Stable isotope and fatty acid biomarkers of seagrass, epiphytic, and algal organic matter to consumers in a pristine seagrass ecosystem

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Abstract. The relative importance of the identity and abundance of primary producers in structuring trophic ecology, particularly in seagrass-dominated ecosystems, remains unclear. We assessed the contributions of seagrass, epiphytes, macroalgae, and other primary producers to the diets of resident animals in the nearly pristine seagrass-dominated environment of Shark Bay, Australia, by combining fatty acid composition with carbon, nitrogen, and sulfur stable isotopes of primary producers and consumers. Overall, mixed inputs of these primary producers fuel secondary production, with tropical detrital seagrass inputs supporting most fish species, likely through benthic intermediates. Epiphytic organic matter inputs were most closely associated with snails, whereas seagrass detritus, macroalgae, gelatinous zooplankton, and/or phytoplankton may all contribute to higher trophic levels including sea turtles and sharks. The fatty acid and isotope data suggest that diets of large-bodied consumers were highly variable—future food web studies need to incorporate large sample sizes to account for this variability.

Additional keywords: epiphytes, fatty acids, food webs, seagrass, Shark Bay, $\delta^{13}$C, $\delta^{15}$N, $\delta^{34}$S.

Introduction

Seagrass meadows are highly productive communities that serve a structural role by providing protection from predation and a trophic role by contributing to ecosystem primary productivity (Duarte 1989; Lee et al. 2001). The trophic importance of seagrass is debated, but generally thought to be minor compared with epiphytic algae, macroalgae, and benthic microalgae (Moncreiff and Sullivan 2001; Jaschinski et al. 2008; Doreopoulos et al. 2009; Lebreton et al. 2011). Grazers of diverse body sizes, however, forage directly on seagrass and can remove considerable proportions of biomass (Lal et al. 2010; Douglass et al. 2011; Unabia 2011). Large grazers such as the dugong, Dugong dugon, have been shown to reduce seagrass shoot density, above ground biomass, and below ground biomass (Delongh et al. 1995; Preen 1995), and green sea turtles, Chelonia mydas, may be responsible for recent declines in seagrass meadows in Bermuda (Fourquarean et al. 2010). Particularly in low latitudes, large grazers have played important roles historically in determining life-history characteristics of seagrasses and resident animals (Heck and Valentine 2006). More recently, some nearshore food webs have undergone a shift from large herbivores that can strongly impact seagrass biomass to smaller herbivores (for example the bucktooth parrotfish, Sparisoma radians and the Salema porgy, Sarpa salpa (Kirsch et al. 2002; Tomas et al. 2005)), which can consume large quantities of seagrass with lower impacts on seagrass density (Heck and Valentine 2006). Seagrass organic matter can also enter food webs via heterotrophic bacteria—in Florida Bay, seagrass-derived organic carbon accounted for 13–67% of bacterial $\delta^{13}$C signatures (Williams et al. 2009). A complexity in determining the relative abundance of seagrass vs other inputs to food webs is that approaches such as stable isotope analysis often cannot provide a great enough distinction between seagrass and epiphyte and/or macroalgal organic matter and may suggest substantial seagrass consumption that is not supported by other methods such as gut content analysis or fatty acid analysis (Jaschinski et al. 2008; Crawley et al. 2009; Douglass et al. 2011).
Fatty acid fingerprinting can be a particularly useful tool for dietary studies where stable isotope composition alone may not provide the necessary resolution between discrete organic matter substrates or trophic levels. Unique, source-specific fatty acids or the general distribution of fatty acids can provide detailed tracking of carbon substrates in food webs because fatty acids from triacylglycerol storage lipids in a food item are taken up into consumer tissue with relatively minor or predictable modifications (Dalsgaard et al. 2003; Iverson et al. 2004; Budge et al. 2006).

The coupling of fatty acid analysis with stable isotope composition has provided additional information on the trophic importance of seagrass, yet results seem to vary greatly with habitat. For example, Kharlamenko et al. (2001) determined that seagrass was an important organic matter substrate, particularly for gastropods and some surface deposit-feeding bivalves in an eelgrass meadow in the Sea of Japan, although the link between seagrass and consumer relied on both detrital and bacterial pathways. Conversely, in south-western Australia, Crawley et al. (2009) determined that brown algae, as opposed to seagrass, disproportionally contributed to consumer food webs in surf-zones. Although brown algae were present in far lower abundances in surf-zone wrack than seagrass, fatty acid analysis demonstrated that allochthonous brown algae subsidised secondary production in these regions (Crawley et al. 2009). Similarly, Doropoulos et al. (2009) determined through laboratory feeding experiments that gastropods, particularly Pyrene bidentata snails, from coastal south-western Australia consumed only minimal amounts of seagrass, preferring instead kelp, red macroalage, and seagrass periphyton.

Here, we explore food web relationships in the relatively undisturbed subtropical seagrass ecosystem of Shark Bay, Western Australia. We couple stable isotope composition with fatty acid analysis to determine the relative inputs of seagrass, epiphytes, and other primary producers to a selected subset of consumers in higher trophic levels as a preliminary study to determine if these molecular approaches can provide sufficient separation of common primary producers in Shark Bay. There are limited studies of the trophic ecology in Shark Bay; however, previous studies using bulk stable isotopes suggest that mangrove-derived productivity contributes relatively little to food webs (Heithaus et al. 2011) and that some individual green sea turtles, often considered to be highly reliant on seagrasses, may in fact rely on other non-seagrass food sources even in extensive seagrass meadows (Burkholder et al. 2011). Furthermore, seagrass productivity is important to food webs in which batoids (rays) and small sharks feed (Vauo and Heithaus 2011). Additionally, a large body of work from south-western Australia suggests that allochthonous organic matter sources, such as kelp and other macroalgae derived from reefs, can subsidise secondary production in seagrass meadows (Wernberg et al. 2006; Doropoulos et al. 2009; Hyndes et al. 2012), surf-zones (Crawley et al. 2009), and beach environments (Ince et al. 2007). Here, we provide what we believe to be the first study to integrate data from fatty acid and stable isotope compositions (particularly sulfur isotopes) of consumers in Shark Bay to gain more detailed insights into trophic structure. Because Shark Bay is characterised by limited anthropogenic influences this study provides a baseline for comparison to more impacted seagrass systems.

Materials and methods

Study area and sampling

Shark Bay is a shallow (average depth 9 m) subtropical embayment, subdivided into Eastern and Western Gulfs by peninsulas and barrier islands (Heithaus 2004). Together with its designation as a United Nations Educational, Scientific and Cultural Organisation (UNESCO) World Heritage Site, the low population of the region and low fishing pressure have preserved Shark Bay as one of the World’s most pristine seagrass ecosystems (Heithaus 2004; Heithaus et al. 2011). This study focussed on the Eastern Gulf of Shark Bay (Fig. 1) where shallow offshore banks (<4 m) are dominated by stands of *Amphibolis antarctica* or occasionally *Posidonia australis*, and deeper regions are sandy or silty with isolated patches of
Seagrass, with more tropical species like *Cymodocea angustata*, *Halophila ovalis*, *Halophila spinulosa*, and *Halodule uninervis*, among others (Heithaus 2004). In addition there are large expanses of nearshore shallow (<3 m) that are largely covered by sand with a strip of seagrass along the deeper portions of the flats (Vaudo and Heithaus 2009).

Samples were collected in March 2011 from nearshore shallows and offshore seagrass bands. We collected three species of seagrass, *A. antarctica*, *H. uninervis*, and *C. angustata* by hand on SCUBA. At least five plants were collected at each site, and multiple leaves were combined for composite samples. Three composites were analysed for each seagrass species. Seagrass was rinsed in DI water and gently scraped with a razor blade to remove epiphytes. Two sediment samples, SOM 63A (25°56′29.94″S, 114°13′39.6″E) and SOM 10 B (25°44′52.98″S, 113°45′18.65″E) were collected using a PVC coring tube. The core was pushed into the sediment 10 cm, recovered, and brought back to the boat. A subsample from the surface of the core (0–2 cm) was collected for analysis. All seagrass, epiphyte, and sediment samples were stored on ice in the field and immediately frozen in the laboratory until processing.

To determine if fatty acids and sulfur isotopes can resolve trophic dynamics in Shark Bay, we sampled a subset of the most common consumers in the study area, rather than comprehensively sampling the consumer community (Table 1). Details of methods for the capture and collection of tissues from sharks can be found in Matich et al. (2011) and for sea turtles in Burkholder et al. (2011). Fish were captured in fish traps (see Heithaus 2004) or by handline and small consumers were collected by hand. Samples were rinsed in ultrapure water, then either frozen intact (*Pyrene* spp. snails, seagrass, epiphytes) or dissected to isolate muscle tissue (fish and oysters), then frozen. Because of the long travel times to the laboratory in the United States, all samples were preserved in a small aliquot of isopropanol and butylated hydroxytoluene (BHT; 100 μg mL⁻¹) was added as an antioxidant (Christie 1989). On return to the laboratory, snails were removed from their shells.

### Table 1. Summary of basal resources and consumers, sample type and number for both fatty acid stable isotope analysis, and lipid-extraction status for stable isotope analysis

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample type</th>
<th>n</th>
<th>Lipid extracted for</th>
<th>Lipid extracted for</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amphibolis antarctica</em></td>
<td>Seagrass blades</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><em>Halodule uninervis</em></td>
<td>Seagrass blades</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><em>Cymodocea angustata</em></td>
<td>Seagrass blades</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Epiphytes</td>
<td>Seagrass blades</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sediments</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sargassum spp.</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctenophores</td>
<td>Whole-body Composite</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pyrene</em> spp.</td>
<td>Whole-body</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><em>Pinctada</em> spp.</td>
<td>Muscle</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Tarwhine (<em>Rhabdosargus sarba</em>)</td>
<td>Muscle</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Western butterfish (<em>Pentapodus vitta</em>)</td>
<td>Muscle</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Yellowtail trumpetfish (<em>Amiatiata caudovittata</em>)</td>
<td>Muscle</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Western school whiting (<em>Sillago vittata</em>)</td>
<td>Muscle</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Striped trumpetfish (<em>Pelates octolineatus</em>)</td>
<td>Muscle</td>
<td>3</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Green sea turtle (<em>Chelonia mydas</em>)</td>
<td>Skin</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loggerhead sea turtle (<em>Caretta caretta</em>)</td>
<td>Skin</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiger shark (<em>Galeocerdo cuvier</em>)</td>
<td>Fin Clp</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Stable isotope and bulk parameter analysis

Lipid extracted residues (see below) of all seagrass, snail, oyster, fish, and sediment samples were rinsed with ultrapure water and allowed to dry overnight at 45°C for stable isotope analysis. Tissue was then homogenised with a mortar and pestle. Sub-samples of snail and oyster tissue were treated with 10% HCl for 24 h to remove inorganic carbon then rinsed to neutral pH with ultrapure H₂O. Stable isotopes of *Sargassum* spp., the ctenophore composite, green sea turtles (*Chelonia mydas*), loggerhead sea turtles (*Caretta caretta*), and tiger sharks (*Galeocerdo cuvier*) were measured on non-lipid extracted tissues from split samples (Table 1). Although lipid extraction has been shown to result in a small (~1%) enrichment in δ¹⁵N and δ³⁴S, these changes have not proven ecologically significant for food web analyses (Sotiropoulos et al. 2004; Murry et al. 2006). Because the compositions of primary producers and consumers vary much more than 1% in this study, particularly for δ¹⁵N, we feel confident that we can compare the relative isotope compositions between our lipid-extracted and non-lipid extracted samples. Stable carbon and nitrogen isotope composition were measured concurrently on the samples with an NA 1500 Elemental Analyser (Carlo Erba, Milan, Italy) coupled to a Finnigan MAT Delta isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) at Florida International University’s Stable Isotope Laboratory. Samples were converted to SO₂ and ³⁴S determinations were performed at the University of Virginia using a Carlo Erba elemental analyzer coupled to an OPTIMA stable isotope ratio mass spectrometer (Isoprime, Inc., Manchester, UK) (see MacAvoy et al. 2000; for method description). The reproducibility of the measurement is typically better than ±0.2‰ for the sulfur isotope abundances and
Table 2. Groups of fatty acids used to partition bacterial, seagrass, diatom, and flagellate organic matter in this study, and their source assignments reported in the literature

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td>C_{16:1}, C_{18:1} odd-chain iso and anteiso branched fatty acids; C_{18:2}, C_{19:1} odd-chain n-alkanoic acids; C_{18}, C_{19}, and C_{15} monounsaturated fatty acids, 14:0, 18:1a7</td>
</tr>
<tr>
<td>Seagrass/Vascular plants</td>
<td>18:2o6, 18:3o3</td>
</tr>
<tr>
<td></td>
<td>C_{22}-C_{32} saturated n-fatty acids (vascular plants only)</td>
</tr>
<tr>
<td>Diatoms</td>
<td>14:0, 16:1a7</td>
</tr>
<tr>
<td></td>
<td>16:2o6, 16:3o4, 16:4o1, 20:5o3</td>
</tr>
</tbody>
</table>

the average isotope laboratory error for replicate glycine internal standards treated identically as the samples was 0.11\% for $\delta^{13}$C and 0.11\% for $\delta^{15}$N. Due to low sample replication, we have not attempted stable isotope mixing models to constrain consumer food sources.

Lipid extraction and analysis

Lipids were extracted from seagrass, epiphytes, and muscle and skin tissues following a modification of Folch et al. (1957) and details are provided in Belicka et al. (2012). Briefly, samples were extracted with a 2:1 mixture (v/v) of methylene chloride: methanol (CH$_2$Cl$_2$:MeOH), with trace amounts of isopropanol remaining from the preservation process. The original storage vials were rinsed three times with the 2:1 CH$_2$Cl$_2$:MeOH solvent mixture, and the rinse was transferred to the extraction tube to ensure no sample material or isopropanol was left behind in the initial storage vial. Ultrapure (milli-Q) water was added to achieve a final ratio of 2:1:0.7 CH$_2$Cl$_2$:MeOH:H$_2$O. The samples were strongly agitated, and the lower organic phase was removed to an evaporation flask. The extraction was repeated two more times, extracts were combined, and excess solvent was removed by rotary evaporation. Total lipid extracts were flushed with nitrogen and stored in CH$_2$Cl$_2$ at $-20^\circ$C. Saponification and derivatisation follow the methods described in Belicka et al. (2012).

Fatty acids were identified and relative abundances were determined using gas-chromatography-mass spectrometry (GC/MS) with an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 5973 mass spectrometer using a Restek FAMEWAX crossbond polyethylene glycol GC column (Restek Corporation, Bellefonte, PA, USA) (30 m length, 0.25 mm internal diameter, 0.25 mm film thickness). GC and MS parameters are as described in Belicka et al. (2012). Identification of fatty acids was performed by comparison of chromatographic retention times with authentic standards (Supelco PUFa No. 3 from menhaden oil), PUFA No. 1 (marine source), Bacterial Acid Methyl Ester (BAME) Mix, and C$_{18}$-C$_{24}$ FAME Mix) and mass spectra of standard and previously reported compounds. Fatty acids are expressed here as a percentage of the total identified. The most abundant five fatty acids in all samples excluding sediments, resulting in a total of 12 fatty acids, are shown in figures here for brevity. Additionally, specific fatty acids were grouped into bacterial, seagrass and/or vascular plant, diatom, and flagellate fatty acids on the basis of source assignments in the literature (Table 2; see also Lebrerton et al. 2011).

Statistical analyses applied for fatty acid fingerprinting used average-linkage hierarchical cluster analysis on Bray–Curtis dissimilarity coefficients of untransformed fatty acid data using the statistical package R (R Foundation, for Statistical Computing, Vienna, Austria). The dataset for the cluster analysis consisted of a total of 27 fatty acids, which included the 10 most abundant fatty acids in each sample, and represented between 84–99\% of the total fatty acids in all samples. Overall, ~100 different fatty acids were identified in the sample set (see Supplementary information), and cluster analyses using the reduced versus full datasets resulted in very similar sample associations. Fatty acids are named here as A:B$_x$, where A refers to the number of carbon atoms in the molecule, B refers to the number of double bonds separated by a single methylene group, with additional double bonds separated by a single methylene group.

Results

Stable carbon, nitrogen, and sulfur isotope composition of basal resources and consumers

Stable carbon and nitrogen isotope compositions for seagrasses, epiphytes, *Pinctada* spp. oysters, turtles, and tiger sharks measured here all fall within previously reported ranges of much larger sample sizes (Fig. 2; see Burkholder et al. 2011 and Matich et al. 2011). Seagrasses were enriched in $^{13}$C compared with most consumers, with $\delta^{13}$C values averaging $-11.1\%$ for *A. antarctica*, $-11.4 \pm 0.5\%$ s.d. for *H. uninervis*, and $-9.8 \pm 0.3\%$ s.d. for *C. angustata* (Fig. 2). Epiphytes and sediments were more depleted in $^{13}$C compared with seagrasses, with average $\delta^{13}$C values of $-12.9\%$ and $-14.9\%$, respectively. Note that due to sample loss, epiphyte replicates 1 and 3 were combined so as to obtain one $\delta^{13}$C measurement. The three
replicates were run individually for other isotope analyses. Values of $\delta^{13}$C in Pyrene spp. oysters were similar to those found in epiphytes, averaging $-12.9\%$ (Fig. 2). The other invertebrates investigated in this study, Pinctada spp. oysters, had much more depleted $\delta^{13}$C values, which averaged $-16.3 \pm 0.2\%$ s.d. Fish species measured here showed a wide range in $\delta^{13}$C values, with the western school whiting (Sillago viitata) having the most enriched values which averaged $-10.3 \pm 1.0\%$ s.d., whereas the striped trumpeter (Pelates octolineatus) had the most depleted average values ($-14.4 \pm 2.3\%$ s.d.) (Fig. 2). Values of $\delta^{13}$C for green sea turtles and loggerhead sea turtles averaged $-17.2\%$ and $-14.5\%$, whereas tiger sharks were more enriched, averaging $-12.4\%$ (Fig. 2).

Stable nitrogen isotope values increased with trophic level, with seagrasses having the most depleted $\delta^{15}$N values which ranged from $-2.5\%$ to $+1.9\%$ (Fig. 2). Halodule uninervis was most depleted in $^{15}$N, and A. antarctica was most enriched. Epiphytes were more enriched than seagrasses, and the three replicates had a wide range of values from $4.6\%$ to $7.1\%$. The invertebrates investigated here were also more enriched than seagrasses, with $\delta^{15}$N for snails averaging $4.8\%$, whereas oysters averaged $3.9\%$ (Fig. 2). Fish species and both species of turtles also had similar ranges of $\delta^{15}$N values, which were intermediate between the invertebrates and tiger sharks (Fig. 2). Values of $\delta^{15}$N for turtles ranged from $7.1\%$ to $8.6\%$, whereas all fish species had $\delta^{15}$N values between $7.1\%$ and $8.9\%$. Tiger sharks had the most enriched $\delta^{15}$N values near $12\%$ (Fig. 2).

Regarding S isotopes, the sediments were quite depleted in $^{34}$S, with average values of $-6.3\%$ (Fig. 2b). The tropical seagrasses H. uninervis and C. angustata had intermediate $\delta^{34}$S values which averaged $5.8 \pm 0.4\%$ s.d. and $4.9 \pm 0.5\%$ s.d., respectively, whereas the temperate seagrass A. antarctica was more enriched, averaging $14.2 \pm 0.6\%$ s.d. (Fig. 2b). All species of fish excepting striped trumpeter had similar $\delta^{34}$S values, which ranged from $1.9\%$ to $5.1\%$ (Fig. 2b). Striped trumpeters were more enriched than other fish species, with $\delta^{34}$S values averaging $9.8 \pm 0.5\%$ s.d. Pyrene spp. had $\delta^{34}$S values similar to the tropical seagrasses, averaging $6.0 \pm 1.3\%$ s.d. (Fig. 2b). Average $\delta^{34}$S values for oysters ($9.6 \pm 0.3\%$ s.d.) and
epiphytes (12.3 ± 1.4‰ s.d.) were similar to δ²⁴S values found for the striped trumpeters. Tiger sharks and turtles were, on average, more enriched in ⁴¹S than fish, with δ²⁴S values averaging 15.4 ± 0.6‰ s.d. for tiger sharks and 15.4‰ for the turtles. The samples most enriched in ⁴¹S in this dataset were Sargassum spp. (19.7‰) and ctenophores (20.6‰).

Fatty acid composition of basal resources and consumers
Each seagrass species was dominated by the same three fatty acids: 18:2ω6 (linoleic acid) and 18:3ω3 (ω-linolenic acid), and 16:0n (palmitic acid), which is common in all organisms (Table 2, Fig. 3). Smaller contributions from 18:0n (stearic acid) and 18:1ω9 (oleic acid) were also present in all three species. Only minor amounts of 20:4ω6 (arachidonic acid) and 20:5ω3 (icosapentaenoic acid) were present, and no C₂₂ polysaturated fatty acids were present in any of the seagrass samples (Fig. 3). Unlike seagrass, epiphytes and Sargassum spp. were instead dominated by C₂₀ polysaturated fatty acids, particularly 20:5ω3 and 20:4ω6 (Fig. 4a). The relative abundance of two of the dominant fatty acids in seagrasses, 18:2ω6 and 18:3ω3, were substantially lower in epiphytes and Sargassum spp., although abundances of 16:0n were similar to levels present in seagrass. Sargassum spp. contained higher abundances of 18:1ω9 and 14:0n compared with epiphytes and seagrass (Fig. 4a).

The fatty acid composition of Pyrene spp. snails was dominated by the following fatty acids: 16:0n, 20:4ω6, 20:5ω3, 22:5ω3, 16:1ω7, 18:0n, and 14:0n (Fig. 4b). Pinctada spp. oysters contained abundant 22:6ω3 (docosahexaenoic acid), as well as the fatty acids that dominated in Pyrene spp., with the exception of 16:1ω7. In general, all fish species contained similar fatty acid compositions, with abundant 16:0n, 22:6ω3, 20:4ω6, 18:0n, 18:1ω9, and 20:5ω3 (Fig. 4c). Striped trumpeter

![Fig. 3](image-url) Average relative abundance and standard deviation (n = 3) of predominant fatty acids in three species of seagrass from Shark Bay, Australia.

![Fig. 4](image-url) Average relative abundance and standard deviation (shown where n = 3) of predominant fatty acids in (a) primary producers and sedentary organic matter (SOM), (b) invertebrates, (c) fish, and (d) turtles and sharks from Shark Bay, Australia.
contained the highest levels of 18:2ω6 and 18:3ω3 out of all fish species, whereas western butterfish (Pentapodus vitta) and tarwhine (Rhabdosargus sarba) instead had high abundances of 22:6ω3 (Fig. 4c). Loggerhead sea turtles, green sea turtles, and tiger sharks all contained similar compositions of fatty acids, with high levels of the ubiquitous fatty acids 16:0, 18:0, and 18:1ω9, plus 20:4ω6 (Fig. 4d). Tiger sharks and loggerhead sea turtles contained higher levels of 22:6ω3 compared with green sea turtles; however, green sea turtles contained higher abundances of 18:2ω6.

When grouped by source (Fig. 5), bacterial fatty acids, especially 15:0i (iso branched) and 15:0a (anteiso branched), were abundant in the sedimentary organic matter, particularly for the sandy sediments that were not collected in the seagrass meadow. Flagellate fatty acids (Table 2) were present in low quantities in sediments and epiphytes, but were high in oysters, suggesting an important planktonic component of flagellates to these filter feeders, as well as western butterfish and tarwhine (Fig. 5b). Fatty acids indicative of diatoms (Table 2) were abundant in sediments and epiphytes, demonstrating the importance of these microalgae to benthic and epiphytic organic matter substrates (Fig. 5c), and also abundant in Pinctada spp., Pyrene spp., and striped trumpeter. The fatty acids most characteristic of seagrass, including 18:2ω6 and 18:3ω3, were present in only low abundances in sediments, striped trumpeter, and green sea turtles (Fig. 5d).

Hierarchical cluster analysis performed on the basis of the Bray–Curtis dissimilarities of fatty acid composition resolved most species into individual clusters (Fig. 6), suggesting low intraspecific variation relative to interspecific variation in fatty acid composition for these sampled organisms. Tarwhine was the only fish to not show this tight clustering, with two individuals clustering with western butterfish, and one individual clustering with western school whiting (Fig. 6). Seagrass, also, was not entirely resolved by species, yet formed its own cluster which was not closely related to any of the other basal resources or consumers (Fig. 6). As expected, the fatty acid composition of Pyrene spp. was closely related to that of epiphytic material (Fig. 6). Fish formed two clusters at the 20% dissimilarity level; the first including western butterfish, the majority of the tarwhine samples, and Pinctada spp. oysters, whereas the second cluster contained western school whiting, one individual tarwhine, yellowtail trumpeter (Amniataba caudavittata), and striped trumpeter. Striped trumpeter were less closely associated with western school whiting and yellowtail trumpeter (Fig. 6).

Discussion

The role of seagrass as a basal resource for consumers in Shark Bay

Evidence from fatty acids, δ¹³C, δ¹⁵N, and δ³⁴S provides conflicting insights into the potential importance of seagrass to consumers in Shark Bay. The fatty acid composition of seagrasses, similar in the three species analysed here and characterised by abundant 18:2ω6 and 18:3ω3 with low abundances of saturated and monounsaturated C₁₈ fatty acids, was not strongly represented in the fatty acid compositions of any

Fig. 5. Average relative abundance and standard deviation (shown where n = 3) of selected groups of fatty acids from typically identified sources. See Table 1 for fatty acid source assignments.
consumers, suggesting that seagrass biomass was not an important component of the diet of any of the consumers. Hierarchical cluster analysis, using a suite of the most abundant fatty acids in all samples, further demonstrated that seagrasses were most dissimilar to all other consumers in terms of fatty acid composition (Fig. 6). Because dietary fatty acids are transferred relatively unchanged from food to consumer tissues with minor composition (Fig. 6). Because dietary fatty acids are transferred

![Image of a graph showing average-linkage hierarchical cluster analysis of Bray–Curtis dissimilarities identified on the basis of relative abundances of fatty acids in primary producers and consumers from Shark Bay, Australia.]

Fig. 6. Average-linkage hierarchical cluster analysis of Bray–Curtis dissimilarities identified on the basis of relative abundances of fatty acids in primary producers and consumers from Shark Bay, Australia.

three seagrass species studied here, and snails and all fish excepting striped trumpeter had $^{34}S$ values similar to the two tropical seagrass species $H. uninervis$ and $C. angustata$ (Fig. 2b). In contrast, snails and oysters are substantially more deplete in $^{13}C$ than seagrass, instead appearing to utilise epiphytic and planktonic resources, respectively (Fig. 2a), and cluster analysis of the fatty acid data also highlighted strong associations between snails and epiphytes (Fig. 6). Our $^{13}C$ and fatty acid data for the snails closely follows feeding experiments of $P. bidentata$ in coastal south-western Australia that found that epiphytes and macroalgae were preferred relative to seagrass in concordance with fatty acid data (Fig. 6).

One possible explanation for the presence of a carbon and sulfur isotope signal with a corresponding lack of fatty acid signature of tropical seagrass in some consumers is the relative importance of a detrital seagrass pathway. Two of the most abundant fatty acids in seagrass were 18:2 $\omega$6 and 18:3 $\omega$3. Their negligible presence in consumers tends to discount direct seagrass grazing, because these markers have been shown to be transferred to lipid depots in green sea turtles grazing on $H. hawaiicensis$ (Seaborn et al. 2005). However,
polyunsaturated fatty acids such as 18:2\(\text{a6}\) and 18:3\(\text{a3}\) degrade faster than saturated fatty acids (Parker and Leo 1965; Nichols et al. 1982), suggesting that their presence in detrital seagrass materials may be significantly less compared with live seagrass. Although we were unable to obtain degraded seagrass samples from this study site for fatty acid and stable isotope analysis, both Kharlamenko et al. (2001) and Harbeson (2010) found that in Zostera marina detritus, these two fatty acids are present in amounts about an order of magnitude less compared with abundances in fresh material. Whether \(\delta^{13}\)C of seagrass detritus is generally distinct from the \(\delta^{13}\)C of living seagrass leaves is unclear. Because the more refractory lignocelluloses are more depleted than bulk material (Benner et al. 1987), one would expect detritus to be more depleted than the original seagrass. Indeed, during the first year of decomposition, seagrass detritus became more depleted by 2\% than the starting material in Florida Bay seagrass meadows dominated by Thalassia testudinum (Fourquean and Schrlau 2003). However, other studies have found no change in \(\delta^{13}\)C during decomposition (e.g. Zieman et al. 1984), or even that dead seagrass leaves were more enriched than the living leaves (Harbeson 2010). Thus, it appears that tropical seagrass detritus, and presumably the microbial fauna colonising the detritus, contribute organic matter to fish species in Shark Bay food webs. Interestingly, the temperate seagrass species A. antarctica had considerably more enriched \(\delta^{34}\)S values compared with H. uninervis and C. angustata, and is not likely to have a major contribution to fish or invertebrate species in Shark Bay food webs, although sulfur isotopes do suggest some contribution to loggerhead and green sea turtles and tiger sharks (Fig. 2b). Although both fish (Burkholder et al. 2012) and dugongs (Wirsing et al. 2007) forage on Amphibolis, experimental studies show that grazers in Shark Bay prefer tropical species greatly over this low-nutrient species (Burkholder et al. 2012).

On the basis of \(\delta^{13}\)C values, Connolly et al. (2005) found seagrass and epiphytes, as detritus in seston, to be important food sources for the yellowfin whiting Sillago schomburgkii in southern Australia through detritivorous and carnivorous intermediaries (polychaetes). Similarly, Vaudo and Heithaus (2011) demonstrated with \(\delta^{13}\)C the importance of seagrass organic matter, through crustacean intermediaries, to elasmobranchs (primarily batooids) in Shark Bay. In our study, the ~8\% enrichment in \(^{15}\)N between seagrass and fish also supports the possibility of an invertebrate trophic intermediary because the average enrichment of \(^{15}\)N between trophic levels is 3–4\% (McCutchan et al. 2003). The use of detrital seagrass as a carbon source by invertebrate prey of fish such as western school whiting and tarwhine can explain the apparent discrepancy between the fatty acid and isotope data, and corresponds to conclusions drawn by Vaudo and Heithaus (2011) discussed above since western school whiting tend to feed in similar sand-flat environments as batooids. Western school whiting and tarwhine contained substantial inputs of microbial fatty acids (Fig. 3a), suggesting that detrital organic matter does contribute to their diet, but stable isotope and fatty acid analysis of degraded seagrass material and benthic invertebrates are necessary for further evaluation of detrital energy pathways and should be included in more comprehensive food web analyses of this region.

The role of epiphytes, plankton, and macroalgae as basal resources for consumers in Shark Bay

Epiphytic organic matter, scraped from the leaves of seagrass, was an important food source for snails, as shown by similarities in their \(\delta^{13}\)C values (Fig. 2a) and fatty acid compositions (Fig. 6). On the basis of their fatty acid composition (i.e. abundant 16:1\(\text{a7}\) and 20:5\(\text{a3}\); Volkman et al. 1989; Dunstan et al. 1993), epiphytes in this region have a large diatom component (Fig. 5c). Flagellate fatty acids were rare in epiphytes (Fig. 5b). Seagrass detritus may also be a dietary component for the snails, as evidenced by the similar \(\delta^{34}\)S values between snails and the tropical seagrasses H. uninervis and C. angustata (Fig. 2b), although as mentioned above, feeding studies on P. hidentata suggested minimal seagrass consumption (Doropoulos et al. 2009). In contrast to the epiphytes, oysters contained abundant flagellate fatty acids, especially the 22:6\(\text{a3}\) fatty acid typically attributed to dinoflagellates (Volkman et al. 1989; Viso and Marty 1993), with additional, but more variable, inputs from diatom fatty acids (Fig. 5b, c). As filter feeders, the fatty acid composition of the Pinctada spp. oysters can be used as a proxy for the plankton community. Although there are few studies characterising phytoplanktonic communities in Shark Bay, diatoms and dinoflagellates were reported to be the main contributors, with proportions varying with salinity (Kimmerer et al. 1985). Diverse communities of heterotrophic dinoflagellates have also been found in Shark Bay plankton (Tong 1997), and could be an important food source for filter-feeding bivalves, like Pinctada spp. oysters, in the study area.

Although not nearly as abundant as seagrasses, macroalgae are common primary producers in the Shark Bay ecosystem, and have been recently shown to be important organic matter sources for green sea turtles (Burkholder et al. 2011). Additionally, multiple studies from coastal south-western Australia have also highlighted the importance of macroalgal subsidy for seagrass and other coastal foodwebs (Wernberg et al. 2006; Ince et al. 2007; Crawley et al. 2009; Doropoulos et al. 2009; Hyndes et al. 2012). Macroalgae of the genera Sargassum, Padina, Dictyota, and Penicillus, ranged in \(\delta^{13}\)C from ~12.04\% to ~17.88\% (Fig. 2b) in the study area (Burkholder et al. 2011). The most enriched macroalgae overlap \(\delta^{13}\)C values for epiphytes, making stable carbon isotope composition alone difficult to distinguish between these two basal resources. The similarities in inputs of fatty acids typically attributed to diatoms (Figs 5c) also make further separation of these two basal resources challenging. In the hierarchical cluster analysis (Fig. 6), Sargassum spp. was not closely associated with a specific consumer, unlike the epiphytes, which were strongly linked to Pyrene spp., but instead was weakly linked to the groups of turtles and sharks, fish and oysters, and snails and epiphytes. Sargassum spp. was more enriched in \(^{34}\)S than epiphytes, with an average value of the two samples analysed here of 19.7\%. The intermediate \(\delta^{34}\)S values of higher consumers (tiger sharks, green sea turtles, and loggerhead sea turtles) between the values for epiphytes and Sargassum spp. imply that both sources of organic matter ultimately contribute to the highest trophic levels in Shark Bay – Matich et al. (2011) also suggested such trophic coupling for tiger sharks. Chemical characterisation of a larger number of epiphyte and macroalgal
samples will be necessary for a more rigorous separation of these two sources to higher trophic levels here.

The values of δ²⁶⁴C suggest that most of the higher trophic level consumers we sampled likely rely on mixed detrital seagrass, epiphytic, macroalgal, and/or planktonic organic matter (Fig. 2a), and serve to integrate multiple trophic pathways, as is common for upper trophic level predators (Rooney et al. 2006). Fatty acid composition suggested that epiphytes are important to all fish in this study, on the basis of abundant 20:4ω6 and 20:5ω3. Fatty acids and stable isotopes also provided more specific information on food preferences for certain fish species. In particular, tarwhine, western school whiting, western butterfish, and yellowtail trumpeter likely utilise seagrass detrital organic matter, as discussed above, likely through benthic intermediates, as indicated by their depleted δ³⁴S signal compared with striped trumpeter, Pinctada oysters, turtles, and sharks (Fig. 2b). This is not surprising because tarwhine, western school whiting, and western butterfish are typically described as benthic carnivores, whereas yellowtail trumpeter is a benthic omnivore. In addition to epiphyte inputs, tarwhine and western butterfish use planktonic organic matter sources, as evidenced by the strong signal from dinoflagellate markers present in their muscle tissue (Fig. 5) and their association with Pinctada spp. oysters in the cluster analysis (Fig. 6). In contrast, the striped trumpeter most likely relies on benthic omnivory to a much lesser degree than other fish.

Values of δ³⁴S in striped trumpeter were more enriched than all other fish in the study, were similar to oysters and epiphytes, and were intermediate between the temperate and tropical seagrass species, whereas δ¹³C was highly variable among the three individual striped trumpeter studied here (Fig. 2). Few published studies exist on the dietary preferences of striped trumpeter; however, Sanchez-Jerez et al. (2002), found a stronger link between a similar species of Pelates (P. sexlineatus) and mobile epifauna, including copepods, ostracods, and amphipods, than with benthic epifauna in seagrass beds of southeastern Australia. Spatial variability in the diets of P. sexlineatus was high though, with little inputs of macroalgae, but higher inputs of plankton to individuals near the entrances of estuaries (Sanchez-Jerez et al. 2002). This contrasts with the findings of Edgar and Shaw (1995), who determined that macroalgae were a predominant food source for P. sexlineatus along coastal southern Australia, as well as the findings of Crawley et al. (2009), who determined that brown algae as opposed to seagrass were a major food source for two predatory fish species (Cnidoglanis macrocephalus and Pelsartia humeralis). These prior studies and the fatty acid and stable carbon isotope data suggest that striped trumpeter diets may vary more among individuals compared with other teleost species we sampled. For example, seagrass inputs were important to the striped trumpeter individual with the most enriched δ¹³C value (Fig. 2), as concentrations of the 18:3ω3 seagrass fatty acid in this fish were 15–23 times higher in abundance than in the other two individuals. In contrast, the individual striped trumpeter with the most depleted δ¹³C value contained higher levels of diatomaceous and flagellate fatty acids, suggesting that epiphytes and/or macroalgae and planktonic energy pathways were more important. For this species, including mobile epifauna and a much larger number of individuals in the analysis would be necessary to fully characterise its diet.

Despite many reports suggesting an ontogenetic shift from carnivory to seagrass herbivory in green sea turtles at an approximate curved carapace length of 40–44 cm (Chaloupka and Limpus 2001; Arthur et al. 2008), recent studies have suggested that macroalgae and gelatinous zooplankton are much more important dietary items than previously believed (Heithaus et al. 2002; Arthur et al. 2007; Burkholder et al. 2011). For the two individuals studied here, δ¹³C and δ³⁴S values and fatty acids suggested little to no seagrass inputs (Figs 2a and 5d). Composition of δ¹³C was very different for each individual, although in general values spanned the range of isotope compositions of macroalgae and gelatinous zooplankton reported by Burkholder et al. (2011). Both species of turtles were less enriched in δ³⁴S compared with Sargassum (δ³⁴S = 19.7‰) and ctenophores (δ³⁴S = 20.6‰), suggesting that mixed inputs from macroalgae and gelatinous zooplankton, along with a source of organic matter more deplete in δ³⁴S, possibly seagrass. Our fatty acid analysis of Sargassum did not find a unique biomarker that could be used to solely trace macroalgae inputs through trophic levels. In general, Sargassum fatty acid distribution was similar to epiphytes, with Sargassum containing more 18:1ω9 and less 20:4ω6 and 20:5ω3, on average, compared with epiphytes. The abundant levels of 18:1ω9 in both species of turtles and in tiger sharks could be indicative of Sargassum inputs to their diets; however, this association should be interpreted with caution as 18:1ω9 is ubiquitous with high relative abundances also found in some species of green algae, cryptophytes and dinoflagellates (Napolitano 1999).

Fatty acids in a composite sample of ctenophores were measured to determine if inputs from gelatinous zooplankton contributed to the diet of the two green sea turtles analysed here. The low molecular diversity of fatty acids (20 fatty acids in ctenophores compared with ~60 in epiphytes) and the fact that three ubiquitous fatty acids (14:0n, 16:0n, and 18:0n) accounted for >75% of the total fatty acids in the ctenophores (see Supplementary information) did not allow for an adequate analysis of the impact of gelatinous zooplankton on consumer diets. Total fatty acid composition of loggerhead sea turtles was most similar to that of green sea turtles (Fig. 6), although loggerheads contained higher inputs of diatom and flagellate fatty acids, suggesting planktonic and epiphytic algae are the basal resources for these consumers. Similarly, tiger sharks were most closely associated with both green sea turtles and loggerhead sea turtles on the basis of fatty acid composition (Fig. 6). A complicating factor for dietary analysis of green sea turtles lies in their ability for hindgut bacterial fermentation (Seaborn et al. 2005). In a typical monogastric consumer, dietary fatty acids are deposited into lipid depots with minor or predictable changes (Iversen et al. 2004; Budge et al. 2006); however, gut fermentation may cause substantial modification of dietary fatty acids before deposition in turtle lipid reserves (Joseph et al. 1985; Seaborn et al. 2005). Fatty acid compositions of green sea turtles must be interpreted with care until further studies on the effect of hindgut fermentation on fatty acid modification are undertaken.

Overall, combined fatty acid and carbon, nitrogen, and sulfur isotope data suggest that detrital, as opposed to live, H. uninervis
and C. angustata inputs likely contribute to the diets of tarwhine, western school whiting, western butterfish, and yellowtail trumpeter, through benthic intermediates, in the Shark Bay ecosystem. Planktonic, epiphytic, and macroalgal organic matter are all important basal resources for turtles and tiger sharks in Shark Bay, Australia. Future comprehensive food web studies should focus on additional characterisations of phytoplankton, zooplankton, and benthic invertebrates to form clearer associations between trophic levels. Stable sulfur isotopes should be included in future food web studies because they provided additional food source information not present from carbon isotopes or fatty acid compositions. Finally, because many species, especially large-bodied consumers, in Shark Bay show considerable variation in stable isotope values (e.g. green sea turtles, Burkholder et al. (2011); loggerhead sea turtles, Thomson et al. (2011); rays, Vauo and Heithaus (2011); and tiger sharks, Matich et al. (2011), Heithaus et al. (2012)) future studies should incorporate a greater sample size of individuals to fully understand trophic structure in Shark Bay.

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