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Functional role of the soft coral *Dendronephthya australis* in the benthic food web of temperate estuaries

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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.

KEY WORDS: 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, Díaz & 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

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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 (SIA) 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}N:^{14}N) in a consumer is enriched in ^{15}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 (≤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, e.g. Opisthobranchia, Pomacentridae, and Chaetodontidae (Fabricius & Alderslade 2001), as do Antarctic species, e.g. Asteroidea and Pycnogonida (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, it 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 this 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 northeast of Sydney in NSW, Australia (32° 42′ 44″ S, 152° 9′ 37″ E; Fig. 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. 2016). Sampling was conducted in both habitat types at 2 locations: Seahorse Gardens, which contained a dense cover of the soft coral *Dendronephthya 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).

Sampling design

Given the close proximity and abiotic similarity of the 2 locations, spatial variation of isotopic signatures between locations was unlikely and not considered.

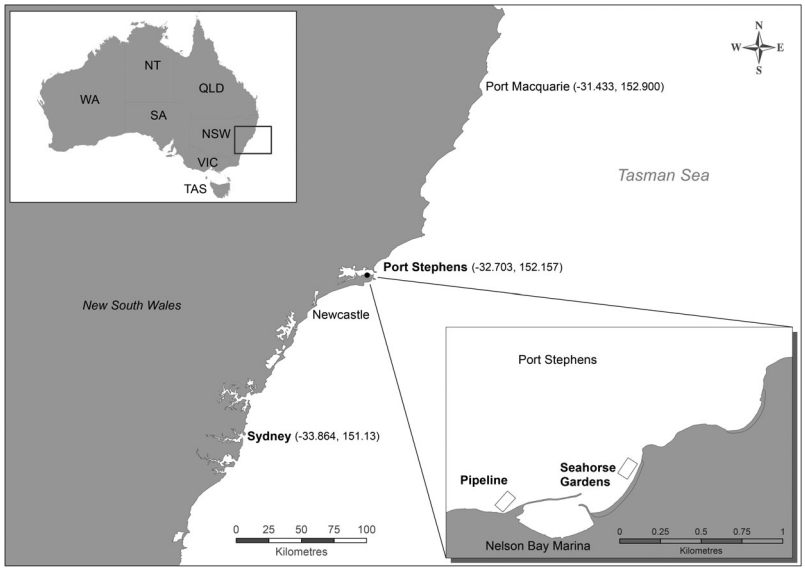

Fig. 1. Lower-right inset: Nelson Bay and Port Stephens estuary, including Seahorse Gardens and Pipeline sampling locations. Main map: central New South Wales coastline. Upper-left inset: Australia

Instead, the sampling design treated the 2 habitat types as part of 1 community represented by 1 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 5 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 December 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 the present 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 autotrophs (seagrass and epiphytes) and >2‰ lower than the $\delta^{15}N$ value of any other primary consumer. All primary source samples were frozen within 6 h from the time of collection and kept at −20°C until preparation and analysis was conducted.

Consumer collection

A knife was used to remove sections (approximately 5 cm^3) from 3 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 macroinvertebrates. All other invertebrates within the habitats with 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 ziplock bags with sufficient water to keep the organisms alive and reduce unnecessary stress until the conclusion of each dive. Samples were then taken to the surface and directly euthanised in an ice water slurry (Blessing et al. 2010). Within 6 h 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 5 replicates of each species. Many mobile species were represented by 1 indi-

vidual (Table S1 in the Supplement at www.int-res.com/articles/suppl/m593p061_supp.pdf) and the lack of replication noted as a study limitation.

SIA

Sediment samples were washed with reverse osmosis (RO) water through a series (4, 1, 0.5, and 0.25 mm) of sieves, and the finest size-fraction (<0.25 mm) was kept as the SOM (Mazumder et al. 2011). Since 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 h to expose the dissolved fraction (DOM). POM was left on the filters for analysis. Sieves were used to separate zooplankton into 3 size classes: 150–250, 250–500, 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 cm^3 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 from) 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 $\delta^{13}C$ 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 0.1 M HCl for 1 h, 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 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:

$$\delta^{13}C{:}N_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C{:}N \quad (1)$$

Powdered samples were stored in sterile 5 ml screwcap vials until being weighed to the nearest µg in tin capsules. The $\delta^{13}C$ and $\delta^{15}N$ signatures were then analysed on a continuous-flow stable isotope ratio mass spectrometer (CF-IRMS), model Delta V Plus (Thermo Scientific), interfaced with an elemental analyser (Thermo Fisher Flash 2000 HT EA, Thermo Electron), 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 2-point calibration was used to normalise the data, using standards that bracket the samples being analysed. Stable isotope values were reported in delta (δ) units, in parts per thousand (‰) relative to the international standard and determined using the equation:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000 \quad (2)$$

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. The IsoSource model calculates the feasible combinations of each source (provided there was at least 1 more source than elements used) that could explain 1 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 5 sources were selected for the analysis of syngnathids diets; however, results were only provided for the top 4 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 $\delta^{13}C$ 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 $\delta^{15}N$ signatures primary sources (Post 2002). Therefore, the $\delta^{15}N$ 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 ($\delta^{13}C$ or $\delta^{15}N$) 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 $\alpha = 0.05$ 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; e.g. 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 enables 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 australis* and 3 species of sponge) and 7 primary sources, spanning a wide range of taxonomic groups and feeding guilds, were sampled from the soft coral and sponge habitats (Table S1 in the Supplement). The isotopic signatures of species collected in December 2014 and in August 2015 were within 1.1‰ for $\delta^{13}C$ and 1.3‰ for $\delta^{15}N$ for all species (Table S2 in the Supplement).

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 (Fig. 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 $\delta^{13}C$ values clustered within a range of 4.3‰. The remaining 16% of consumer species were higher for $\delta^{13}C$ with no distinct cluster. The nitrogen isotope values for all consumers had a range of 5.6‰. The $\delta^{15}N$ value of the bivalve *Fulvia tenuicostata*, the chosen trophic

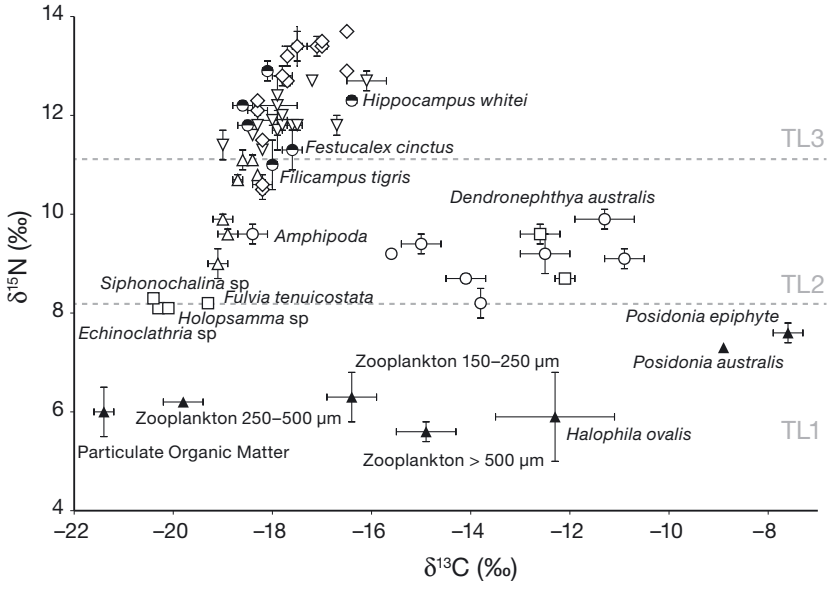

Fig. 2. Mean (±SE) carbon and nitrogen isotope values of *Dendronephthya australis*, sponges (*Echinoclathria* sp., *Holopsamma* sp., and *Siphonochalina* sp.), potential dietary sources, and consumers (n = 1–17; data points without error bars indicate a sample size of 1, or very small variability in the isotope values of a species). Dashed lines represent estimates of trophic levels (TL) considering a 2.9‰ trophic enrichment factor. Symbols: primary source (▲), filter feeder (□), deposit feeder (○), grazer (△), omnivore (▽), planktivore (◓), carnivore (◇)

baseline species, established the base of trophic level 2 (TL2) at 8.2‰. The designated 2.9‰ enrichment per trophic level meant all consumer species were within 2 trophic levels.

Filter feeder, deposit feeder, and grazer feeding groups occupied trophic level 2 and were all significantly less for $\delta^{15}N$ ($F_{6,230}$ = 81.8, p < 0.001, Table S3 in the Supplement) compared with omnivore, planktivore, and carnivore feeding groups in trophic level 3 (TL3). 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 (Fig. 2). In contrast, deposit feeder and filter feeder groups (TL2) had a wider range of $\delta^{13}C$ values.

Soft coral and sponges

The isotopic signature of the soft coral *D. australis* was significantly higher than the 3 species of sponges (*Echinoclathria* sp., *Holopsamma* sp., and *Siphonochalina* sp.) for both $\delta^{13}C$ ($F_{3,20}$ = 197.5, p < 0.01, Table S4 in the Supplement) and $\delta^{15}N$ ($F_{3,20}$ = 55.6, p < 0.01, Table S5 in the Supplement). There was no significant difference among the 3 species of sponge for $\delta^{13}C$ or $\delta^{15}N$. Both pelagic and benthic primary sources supported the diet of the soft coral (Fig. 3). The smallest fraction of zooplankton (150–250 µm) 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% (Fig. 4). The feasible contribution of all other pelagic sources was <4%.

Trophic reliance: syngnathids

No species in TL3 had high $\delta^{13}C$ values and all secondary consumers were at least 3.5‰ less for $\delta^{13}C$ than the soft coral (Fig. 2). Instead, the low $\delta^{13}C$ values of secondary consumers aligned closely to those of the sponges. Amongst the assemblage of secondary consumers were 3 species of protected Syngnathidae: the pipefishes *Filicampus tigris* and *Festucalex cinctus* and the seahorse *Hippocampus whitei*. All 3 species were more than 3.7‰ lower for $\delta^{13}C$ compared to soft coral. The amphipods collected from within soft coral branches occupied TL2 and were low for $\delta^{13}C$ (–18.4‰). The isotopic signatures of both pipefish were within the level of trophic enrichment (<1‰ for $\delta^{13}C$ and <2.9‰ for $\delta^{15}N$) that would be expected for consumers of amphipods in this system.

IsoSource mixing model simulations determined amphipods as the dominant dietary contributor for all 3 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. cinctus*, respectively (Fig. 5). Zooplankton (250–500 µm) was also a major energy source, with an estimated average feasible contribution of 40% for *F. tigris* and 22% for *F. cinctus*. The estimated average feasible contribution of amphipods to the seahorse *H. whitei* was 77% (Fig. 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.

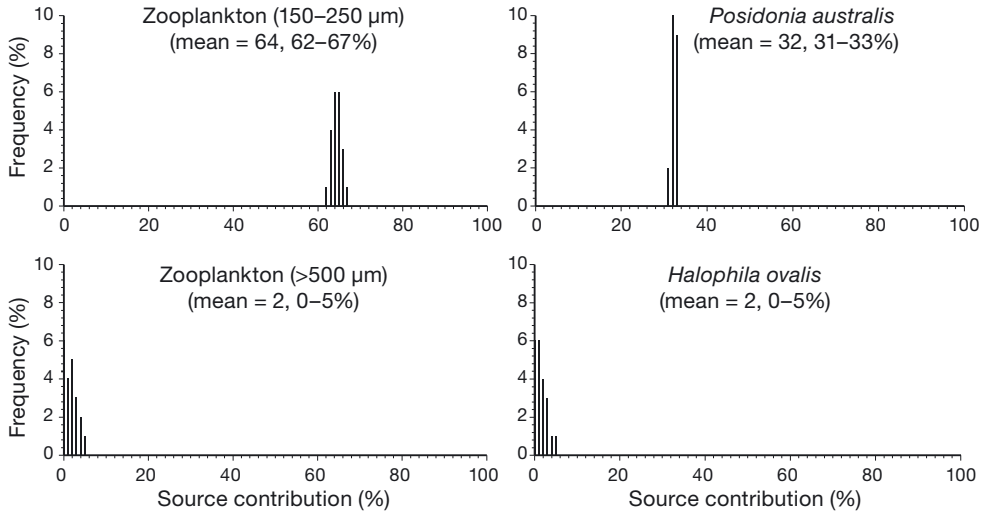

Fig. 3. Distribution of feasible contributions of dietary sources (zooplankton 150–250 and >500 µm, *Posidonia australis*, and *Halophila ovalis*) for *Dendronephthya australis* after correcting for trophic enrichment (1‰ for $\delta^{13}C$ and 2.9‰ for $\delta^{15}N$). Values in brackets are mean and 1st–99th percentiles for the distributions

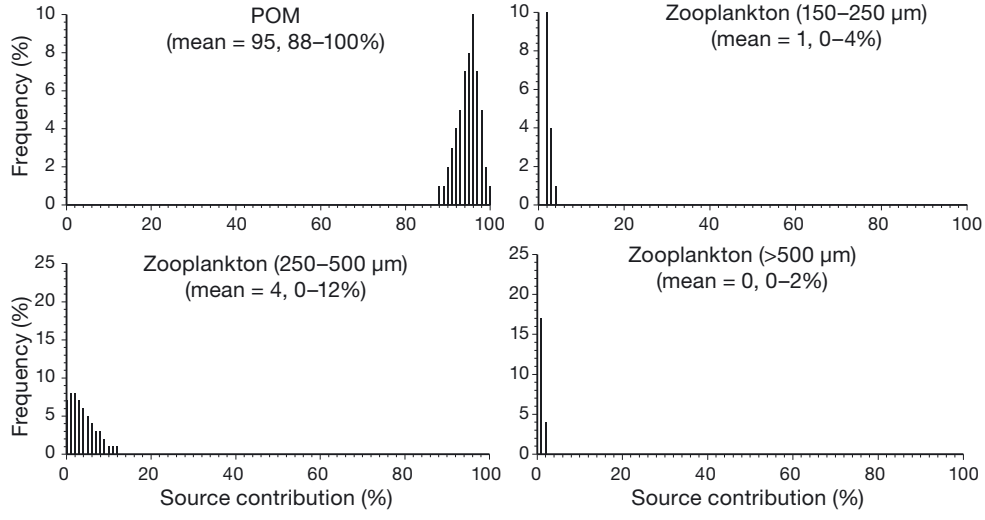

Fig. 4. Distribution of feasible contributions of dietary sources (particulate organic matter [POM] and zooplankton 150–250, 250–500, and >500 µm) for sponges after correcting for trophic enrichment (1‰ for $\delta^{13}C$ and 2.1‰ for $\delta^{15}N$). Values in brackets are mean and 1st–99th percentiles for the distributions

DISCUSSION

Distinct primary sources were identified using SIA, 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 $\delta^{13}C$ 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 $\delta^{13}C$ values. Soft coral and sponges were isotopically distinct and therefore accessed different primary sources for their energy. The $\delta^{13}C$ values indentified sponges as possible prey for secondary consumers but not the soft coral. The diet of 3 protected Syngnathidae species was substantially (>60%) supported by amphipods found among *Dendronephthya australis* branches, suggesting that these animals used the soft coral habitat to feed on the small invertebrates.

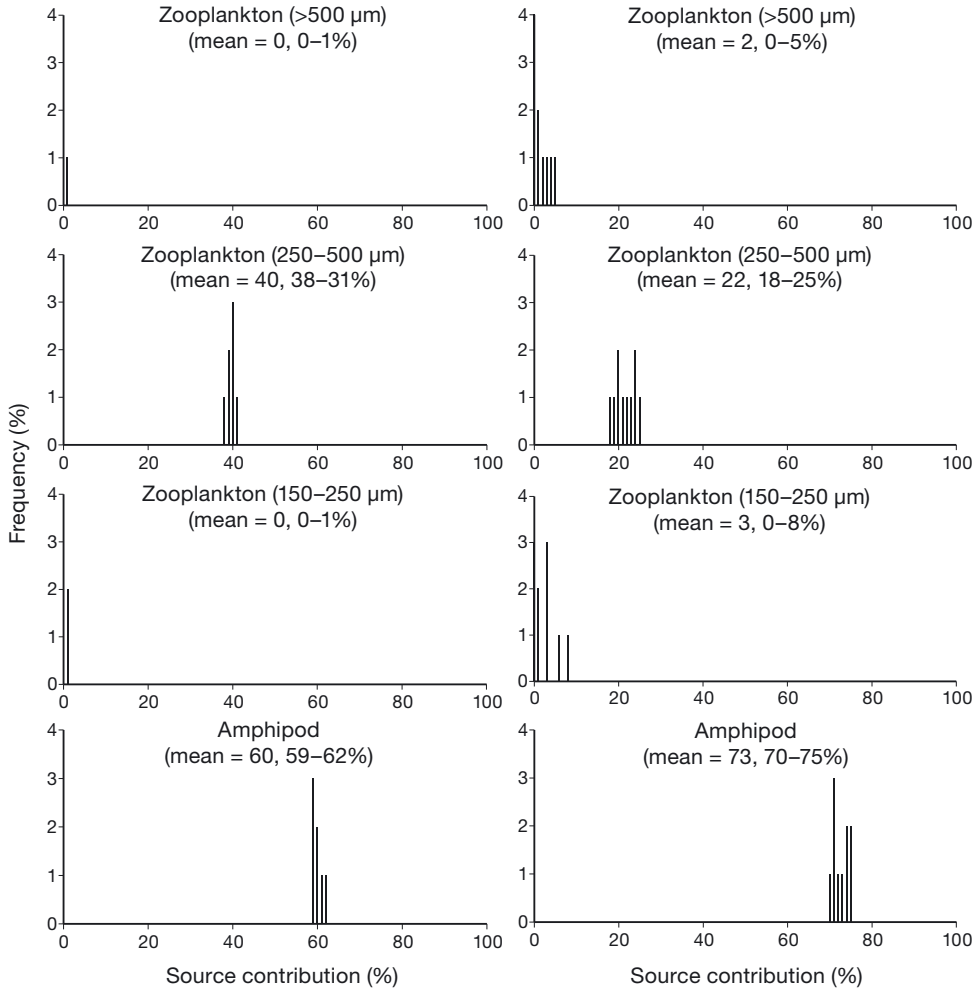

Fig. 5. Distribution of feasible contributions of 4 dietary sources (zooplankton >500, 250–500, and 150–250 µm and amphipods) for *Filicampus tigris* (left) and *Festucalex cinctus* (right) after correcting for trophic enrichment (1‰ for $\delta^{13}C$ and 2.9‰ for $\delta^{15}N$). Values in brackets are mean and 1st–99th percentiles for the distributions

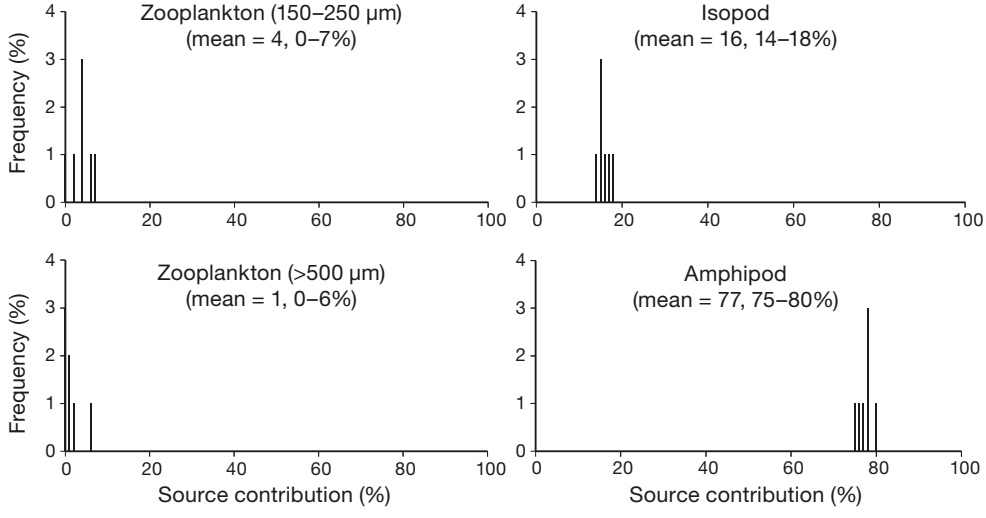

Fig. 6. Distribution of feasible contributions of 4 dietary sources (zooplankton 150–250 and >500 µm, isopods, and amphipods) for *Hippocampus whitei* after correcting for trophic enrichment (1‰ for $\delta^{13}C$ and 2.9‰ for $\delta^{15}N$). Values in brackets are mean and 1st–99th percentiles for the distributions

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

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. *D. australis* fed mainly on small (150–250 µm) 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 (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 2 benthic feeders accounts for the difference in their prey selection.

There was no evidence from the stable isotope data that *D. 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 3 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 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 that 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 feed on

sponges. For example, isotope values of the omnivorous leatherjacket *Nelusetta ayraudi* were 1‰ higher for $\delta^{13}C$ and 3.3‰ for $\delta^{15}N$ than that of the sponge *Holopsamma* 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) and *Filicampus tigris* and *Festucalex cinctus* (both pipefish) closely aligned with amphipods, and it was estimated that amphipods contributed at least 60% to the diet of the 3 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, our 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 the present 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 this should be the focus of future investigation.

CONCLUSIONS

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 2 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 that suitable decisions are made regarding the conservation management of *D. australis*.

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