diets; however, results were only provided for the top four contributors. The Isosource method examines all possible combinations of primary source potential contribution (0-100%) in small increments (1%). Combinations that summed to within 0.01‰ of the consumer signature were considered feasible contributions (i.e. they explain the consumer signature); if mixture isotope values were outside model limits (no contribution could be determined), the tolerance value was increased incrementally up to a maximum of 0.05‰ (Benstead et al. 2006). Results were reported as the distribution of feasible solutions for each source. The mean and 1st percentile to 99th percentile ranges was also given, rather than the full range which is sensitive to small numbers of observations on the tails of the distribution (Melville & Connolly 2003). To account for $\delta^{15}N$ fractionation, we subtracted 2.9‰ from
the signature of consumer species (Zanden & Rasmussen 2001), except for sponges in which 2.1‰ (Vanderklift & Ponsard 2003) was subtracted since estimates were not possible with the 2.9‰ value. To account for δ^{13}C fractionation, we subtracted 1‰ from the signature of all consumer species (Peterson & Fry 1987).

Estimates of consumer trophic position can be influenced by temporal variation in the δ^{15}N signatures primary sources (Post 2002). Therefore, the δ^{15}N value of a bivalve *Fulvia tenuicostata* was used as a baseline to estimate trophic position because longer lived primary consumers, such as bivalves, show far less temporal variation than primary producers (Zanden & Rasmussen 2001). We assumed a trophic fractionation of 2.9‰ for each trophic level in the food web (DeNiro & Epstein 1981, Minagawa & Wada 1984).

**Statistical analysis**

One way ANOVA and Tukey’s *post hoc* HSD were used to test for differences in the isotopic signatures (δ^{13}C or δ^{15}N) among consumer feeding groups, and between soft coral and sponges. The assumption of normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene’s test (Zar 1999). Type I error for statistical tests was set at α = 0.05 and all statistical analyses were performed using SPSS version 22.

To facilitate the analysis of the food web structure, individual taxa were categorised *a priori*, using FishBase (Froese & Pauly 2016) into several feeding (trophic) groups based on common feeding mode: filter feeder, deposit feeder, grazer, planktivore, omnivore, and carnivore. We acknowledge that the feeding categories assigned to species are not definitive; for example, the feeding strategies of fish may be different between juvenile and adult stages (Benavides et al. 1994) or may be influenced by changes in environmental conditions (Behrens et al. 2012). However, separation of species into trophic groups enable
the assessment of carbon flow and trophic structure in the wider context of a food web (Gillies et al. 2012).

Results

In total, 64 consumer species (including *Dendronephthya austalis* and three species of sponge) and seven primary sources, spanning a wide range of taxonomic groups and feeding guilds, were sampled from the soft coral and sponge habitats (Supplement Table 1). The isotopic signatures of species collected in December 2014 and in August 2015 were within 1.1 ‰ for δ^{13}C and 1.3‰ for δ^{15}N for all species (Supplement Table 2).

Food web structure

The mean carbon isotope values of primary sources spanned a large range (13.8‰) with benthic sources such as seagrass (e.g. *Posidonia australis*, -8.2‰) ^{13}C enriched and pelagic planktonic sources (e.g. POM, -21.4‰) ^{13}C depleted (Figure 2). Conversely, the mean nitrogen values of primary sources had a much smaller range of 2.2‰. Carbon isotope values of all consumer species covered a range of 9‰ and were within the range of values of primary sources; however, 84% of consumer species had low δ^{13}C values clustered within a range of 4.3‰. The remaining 16% of consumer species were higher for δ^{13}C with no distinct cluster. The nitrogen isotope values for all consumers had a range of 5.6‰. The δ^{15}N value of the bivalve *Fulvia tenuicostata*, the chosen trophic baseline species, established the base of trophic level 2 at 8.2‰. The designated 2.9‰ enrichment per trophic level meant all consumer species were within two trophic levels.

Filter feeder, deposit feeder and grazer feeding groups occupied trophic level 2 and were all significantly less for δ^{15}N (F(6,230) = 81.8, p < 0.001, Supplement Table 3) compared with omnivore, planktivore, and carnivore feeding groups in trophic level 3.
Carnivore (TL3), planktivore (TL3), omnivore (TL3), and grazer (TL2) feeding groups all had very narrow ranges (< 3‰) of low $\delta^{13}C$ values (< -16‰) that were similar to those of the pelagic primary sources (Figure 2). In contrast, deposit feeder and filter feeder groups (TL2) had a wider range of $\delta^{13}C$ values.

Fig. 2 Mean (±SE) carbon and nitrogen isotope values of *Dendronephthya australis*, sponges (*Echinoclathria sp*, *Holopsamma sp*. and *Siphonochalina sp*), potential dietary sources and consumers (n = 1-17, no error bars indicate a sample size of one, or very small variability in the isotope values of a species). Dashed lines represent estimates of trophic levels (TL) considering a 2.9‰ trophic enrichment factor. Black triangle - primary source; square - filter feeder; circle - deposit feeder; up-pointed triangle - grazer; down-pointed triangle - omnivore; half black circle - planktivore; diamond - carnivore.
Soft coral and sponges

The isotopic signature of the soft coral (*Dendronephthya australis*) was significantly higher than the three species of sponges (*Echinoclathria* sp., *Holopsamma* sp. and *Siphonochalina* sp.) for both $\delta^{13}C$ ($F(3, 20) = 197.5$, $p < 0.01$, Supplement Table 4) and $\delta^{15}N$ ($F(3, 20) = 55.6$, $p < 0.01$, Supplement Table 5). There was no significant difference among the three species of sponge for $\delta^{13}C$ or $\delta^{15}N$. Both pelagic and benthic primary sources supported the diet of the soft coral (Figure 3). The smallest fraction of zooplankton (150-250$\mu$m) had the greatest estimate of feasible contribution (mean 64%) of dietary sources for *D. australis* followed by 32% for *P. australis* seagrass. Unlike soft coral, the diet of sponges was almost solely supported by POM (pelagic source) with the greatest feasible contribution of 95% (Figure 4). The feasible contribution of all other pelagic sources was less than 4%.

Fig. 3 Histograms of the distribution of feasible contributions of dietary sources for *Dendronephthya australis* after correcting for trophic enrichment (1‰ for $\delta^{13}C$ and 2.9‰ for $\delta^{15}N$). Values in brackets are mean, 1-99 percentiles for the distributions.
Fig. 4 Histograms of the distribution of feasible contributions of dietary sources for sponges after correcting for trophic enrichment (1‰ for δ$^{13}$C and 2.1‰ for δ$^{15}$N). Values in brackets are mean and 1-99 percentiles for the distributions.

*Trophic reliability - syngnathids*

No species in trophic level 3 had high δ$^{13}$C values and all secondary consumers were at least 3.5‰ less for δ$^{13}$C than the soft coral (Figure 2). Instead, the low δ$^{13}$C values of secondary consumers aligned closely to those of the sponges. Amongst the assemblage of secondary consumers were three species of protected syngnathidae, *Filicampus tigris*, *Festaculex cintus* (pipefish), and the seahorse *Hippocampus whitei*. All three species were more than 3.7‰ lower for δ$^{13}$C compared to soft coral. The amphipods collected from within soft coral branches occupied trophic level 2 and were low for δ$^{13}$C (-18.4‰). The isotopic signatures of both pipefish were within the level of trophic enrichment (<1‰ for δ$^{13}$C and <2.9‰ for δ$^{15}$N) that would be expected for consumers of amphipods in this system.
Isosource mixing model simulations determined amphipods as the dominant dietary contributor for all three species of syngnathid. For pipefish, the estimate of feasible contribution of amphipods was, on average, 60% and 73% to the diets of *F. tigris* and *F. cintus* respectively (Figure 5). Zooplankton (250-500µm) was also a major energy source with an estimated average feasible contribution of 40% for *F. tigris* and 22% for *F. cintus*. The estimated average feasible contribution of amphipods to the seahorse *H. whitei* was 77% (Figure 6). Unlike pipefish, the remaining portion of the *H. whitei* diet was probably supported by isopods, who were estimated to have a feasible contribution of 16%, and a smaller (< 5%) contribution from zooplankton sources.
Fig. 5 Histograms of the distribution of feasible contributions of four dietary sources for *Filicampus tigris* (left) and *Festaculex cintus* (right) after correcting for trophic enrichment (1‰ for δ¹³C and 2.9‰ for δ¹⁵N). Values in brackets are mean and 1-99 percentiles for the distributions.
Fig. 6 Histograms of the distribution of feasible contributions of four dietary sources for *Hippocampus whitei* after correcting for trophic enrichment (1‰ for δ\(^{13}\)C and 2.9‰ for δ\(^{15}\)N). Values in brackets are mean and 1-99 percentiles for the distributions.

**Discussion**

Distinct primary sources were identified using stable isotope analysis allowing the elucidation of energy contribution to soft coral and sponges, and other species in the southern temperate estuarine food web. Consumers were grouped according to their feeding type and main food source which, for primary consumers, was considerably varied since δ\(^{13}\)C values spanned the full range of primary source values. Secondary consumers; however, were highly dependent on pelagic food sources since all groups were clustered within a narrow range of low δ\(^{13}\)C values. Soft coral and sponges were isotopically distinct and therefore accessed different primary sources for their energy. The δ\(^{13}\)C values indentified sponges as possible prey for secondary consumers but not the soft coral. The diet of three protected Syngnathidae species was substantially (> 60%) supported by
amphipods found among *D. australis* branches suggesting that these animals used the soft coral habitat to feed on the small invertebrates.

**Food web structure**

The $\delta^{13}C$ values of the pelagic and benthic primary sources were different and it was possible to identify the contribution of potential energy sources to individual consumer species, as well as the contribution of pelagic and benthic carbon to the overall food web. The primary consumers (filter feeders, deposit feeders and grazers) in this estuary derived their energy needs from multiple sources and from both benthic and pelagic environments, given their large range of $\delta^{13}C$ values. Dietary variation among primary consumers reflects the variability of primary food sources (Coma et al. 2001) and this is a common observation in shallow water benthic communities (Hobson et al. 2002, Le Loc'h et al. 2008, Gillies et al. 2012).

In contrast, the secondary consumer groups (carnivore, omnivore, and planktivore) accessed sources of energy from the pelagic environment (POM and zooplankton) only, given all groups had a similar and restricted range of low $\delta^{13}C$ values. It is common for secondary consumers in benthic coastal communities, especially fish, to access their dietary requirements from pelagic derived sources (Gillies et al. 2012), even in environments rich in benthic production (Shahraki et al. 2014). However, conflicting with our observations, pelagic components of secondary consumer diets are often supported by contributions from benthic derived sources (Nyunja et al. 2009, Wyatt et al. 2012). The results strongly suggest that in this system, carbon and energy inputs derived from benthic sources fail to be transferred onto secondary consumer groups and as such, higher consumers were very reliant upon pelagic energy sources.
Soft coral and sponges fed on different components of the seston and soft coral occupied a higher trophic level in the food web as evidenced by the separated $\delta^{13}C$ and $\delta^{15}N$ values of these organisms. *Dendronephthya australis* fed mainly on small (150 - 250 $\mu$m) zooplankton and similar results have been observed for northern hemisphere species (*Sebens & Koehl 1984*). Seagrass was also suggested to have a considerable contribution (32%) to the soft coral diet. Although seagrass is not considered food for soft corals (*Fabricius & Alderslade 2001*), detritus has been established as a dietary source for the soft coral *Alcyonium siderium* (*Sebens & Koehl 1984*) and it is possible that seagrass in the detritus is substantially contributing to the diet of *D. australis*. Further studies in the estuary are recommended to investigate the seagrass detritus link with *D. australis* to determine the importance of this source to the persistence of soft coral populations. Unlike *D. australis*, sponges derived their energy almost exclusively from phytoplankton (POM) and this was consistent with other studies (*Pile et al. 1997, Lesser 2006*). Sponges lack tentacles and rely on filtering large volumes of water to feed (*Reiswig 1971*) and it is likely that the difference in morphology of the two benthic feeders accounts for the difference in their prey selection.

There was no evidence from the stable isotope data that *Dendronephthya australis* was a direct food source for secondary consumers as the $\delta^{13}C$ values of the soft coral were substantially higher than those of all other consumer species. Soft corals are not usually a food source for generalist predators, and only specialist consumers (pycnogonids and opisthobranchs) readily feed on them (*Sammarco & Coll 1992, Avila et al. 1999*). The results do however conflict with observation of direct predation by the nudibranch *Dermatobranchus* sp on *D. australis* (*Davis et al. 2017*). A secondary consumer,
*Dermatobranchus* sp., as well as three other nudibranch species, had low $\delta^{13}$C values and their $\delta^{15}$N values were not higher than *D. australis*, indicating no trophic link to the soft coral. The disparity between the direct observation of feeding on soft corals by *Dermatobranchus* sp. and stable isotope values suggests conventional isotopic enrichment factors may not be applicable for this group of organisms. Nudibranchs possess the ability to shift organic material to specific regions of their body as part of a chemical defence strategy (Penney 2002) and this may interfere with the way carbon and nitrogen isotopes fractionate in their tissues but it this is yet to be tested. Since nudibranchs have no known predators in these environments it is unlikely energy gained from *D. australis* is transferred further up the food web; therefore, the claim that the soft coral is not an important food source still holds.

In contrast to *D. australis*, it was possible that the sponges were used as a direct food source by consumer species given their isotopic values aligned with those of secondary consumers in the pelagic pathway. Various predators (sea stars, fish and sea turtles) actively consume sponges (Lesser 2006), particularly in tropical environments (Hill 1998). Within the temperate food web, we collected fish from families (Monocanthidae and Pomacentridae) that consume sponges in tropical environments (Ruzicka & Gleason 2009), and their isotopic values were within the range expected for fish that fed on sponges. For example, isotope values of the omnivorous leatherjacket *Nelusetta ayraudi* were 1‰ higher for $\delta^{13}$C and 3.3‰ for $\delta^{15}$N to that of the sponge *Holopsammona sp*. This is consistent with established enrichment factors (Post 2002). Therefore, it is possible that sponges contributed to the diet of the leatherjacket, and many other fish species with similar isotope values. While gut content analysis complementing the SIA would support this conclusion, the isotope data suggest that it is likely that sponges provide one possible
link in the energy pathway between primary sources and secondary consumers within this temperate estuarine system. Given the dependence of secondary consumers on pelagic derived energy, the results suggest sponges play a very important functional role in the transfer of pelagic energy to secondary consumers in this system.

Trophic reliance - syngnathids

The $\delta^{13}C$ value of the amphipods, collected only from soft coral habitat, linked these small invertebrates to secondary consumers. In particular, the $\delta^{13}C$ values of protected syngnathids *Hippocampus whitei* (seahorse), *Filibampus tigris* and *Festucalex cintus* (pipefish) closely aligned with amphipods and it was estimated that amphipods contributed at least 60% to the diet of the three syngnathid species. The seahorse *H. whitei* displays a preference for *D. australis* which was thought to provide a habitat to hide from predators (Harasti et al. 2014). However, this study suggests that the seahorses and pipefish also feed within the *D. australis* habitat; linking the soft coral indirectly to the energy pathway of these protected species. This energy link may not be limited to syngnathids alone. Amphipods are also a major dietary component for many other marine consumers (Morton et al. 2016) and isotope signatures of secondary consumers indicate that it is highly likely many of these species feed on amphipods. Therefore, soft corals in this system could be considered critical habitat since their structure supports amphipod communities that are important prey for protected syngnathids and many other fish consumers. While the results describe the indirect link between soft coral and pelagic energy transfer, it is important to consider that amphipods also reside in many other marine habitats (Stoner 1980), including sponges (Poore et al. 2000). It is evident from this study that the importance of the role of soft corals in linking amphipods and syngnathids can only be recognised by comparing the isotopic signatures of amphipods from soft coral
habitat to amphipods from other habitat types within this estuary and should be the focus of future investigation.

Conclusion

Analysis of $\delta^{13}C$ and $\delta^{15}N$ in the tissue of organisms within the temperate estuarine environment revealed the structure of the benthic community and identified the importance of pelagic energy sources. In this system, soft coral indirectly influences the transfer of energy between primary and secondary consumers. *Dendronephthya australis* branches provide habitat for amphipods that were the largest contributor to the diets of protected syngnathids and possibly the diets of many other consumers in the community. Soft corals occupy a different ecological niche to sponges as the two filter feeders accessed different planktonic sources for their energy and were linked differently to secondary consumers. The functional role of the soft coral was therefore different to that of the sponges in this estuarine system and as a consequence, sponges may not be able to compensate for the ecosystems services lost should *D. australis* habitat continue to decline. Given that *D. australis* has a functional role as a critical habitat that is indirectly linked to the diet of protected syngnathids, this study suggests that in order to maintain the biodiversity value of this ecosystem both filter feeders require protection. It is hoped that data obtained from this study can be used to ensure suitable decisions can be made regarding the conservation management of *D. australis*. 
Acknowledgements

We are especially thankful to Christopher Gallen and Roger Laird for their sustained support with sampling, and to Barbara Gallagher and Scott Allchin who expertly performed the stable isotope analysis. This research was supported by the Fisheries Scientific Committee’s Student Research Grant for threatened and rare fish and marine vegetation in NSW.

Ethics: Sampling was conducted under the approval of the NSW Department of Primary Industry Animal Research Authority (ACEC 14-05) and The University of Newcastle Animal Ethics Approval A-2014-438.

The authors declare that they have no conflict of interest.

References


Lesser MP (2006) Benthic–pelagic coupling on coral reefs: feeding and growth of

Mazumder D, Saintilan N, Williams RJ, Szymczak R (2011) Trophic importance of a
temperate intertidal wetland to resident and itinerant taxa: evidence from multiple

primary sources of nutrition for fish. Oecologia 136:499-507

evidence and the relation between $\delta^{15}$N and animal age. Geochim Cosmochim Acta
48:1135-1140

Morton DN, Bell TW, Anderson TW (2016) Spatial synchrony of amphipods in giant kelp


supporting a diverse fish community in a tropical coastal ecosystem (Gazi Bay,
Kenya). Estuar Coast Shelf Sci 83:333-341

Oecologia 132:411-418

18:293-320

many sources. Oecologia 136:261-269

Pile A, Patterson M, Witman J Finding Reiswig’s missing carbon: quantification of sponge
feeding using dual-beam flow cytometry. Proc Proceedings of the 8th international
Coral Reef Symposium


Power ME (1992) Top-down and bottom-up forces in food webs: do plants have primacy. Ecology 73:733-746


Zanden M, Rasmussen JB (2001) Variation in $\delta^{15}$N and $\delta^{13}$C trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 46:2061-2066

Zar JH (1999) Biostatistical analysis. Pearson Education India