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Investigating diet and diet switching in green turtles (*Chelonia mydas*)

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Abstract. Understanding the dietary ecology of animals provides information about their habitat requirements, facilitating informed conservation. We used last-bite diet and stable isotope analysis to assess the diet of juvenile and adult green turtles (*Chelonia mydas*) at two different habitats located 10 km apart within Port Curtis, Queensland, Australia. Last-bite diet analysis indicated that turtles had distinctly different diets in these two habitats: in one the diet was dominated by red macroalgae and in the other the diet was dominated by seagrass. Only juveniles ($n = 12$) were caught in the habitat where red macroalgae dominated the diet, while both juveniles ($n = 9$) and adults ($n = 38$) were captured in the habitat where seagrass dominated the diet. In the seagrass habitat there was no difference in diet between juveniles and adults, and no difference in diet between adult males ($n = 17$) and females ($n = 21$).

Because the red macroalgae and seagrass had distinctly different carbon stable isotope ratios, it was possible to detect a change in diet by comparing the carbon stable isotope ratio between serum and epidermal tissue sampled from the same turtle. In this region, a switch in diet would reflect a shift in foraging habitat. Such comparisons indicate that ~50% of turtles switched diet, and therefore changed foraging habitat between the time when blood serum and epidermis were formed. This implies that switching foraging habitat by green turtles within this region is a common occurrence, which is somewhat surprising because previously it was thought that foraging green turtles had high site fidelity with relatively small home ranges.

Additional keywords: foraging, sea turtle, SIA, stable isotopes.

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Introduction

Green turtles (*Chelonia mydas*) are large marine herbivores that inhabit inshore tropical and semitropical waters throughout the world (Bowen *et al*. 1992). Juveniles, once they have grown out of the open ocean planktonic stage, and adults, are generally nearshore benthic feeders that feed principally on seagrass and macroalgae (Bjorndal 1997) although where is evidence that a few adult individuals may remain pelagic feeders (Seminoff *et al*. 2008). Because macroalgae typically grows on solid substrate and seagrass typically grows on soft sediments and these habitat types are usually not close to each other, an individual green turtle's diet is often dominated by either seagrass or macroalgae depending upon which habitat they come from (e.g. Bjorndal 1980; Arthur *et al*. 2009; Reisser *et al*. 2013). Like other marine turtle species, adult green turtles are thought to show high fidelity to a particular foraging area where they spend most of their life, only leaving this area briefly to migrate to and from breeding grounds every 3–6 years (Chaloupka *et al*. 2004; Broderick *et al*. 2007; Schofield *et al*. 2010). Home ranges of juvenile green turtles are reported to be between 0.7 and 5 km^2 in Florida, USA (Makowski *et al*. 2006), and between 0.5 and 1.0 km^2 in the Great Barrier Reef, Australia (Hazel *et al*. 2013), while foraging activity centres of between 0.4 and 6.4 km^2 were observed in the gulf of California, Mexico (Seminoff *et al*. 2002). Foraging activity centres for juvenile and adult green turtles from within Port Curtis, Australiam range between 2.0 and 13.8 km^2 (Babcock *et al*. 2015).

Stable isotope analysis (SIA) quantifies the stable isotope ratio of elements (typically carbon and nitrogen) that are incorporated into animal tissues, the ratios of which are slightly enriched in comparison to that of the dietary items consumed (Tieszen *et al*. 1983; Peterson and Fry 1987). Stable carbon isotope values ($\delta^{13}C$) indicate the relative contributions of primary producers to a consumer's diet (Peterson and Fry 1987), so they provide information on the types of food consumed, while stable nitrogen isotope values ($\delta^{15}N$) are indicative of the trophic level at which the animal has been feeding (Deniro and Epstein 1981). Recently, stable isotope values sampled from the epidermis of loggerhead turtles nesting on the east coast of the USA have been used to identify the feeding ground locations of these turtles (Vander Zanden *et al*. 2014*a*; Ceriani *et al*. 2015), which can help in planning high-priority conservation reserves for this endangered species.

Stable isotope values typically differ between tissues taken from the same animal. This can be a result of various tissues having different synthetic pathways, resulting in different fractionation rates of isotopes (Tieszen *et al*. 1983; Peterson and Fry 1987; Hobson and Clark 1992) and because diet varied in composition at the times when different tissues were formed (Hobson and Clark 1993; Vander Zanden *et al*. 2014*b*). For example, in hatchling and juvenile loggerhead turtles (*Caretta caretta*), incorporation rates of carbon isotopes differ between blood cells, plasma and epidermis, with plasma and epidermis showing the greatest difference (Reich *et al*. 2008). Hence differences in the carbon stable isotope values for epidermis and plasma are expected if the stable isotope composition of the diet changed within the time frame of the carbon residence times of these tissues. In marine environments seagrass and macroalgae have different $\delta^{13}C$ values even if they grow close to each other (Raven *et al*. 2002; Connolly 2003) (Table 1), so diet switching between a primarily seagrass and a primarily macroalgae diet should be easily detectable using SIA if tissues that differ in their turnover rate, such as blood serum and epidermis, are sampled.

The current study examined the last-bite diet of juvenile and adult green turtles within Port Curtis, Queensland, Australia (Fig. 1). Within Port Curtis, green turtles feed in estuarine habitats dominated by mangrove macroalgae, such as those found at Wiggins Island, and on seagrass-dominated flats, such as those found at Pelican Banks. The aims of this study were (1) to use last-bite dietary analysis to assess the diet of green turtles from within these two different habitats, and (2) to use stable isotopes to investigate whether there is evidence that individual green turtles switch between different feeding

Table 1. Stable isotope $\delta^{13}C$ and $\delta^{15}N$ values of plant material sampled from Port Curtis, 4–8 November 2013
Only one sample per plant species was analysed

Plant type	Species	$\delta^{13}C$	$\delta^{15}N$
Mangrove	*Rhizophora stylosa* (leaves)	–27.4	3.7
	Avicennia marina (leaves)	–27.2	2.2
	Aegicera corniculatum (leaves)	–26.5	3.7
Seagrass	*Zostera capricorni*	–8.6	1.5
	Halophila ovalis	–9.6	5.3
	Halodule uninervis	–11.7	2.1
Red algae Group 1	*Catenella nipae*	–28.6	3.2
	Spyridia filamentosa	–27.8	2.9
Red algae Group 2	*Chondria* sp.	–19.0	2.5
	Laurencia sp.	–18.2	3.4
Brown algae	*Sargassum flavicans*	–15.6	3.1
	Dictyota ligulata	–21.6	7.5
Green algae	Green filamentous	–22.1	3.8

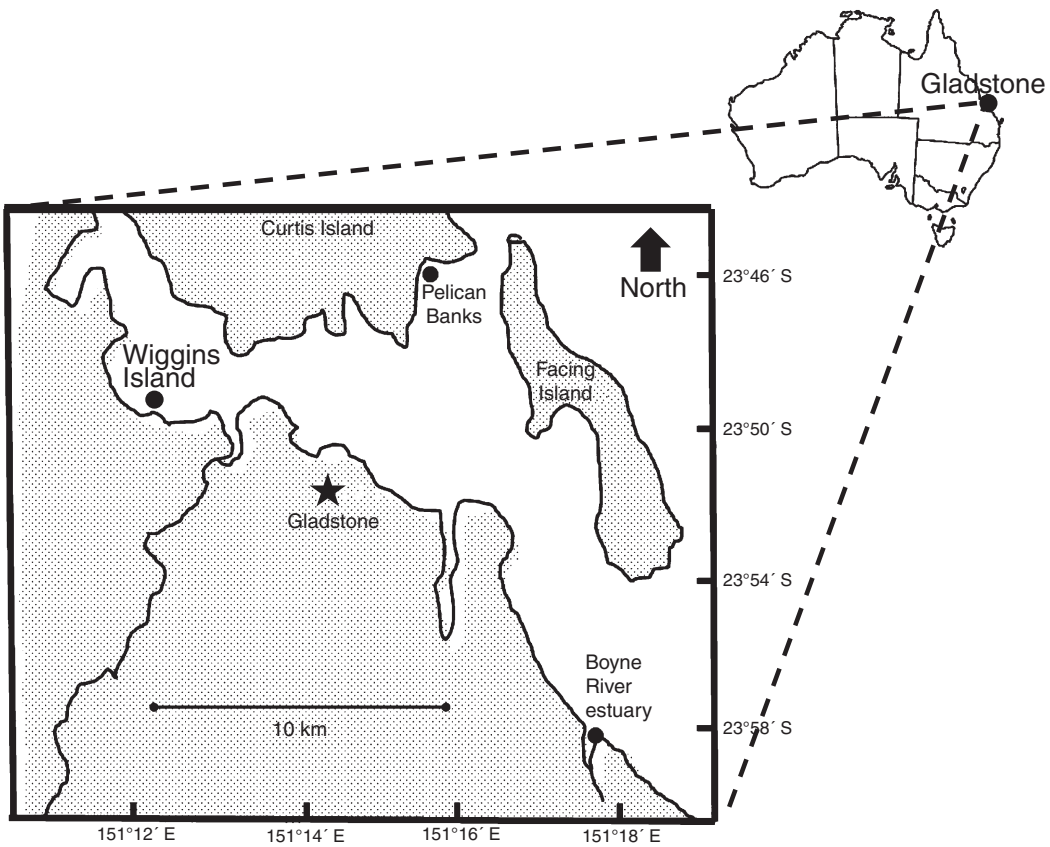

Fig. 1. The location of the green turtle sampling sites, Wiggins Island and Pelican Banks, at Port Curtis, Queensland, Australia.

habitats within this region. A third aim was to determine whether stable isotope signals determined from plasma and serum samples are equivalent and if the same is true for blood cells separated from plasma and blood clots separated from serum. As such, this study will further validate the use of stable isotopes as a tool to infer diet and the occurrence of changes in diet of sea turtles without having to observe feeding behaviour and/or determine gut contents.

Materials and methods

Study area

Port Curtis is located adjacent to the Great Barrier Reef Marine Park and includes large shallow banks and deep channels with fringing mangroves. Between 4 and 8 November 2013, green turtles were captured from two different sites within Port Curtis, Pelican Banks (23°45′47″S,151°17′45″E) and Wiggins Island estuary (23°48′32″S, 151°10′12″E), which are located 10 km apart (Fig. 1). Samples of potential food plants were also collected from these sites at this time.

Sampling of potential dietary items

At each sampling site, samples of plants that had the greatest substrate surface coverage were collected by cutting material above the substratum for determining reference $\delta^{13}C$ and $\delta^{15}N$ values. No animal material was collected because none was obvious during plant collection. Fresh samples of collected plants were rinsed in freshwater and stored at 14°C for up to 24 h until identified and then stored at −17°C until isotope analysis.

Turtle capture

Capture and handling of green turtles was approved by the University of Queensland Animal Ethics Committee (approval no. SBS/229/13/EHP). Green turtles at the Pelican Banks site were captured using the rodeo method (Limpus and Reed 1985) while gill-nets were used at the Wiggins Island site because the turbid water precluded the use of the rodeo method. Differences in capture techniques are unlikely to influence diet assessment because previous studies have found that it is the food availability in the foraging area that determines green turtle diet (e.g. Garnett *et al*. 1985; Seminoff *et al*. 2002; Arthur *et al*. 2009). All turtles were transported to shore for processing where they were flipper tagged, weighed and curved carapace length (CCL) measured. Turtles with a CCL <65 cm were assumed to be juveniles (Limpus and Reed 1985) and were not sexed. Individuals with a CCL >65 cm were assumed to be adults, and males were distinguished from females by differences in tail length (Limpus and Reed 1985). Skin, blood and last-bite samples were then taken and turtles released at their site of capture within 12 h of initial capture.

Last-bite sampling

Oesophageal lavage was used to obtain last-bite diet samples of material contained within the anterior crop and oesophagus of each turtle; this was assumed to consist of material from the most recent feeding event (Forbes and Limpus 1993). Turtles were inverted on a modified wheelbarrow so the head was lower than the body. The mouth of the turtle was opened using an aluminium pry bar and held open with a veterinary gag. A lubricated plastic tube was inserted beyond the epiglottis and water was introduced at low pressure via a second plastic tube attached to a tap line to flush out food particles. Flushed food particles were collected in a fine-meshed bag. This process was performed for ~3–5 min per turtle and food samples were stored in 70% ethanol until laboratory analysis.

In the laboratory last-bite contents from each turtle were separated into different food item categories and each component weighed. Diet items were assigned to the lowest taxonomic category using a dissecting microscope and taxonomic keys (seagrass: Lanyon 1986; macroalgae: Cribb 1996; mangrove: Duke 2006).

For each site a frequency of occurrence (FO) was calculated as the overall proportion of samples in which a food item was detected, and a proportion by wet mass (M) of each component within a sample was also calculated. An Index of Relative Importance (IRI) for food items was calculated. IRI overcomes potential bias resulting from extreme samples where a high mass of a single food type was found in a limited number of turtles, or an insignificant amount of a food type is found in a high frequency of turtles when evaluating diet (Bjorndal *et al*. 1997). For each sampling site,

$$\%\mathrm{IRI} = (\mathrm{FO_i} \times \mathrm{M_i}/({}^{n}\Sigma_{i=1}(\mathrm{FO_i} \times \mathrm{M_i}))) \times 100,$$

where FO = fractional frequency of occurrence, M = fractional mass, n = number of food types, and i = food type.

Stable isotope analysis

Tissue samples were procured immediately after last-bite dietary samples were taken. Following swabbing with 70% ethanol, epidermal tissue from the region behind the fore flippers was collected using a scalpel and immediately frozen at −17°C. A 23-gauge needle and a 10-mL syringe were used to extract between 1 and 3 mL of blood from a dorsal branch of a jugular vein. Blood from all turtles sampled was placed into a 1.5-mL Eppendorf tube and after 24 h the serum separated from blood clots in the tubes. In a subsample of 14 turtles, some blood was also placed into a 1.5-mL Eppendorf tube containing a crystal of sodium heparin (as recommended by Lemons *et al*. 2012). These tubes were shaken and then spun at 5000 rpm for 15 min and the plasma separated from blood cells. Serum, blood clots, plasma and blood cells were then stored frozen at −17°C until isotope analysis.

Epidermis samples were rinsed with distilled water and cleaned with alcohol swabs. Connective tissue was removed, and surface epidermis was diced with a scalpel blade or scissors. All samples were oven-dried at 55−60°C for 24 h and ~1–2 mg of each epidermal, plasma, serum, blood cells or blood clot sample and 5–7 mg of vegetation samples were used for SIA. Dried tissues were then ground using a mortar and pestle until a homogenous powder was obtained; these were sealed in tin capsules that were loaded into an elemental analyser. Lipids were not removed from blood or epidermal tissues before analysis because of their low lipid content, as indicated by a C : N ratio <3.5 (Post *et al*. 2007) and the fact that lipid extraction does not change the $\delta^{13}C$ values of these tissues in sea turtles (Vander Zanden *et al*. 2012; Carpentier *et al*. 2015). Following the

protocol of Carpentier *et al.* (2015), SIA was performed using a EuroEA 3000 (EuroVector, Italy) elemental analyser and an IsoPrime (Micromass, UK) isotope ratio mass spectrometer. Technical standards were interspersed after every 20th sample throughout these analyses, and the precision of these analyses was ±0.1‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$. Stable isotopes were quantified using δ notation defined as part per thousand (‰) relative to the standard, where $\delta = ((R_{sample}/R_{standard}) - 1) \times 1000$, R_{sample} is the ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample and $R_{standard}$ is the isotope ratio of the corresponding international standard (PeeDee Belemnite (PDB) for carbon, and atmospheric air for nitrogen: Peterson and Fry 1987).

Statistical analysis

Comparisons of the frequency of occurrence of dietary items between Pelican Banks and Wiggins Island locations and between adult males and females from Pelican Banks were performed using a Fisher's Exact test. Pearson correlation and paired Student's *t*-tests were used to compare $\delta^{13}C$ and $\delta^{15}N$ values of blood clots/blood cells and blood plasma/blood serum of individual turtles. Between-site values of $\delta^{13}C$ and $\delta^{15}N$ were compared using one-way ANCOVA, with CCL as the covariate, and are reported as least-square means (adjusted to a CCL of 73.9 cm, which was the mean CCL of sampled turtles).

When comparing $\delta^{13}C$ values between blood cells and serum, and epidermis and serum from the same individuals to test whether there were indications of diet switching, a tissue-to-tissue conversion factor (Vander Zanden *et al.* 2014*b*) was used to generate a theoretical 'no change in diet' line. This conversion factor was based on the fact that in adult and juvenile green turtles fed a constant diet, the serum $\delta^{13}C$ values were enriched by, on average, 0.30‰ compared with blood cells, and depleted by an average of 1.04‰ compared with epidermis (Vander Zanden *et al.* 2012).

Statistical differences were concluded if $P \leq 0.05$ and results are presented as least-square covariate means ± standard deviation. Statistical analyses were performed with Statistica 7.01 software.

Results

Reference plant stable isotope values

Thirteen different potential plant food species were sampled for reference $\delta^{13}C$ and $\delta^{15}N$ values (Table 1). The four species of red macroalgae sampled were broken into two groups based on differences in their $\delta^{13}C$ values. The $\delta^{13}C$ values of seagrass were the most enriched, –10.0 ± 1.6‰, and the mangroves and red macroalgae Group 1 the least enriched, –27.0 ± 0.5‰ and –28.2 ± 0.5‰ respectively. Red macroalgae Group 2, –18.6 ± 0.6‰, brown algae, –18.6 ± 4.2‰, and green filamentous algae, –22.1‰, were intermediate between these two extremes. The $\delta^{15}N$ values from the reference plant collection had a much narrower range (1.5 to 7.5‰) than did the $\delta^{13}C$ values (–28.6 to –9.6‰) (Table 1, Fig. 2). These stable isotope values are similar to values obtained from the same plant species sampled in other years and at other locations along the southern Queensland coast (Table 2).

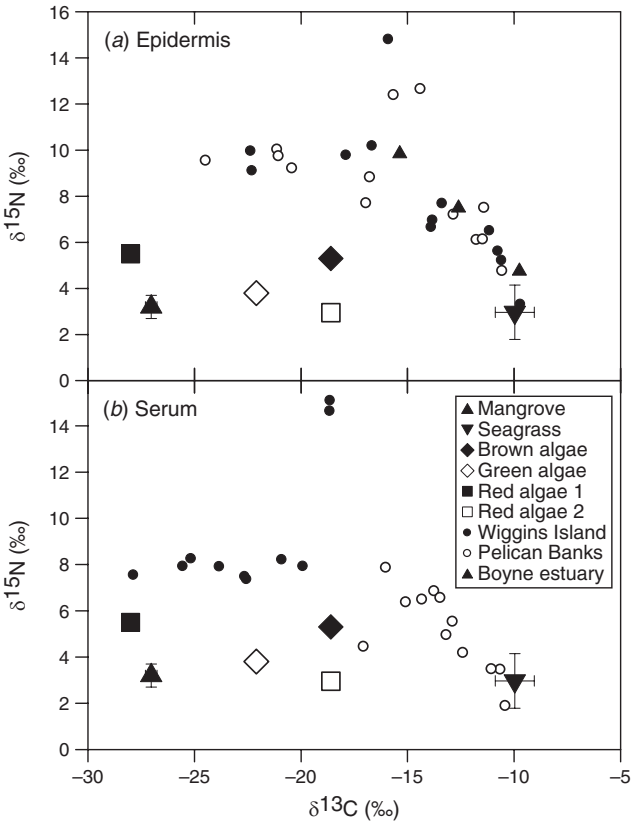

Fig. 2. Plots of $\delta^{15}N$ values against $\delta^{13}C$ values of food items (large symbols) and green turtle tissues (small symbols) sampled from Port Curtis, Queensland, Australia. (*a*) Epidermal tissue ($n = 25$), (*b*) blood serum ($n = 19$). Solid triangle = mangrove, solid inverted triangle = seagrass, solid diamond = brown algae, open diamond = green algae, solid square = red algae Group 1 (*Spyridia* sp. and *Catenella nipae*), open square = red algae Group 2 (*Chondria* sp. and *Laurencia* sp.). Error bars represent standard deviations. Solid circles = turtles sampled from Wiggins Island, open circles = turtles sampled from Pelican Banks.

Last-bite samples

Last-bite samples were taken from 12 turtles from Wiggins Island and 47 turtles from Pelican Banks. Turtles from Wiggins Island (CCL = 48.2 ± 5.5, range = 42.7–60.0 cm; mass = 12.8 ± 5.2 kg, range = 6.2–22.8 kg) were smaller in CCL and mass (both, $P < 0.001$) than turtles from Pelican Banks (CCL = 87.2 ± 20.0, range = 42.1–114.3 cm; mass = 86.2 ± 6.1, range = 7.5–180.0 kg). All turtles caught from Wiggins Island were juvenile, while at Pelican Banks nine (19%) turtles caught were juvenile, 21 (45%) were female and 17 (36%) were male. Last-bite samples consisted almost exclusively of plant material, with the dominant plant type depending on their capture site (Table 3).

The 12 last-bite samples from Wiggins Island consisted predominantly of red macroalgae (%IRI = 87.6%) with *Catenella nipae*, *Chondria* sp. and *Spyridia* sp. having the highest relative abundances (Table 3). Seagrass was found in 46% of samples, but due to its low proportion in these samples (14.3% M_{tot}, where M_{tot} is total mass of the sample) it was of low dietary importance (%IRI = 9.2%). Mangrove material was

Table 2. Stable isotope $\delta^{13}C$ and $\delta^{15}N$ values (‰) of the same plant species that were sampled in the current study and have been collected from other marine environments in south-east Queensland

Plant type	Species	$\delta^{13}C$	$\delta^{15}N$	Location and reference
Mangrove	*Rhizophora stylosa* (leaves)	–26.5	1.3	Moreton Bay (Brine 2008)
	Rhizophora stylosa (leaves)	–27.1	0.8	Hervey Bay (Cameron 2007)
	Rhizophora stylosa (leaves)	–26.6	0.9	Shoalwater Bay (Arthur *et al*. 2009)
	Avicennia marina (propagule)	–27.4	1.4	Hervey Bay (Cameron 2007)
Mean		–26.9 ± 0.4	1.1 ± 0.3	
Seagrass	*Zostera capricorni*	–10.8	1.6	Hervey Bay (Cameron 2007)
	Zostera capricorni	–10.4	0.5	Shoalwater Bay (Arthur *et al*. 2009)
	Halophila ovalis	–12.4	0.5	Hervey Bay (Cameron 2007)
	Halophila ovalis	–12.2	0.5	Moreton Bay (Brine 2008)
	Halodule uninervis	–11.6	2.6	Hervey Bay (Cameron 2007)
	Halodule uninervis	–10.3	3.2	Moreton Bay (Brine 2008)
Mean		–11.3 ± 0.9	1.5 ± 1.2	
Brown algae	*Sargassum* sp.	–17.4	4.4	Moreton Bay (Brine 2008)

found in trace amounts in 38.5% of samples and thus was an insignificant dietary item (%IRI = 0.4%). A green filamentous alga was found in large proportions in two turtles (16% FO), resulting in a %IRI of 2.4%. In contrast to the turtles from Wiggins Island, the 47 last-bite samples from Pelican Banks were almost exclusively seagrass (%IRI = 99.5%) (Table 3). Only two of the three sea grass species found in Port Curtis, *Zostera capricorni* and *Halophila ovalis*, were identified in last-bite samples (100% and 74% FO respectively). Animal material was present in trace amounts in 70% of samples (%IRI = 0.3%). Within the adult turtles there was no apparent difference in occurrence of dietary items between males and females (Fisher's Exact test, $P = 0.763$), and no apparent difference between adults and juveniles (Fisher's Exact test, $P = 0.483$). The frequency of occurrence of dietary items was significantly different between the Pelican Banks and Wiggins Island sites (Fisher's Exact test, $P = 0.004$), with red macroalgae dominating diets of turtles from Wiggins Island, and seagrass dominating the diet of turtles from Pelican Banks.

Comparing $\delta^{13}C$ and $\delta^{15}N$ values within blood samples

The $\delta^{13}C$ and $\delta^{15}N$ values of the plasma and serum samples collected from the same turtles were tightly correlated and were not different ($\delta^{13}C$: $r^2 = 1.00$, paired t-test $P = 0.504$, $n = 14$; $\delta^{15}N$: $r^2 = 0.99$, paired t-test $P = 0.837$, $n = 14$; 3 from Wiggins Island, 11 from Pelican Banks). Likewise, the $\delta^{13}C$ and $\delta^{15}N$ values from blood cells separated from plasma and blood clots separated from serum were tightly correlated and not different ($\delta^{13}C$: $r^2 = 1.00$, paired t-test $P = 0.964$, $n = 14$; $\delta^{15}N$: $r^2 = 0.92$, paired t-test $P = 0.124$, $n = 14$). As values from plasma and serum, and values from blood cells and blood clots were essentially identical, we only use values derived from the serum and blood clots in subsequent analyses because we collected more of these types of samples.

Comparing $\delta^{13}C$ and $\delta^{15}N$ values across sampling sites

Epidermal tissue was taken from all 12 turtles from Wiggins Island and from a subsample of 13 turtles from Pelican Banks, and blood samples were successfully collected from 7 turtles from Wiggins Island and 12 turtles from Pelican Banks. There was no correlation between turtle size (CCL) and $\delta^{13}C$ ($r^2 = 0.02$, $n = 25$, $P = 0.477$) or size and $\delta^{15}N$ ($r^2 = 0.02$, $n = 25$, $P = 0.510$) of epidermis. The $\delta^{13}C$ values for epidermis of turtles from Wiggins Island (–14.9 ± 4.3 ‰) and Pelican Banks (–16.1 ± 4.5‰) overlapped each other (Fig. 2*a*) and were not different (Student's t-test, $P = 0.506$). The $\delta^{15}N$ values for epidermis tissue of turtles from Wiggins Island (8.0 ± 3.0‰) and Pelican Banks (8.6 ± 2.4‰) overlapped each other (Fig. 2*a*) and were not different (Student's t-test, $P = 0.572$). In contrast to epidermis, serum $\delta^{13}C$ values from Wiggins Island turtles (–22.6 ± 3.1‰) did not overlap and were significantly depleted (Student's t-test, $P < 0.001$) compared with those from Pelican Banks (–13.4 ± 2.1‰) (Fig. 2*b*). Serum $\delta^{15}N$ values from Wiggins Island turtles (9.2 ± 3.0‰) were significantly enriched (Student's t-test, $P < 0.001$) compared with those from Pelican Banks (5.2 ± 1.7‰) (Fig. 2*b*).

Relationship between $\delta^{13}C$ serum and epidermis values and last-bite diet samples and evidence of diet switching within individual turtles

Because last-bite diet analysis revealed that green turtle diet was essentially dichotomous within Port Curtis, either being dominated by red macroalgae at the Wiggins Island site or seagrass at the Pelican Banks site, and because the $\delta^{13}C$ isotope values from red macroalgae and seagrass were so different, we predicted that there would also be a dichotomous relationship between tissue $\delta^{13}C$ isotope values in turtles sampled from the two sites. To test this hypothesis we plotted collection site against $\delta^{13}C$ values of serum and epidermis (Fig. 3*a*, *b*). There was a clear dichotomous pattern in the distribution of serum $\delta^{13}C$ values (Fig. 3*a*), but no dichotomous distribution pattern in epidermal $\delta^{13}C$ values (Fig. 3*b*).

To indicate whether there was evidence of diet change between the time that blood plasma, blood cells and epidermal tissues were formed, $\delta^{13}C$ serum values were compared with $\delta^{13}C$ values for blood cells and epidermis after tissue-specific corrections had been applied (Fig. 4*a*, *b*). Turtles were classified

Table 3. Composition of oesophageal samples from green turtles from Port Curtis, 4–8 November 2013

%FO, frequency of occurrence (%); $\%M_{tot}$, total mass of sample; %IRI, index of relative importance (%)

Location	Food item	%FO	$\%W_{tot}$	%IRI
Wiggins Island	Phylum Rhodophyta	100.0	68.2	87.6
(*n* = 12)	*Catenella nipae*[A]	50.0	34.3	
	Hypnea sp.	33.3	4.6	
	Soliera robusta	33.3	0.3	
	Bostrychia tenella	25.0	0.4	
	Chondria sp.[A]	16.7	18.4	
	Spyridia filamentosa[A]	16.7	9.3	
	Gracilaria sp.	16.7	0.5	
	Amansia glomerata	8.3	0.2	
	Dasya naccarioides	8.3	0.2	
	Polysyphonia spp.	8.3	0.1	
	Gelidopsis variabilis	8.3	<0.1	
	Seagrass	50.0	14.3	9.2
	Halophila ovalis[A]	41.7	11.1	
	Zostera capricorni[A]	33.3	7.8	
	Mangrove	41.7	0.7	0.4
	Mangrove bark	33.3	0.4	
	Mangrove leaf[A]	16.7	0.3	
	Phylum Ochrophyta	25.0	0.1	0.0
	Sargassum flavicans[A]	16.7	0.1	
	Dictyota ligulata[A]	16.7	<0.1	
	Zonaria diesingiana	8.3	<0.1	
	Phylum Chlorophyta	16.7	11.3	2.4
	Green filamentous[A]	16.7	11.3	
	Animal material	8.3	<0.1	0.0
	Amphipod	8.3	<0.1	
	Other	75.0	0.4	0.4
	Seed	16.7	<0.1	
	Shell	75.0	0.4	
Pelican Banks	Seagrass	97.9	99.0	99.5
(*n* = 47)	*Zostera capricorni*[A]	97.9	93.5	
	Halophila ovalis[A]	72.3	4.8	
	Seagrass roots	31.9	0.6	
	Phylum Rhodophyta	23.4	0.1	0.0
	Dictyota ligulata	23.4	0.1	
	Gracilaria sp.	10.6	0.1	
	Catenella nipae[A]	6.4	<0.1	
	Polysyphonia spp.	2.1	<0.1	
	Halymenia floresii	2.1	<0.1	
	Phylum Ochrophyta	25.53	0.127	0.0
	Dictyota ligulata[A]	23.4	0.1	
	Sargassum flavicans[A]	4.3	<0.1	
	Animal material	70.2	0.4	0.3
	Amphipod	59.6	0.4	
	Fluke worm	6.4	<0.1	
	Leech	4.3	<0.1	
	Other	36.2	0.3	0.1
	Shell	19.2	0.3	
	Seed	17.0	0.1	
	Mangrove	14.9	0.1	0.0
	Mangrove leaf	10.6	0.1	
	Mangrove bark	4.26	<0.1	

[A]Indicates species for which the stable isotope $\delta^{13}C$ and $\delta^{15}N$ values were determined.

as having switched their diet if the difference between a measured value and the theoretical same diet line value was greater than 30% of the predicted value. This analysis indicated that no turtles had changed diet between the time when blood cells and blood serum were formed (Fig. 3*a*), but five turtles from Wiggins Island and six turtles from Pelican Banks switched diets between the time when epidermis and blood serum were formed (Fig. 3*b*).

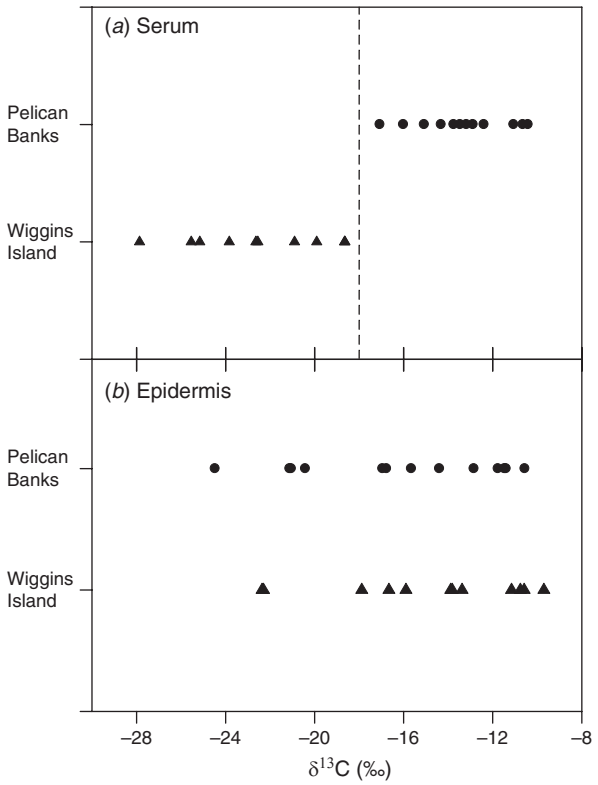

Fig. 3. Plots of capture location against serum and epidermis $\delta^{13}C$ values for green turtles from Port Curtis, Queensland, Australia. Serum $\delta^{13}C$ values indicate a dichotomous distribution, with turtles from Pelican Banks having values greater than –18% and turtles from Wiggins Island having values less than –18% (*a*). There is no evidence of a dichotomous distribution of epidermis $\delta^{13}C$ values (*b*).

Discussion

Both last-bite diet analysis and SIA indicate that green turtles in Port Curtis are highly herbivorous. Last-bite samples indicated that diet composition was site specific, with green turtles eating almost exclusively seagrass at the Pelican Banks site, and predominantly red macroalgae at the Wiggins Island site. SIA of blood serum supported these findings, but SIA of epidermis was not entirely consistent with this conclusion, with the $\delta^{13}C$ epidermal samples of ~50% of turtles indicating a diet composition different to that of the last-bite sample. This change in diet between the time when epidermal tissue was synthesised and the last-bite sample taken, most probably occurred as the result of turtles moving between seagrass and red macroalgae dominated areas within Port Curtis rather than selectively feeding on different plants within a fixed home range. This conclusion is supported by the recent finding that some acoustically tagged green turtles moved between Pelican Banks and Wiggins Island (Babcock *et al*. 2015). These findings have important implications for sea turtle foraging ecology

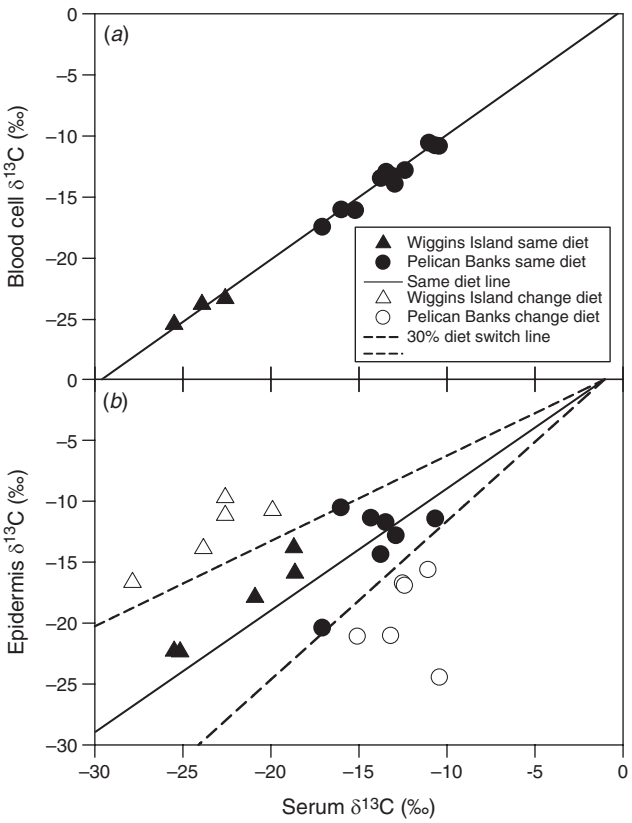

Fig. 4. (*a*) Blood cell and (*b*) epidermis $\delta^{13}C$ values plotted against serum $\delta^{13}C$ values taken from the same individual green turtles. The same diet line was calculated using tissue conversion factors averaged for juvenile and adult green turtles (Vander Zanden *et al*. 2012). A 30% change in the observed $\delta^{13}C$ value from the predicted diet line was chosen as a break-point to distinguish a switch in diet. Using this criterion no turtles changed diet between the time blood cells and blood serum were formed (*a*). However, five turtle from Wiggins Island and six turtles from Pelican Banks appeared to switch diet between the time epidermis and blood serum were formed (*b*).

because they indicate that (1) even within a relatively small region an individual's diet can vary remarkably, which may have implications for growth rates and reproductive output, and (2) that at least some individuals shift their foraging home range within this region on a regular basis.

Reference plant stable isotope values

The $\delta^{13}C$ values of seagrass and mangrove found in the current study (Table 1) agree well with those from previous studies from the south-east Queensland coast (Table 2). This indicates that different food plant types have stable $\delta^{13}C$ values through time and location and thus can be reliably used in the interpretation of stable isotope values from tissues of marine herbivores in this region. With the exception of two red macroalgae species (Table 1, Group 1), the current study found $\delta^{13}C$ macroalgae values to be variable and intermediate between seagrass (–11‰) and mangrove (–27‰). These findings are consistent with those of previous studies. For example, Winemiller *et al*. (2007) reported the mean $\delta^{13}C$ value of macroalgae to be –18.7‰ ± 0.3 and Burkholder *et al*. (2011) found the $\delta^{13}C$ value of macroalgae to range between –24‰ and –12‰, depending upon species. Raven *et al*. (2002) found red macroalgae generally had more negative $\delta^{13}C$ values than other algae, with most species having values less than –25‰. Differences in primary producer $\delta^{13}C$ values can be attributed to differences in biogeographic region, differences in taxonomic group, or differences associated with the way in which carbon isotopes are fractionated in biosynthetic pathways, particularly differences in photosynthetic pathways (Peterson and Fry 1987; Hobson and Clark 1993; Raven *et al*. 2002). Because all plants came from the same biogeographic region in the current study, differences in $\delta^{13}C$ values must be due to differences in carbon fractionation associated with taxonomic group and/or biosynthetic pathways. The fact that seagrass, which dominated the diet of turtles at the Pelican Banks site, and red macroalgae Group 1, which was prominent in the diet of turtles from the Wiggins Island site, have very different $\delta^{13}C$ values makes inferences about diet based on turtle tissues $\delta^{13}C$ values easier. In contrast to $\delta^{13}C$ values, $\delta^{15}N$ values of reference plants had a relatively narrow range, which reflects the fact that all are primary producers sitting at the lowest level of the trophic chain.

Last-bite samples

Last-bite samples give an indication of what a turtle has been feeding on immediately before capture and provide quantitative and qualitative information on ingested food. Our results indicated that there was a distinct difference in the dominant food types consumed by turtles at different sites within Port Curtis. The turtles from Pelican Banks fed almost exclusively on the seagrasses *Z. capricorni* and *H. ovalis*, with *Z. capricorni* being the species most eaten, reflecting its greater abundance in this area (McCormack *et al*. 2013). Red macroalgae, particularly *Catenella nipae* and *Chondria* sp., dominated the last-bite samples taken from turtles captured at the Wiggins Island site, which consists of mangrove-lined estuaries. These algae species are frequently associated with mangrove trunks and pneumatophores (Cribb 1996) and so it would appear that green turtles were grazing on these during high tide. Interestingly, two turtles had last-bite samples consisting almost exclusively of green filamentous algae. Few studies have reported green algae as being common in green turtle diets; however, the green macroalgae *Codium* sp. was prominent in the diet of green turtles from rocky shores in Avoredo, Brazil (Reisser *et al*. 2013). To summarise, last-bite samples indicate that green turtles fed on the most common plants in their habitat, which is consistent with previous dietary studies that also found green turtle diet to be dominated by the most abundant plant species present in their feeding area (e.g. Garnett *et al*. 1985; Seminoff *et al*. 2002; Arthur *et al*. 2009).

Comparing $\delta^{13}C$ and $\delta^{15}N$ values within blood samples

It has been assumed that in sea turtles $\delta^{13}C$ and $\delta^{15}N$ values for plasma and serum should be identical, and that $\delta^{13}C$ and $\delta^{15}N$ values for blood cells and blood clots should be identical (Vander Zanden *et al*. 2012) and Lemons *et al*. (2012) provided

data that support this assumption. We found no difference between the serum and plasma $\delta^{13}C$ and $\delta^{15}N$ values or between blood cells and blood clots, confirming that results obtained from the serum–blood clot and the plasma–blood cell methodologies are directly comparable in green turtles.

Comparing $\delta^{13}C$ and $\delta^{15}N$ values across sampling sites

At ~35–40 cm CCL east Australian green turtles convert from an oceanic planktonic feeding habit to an inshore benthic feeding habit (Limpus and Chaloupka 1997) and, as such, there is a distinct change in $\delta^{13}C$ and $\delta^{15}N$ of food items (Arthur *et al*. 2008). All of the turtles sampled in the current study were larger than this threshold and even the smallest turtle sampled (CCL = 43 cm) had most likely been in the inshore habitat for more than 12 months. At the Pelican Banks site, where both juvenile and adults were sampled, there was no correlation between either epidermal $\delta^{13}C$ and CCL or $\delta^{15}N$ values and CCL (CCL range 43–107 cm) and the same was true for serum stable isotope samples. A similar conclusion was made for a Shark Bay, Western Australia, population of green turtles (CCL range 40–120 cm) (Burkholder *et al*. 2011). Hence, on the basis of stable isotope data, there is no evidence of size-specific differences in diet of green turtles, a conclusion consistent with our last-bite sampling and with a previous study that used last-bite diet sampling from a Shoalwater Bay, Queensland, population of green turtles (Arthur *et al*. 2009).

The range of epidermal $\delta^{15}N$ values of green turtles in the current study (3–15‰) was slightly larger than that reported for a foraging aggregation in Shark Bay, Western Australia (5–11‰) (Burkholder *et al*. 2011) and wider than than reported for a San Diego Bay, California, USA, population (11.0–19.3‰) (Lemons *et al*. 2011). Being primarily herbivorous, green turtles are expected to be one trophic level above primary producers, which should be reflected by tissue $\delta^{15}N$ enrichment of ~2.8‰ (Seminoff *et al*. 2006). Given that the mean $\delta^{15}N$ values of seagrass and algae from this study were 2.0 and 3.5‰, respectively, it was expected that the $\delta^{15}N$ values would be ~4.8–6.3‰ if turtles were exclusively herbivores. Serum $\delta^{15}N$ values of turtles from Pelican Banks were in this range; however, serum $\delta^{15}N$ values from Wiggins Island turtles were above this range. The turtles with elevated $\delta^{15}N$ values may have been feeding on foods from trophic levels higher than primary producers (e.g. jellyfish). Wiggins Island turtles were also juveniles so their foraging behaviour with respect to the amount of animal material may differ from that of the larger turtles sampled from Pelican Banks. Because soft-bodied animal material is highly digestible, diet studies based on gut or faecal samples are likely to under-represent the amount consumed and, therefore, soft-bodied animals may have greater prominence in green turtle diets than previously thought. For example, individual green turtles have been observed selectively feeding on gelatinous macroplankton (Heithaus *et al*. 2002; Arthur *et al*. 2007; Burkholder *et al*. 2011; Reisser *et al*. 2013). Similarly, studies using SIA have suggested that green turtles often consume large amounts of gelatinous macroplankton (Seminoff *et al*. 2006; Burkholder *et al*. 2011; Carman *et al*. 2014).

Relationship between $\delta^{13}C$ serum and epidermis values and last-bite diet samples and evidence of diet switching within individual turtles

If a turtle feeds on a diet of constant stable isotope composition all tissues in its body will eventually assume a constant stable isotope composition. This composition will vary slightly between tissue types because of tissue-specific isotopic fractionation (Peterson and Fry 1987; Hobson and Clark 1992). Hence, within the Port Curtis area, tissue $\delta^{13}C$ values must, in theory, lie between the extremes of an all-red-macroalgae Group 1 diet (~–27 ‰) and an all-seagrass diet (~–11 ‰), which was indeed the case. Turtles with tissue $\delta^{13}C$ values near the seagrass extreme must have been feeding predominantly on seagrass; likewise, turtles with tissue $\delta^{13}C$ values near the red macroalgae Group 1 extreme must have been feeding predominantly on red macroalgae Group 1. If a change of stable isotope composition in the diet occurs (such as switching from a red macroalgae Group 1 to a seagrass diet) then the stable isotope of the animal's tissues will also change, and the change will be fastest in tissues that have faster turnover rates. Blood serum has the fastest stable isotope turnover rates of the regularly non-destructively sampled tissues in mammals and birds (Hobson and Clark 1992, 1993). Unfortunately, no data are available for adult sea turtles, but it is assumed that blood serum has a faster turnover rate than epidermis (see discussion below). For this reason, stable isotope values of serum are more likely to reflect the last-bite diet than that of epidermis, if the probability of a turtle shifting habitats increases as time progresses. The dichotomous distribution of serum $\delta^{13}C$ values reflects this prediction (Fig. 3*a*): all turtles sampled from Wiggins Island had values more depleted than –18‰, reflecting a diet dominated by red macroalgae Group 1, as indicated by last-bite sampling, and all turtles sampled from Pelican Banks had values more enriched than –18‰, reflecting a diet dominated by seagrass, as indicated by last-bite sampling. In contrast, the epidermis $\delta^{13}C$ values had a seemingly random distribution (Fig. 3*b*), with ~50% of the turtles having values reflecting a diet in the habitat opposite to that in which they were caught. The simplest explanation for this phenomenon is that ~50% of the turtles shifted habitats between the time that epidermal and serum tissues were formed.

Directly comparing $\delta^{13}C$ values from different tissues from the same individual turtle also provides evidence that some individuals have changed their diet, most likely by shifting foraging area within the timeframe of tissue turnover half-lives. Once a tissue conversion factor was applied, serum and blood clot $\delta^{13}C$ values of all turtles sampled were virtually identical (Fig. 4*a*), indicating that their diet had a similar stable isotope composition when these tissues were formed. This is consistent with data from juvenile loggerhead turtles, which indicate that the half-life of plasma and blood cells is similar (27.7 and 27.3 days respectively: Reich *et al*. 2008) and therefore are formed at very similar times. In 12 of the 23 turtles sampled, serum and epidermis $\delta^{13}C$ values were similar (Fig. 4*b*), also indicating that their diet had a similar stable isotope composition when these tissues were formed. However, in 11 turtles, serum and epidermis $\delta^{13}C$ values were different (Fig. 4*b*), indicating that the stable isotope composition of their diet was different

when these tissues were formed. Last-bite and serum $\delta^{13}C$ values indicate that all turtles from Wiggins Island have diets dominated by red macroalgae Group 1. In the five turtles from this site that switched diet, the epidermis $\delta^{13}C$ values were enriched compared with the serum $\delta^{13}C$ values, indicating that during the time that epidermis was manufactured their diet was dominated by seagrass. Likewise, last-bite and serum $\delta^{13}C$ values indicate that all turtles from Pelican Banks have diets dominated by seagrass. In the six turtles from this site that switched diet, the epidermis $\delta^{13}C$ values were depleted compared with the serum $\delta^{13}C$ values, indicating that during the time that epidermis was manufactured their diet was dominated by red macroalgae Group 1. Hence there is clear evidence that green turtles regularly switch between red macroalgae Group 1 and seagrass-dominated habitats within Port Curtis. Recent acoustic tagging of green turtles in this region also indicates that some turtles switch from sea grass–dominated to red algae–dominated feeding grounds (Babcock *et al*. 2015).

Unfortunately, we cannot put an accurate timeframe around these apparent shifts in feeding habitat because definitive data on stable isotope turnover rates in serum, blood clots and epidermis are not available for juvenile and adult green turtles. In juvenile loggerhead turtles the serum turnover rate of carbon (half-life 27.3 days) was only slightly faster than that of epidermis (half-life of 31.8days), but this lack of apparent difference in tissue carbon turnover rates was attributed to a masking effect caused by rapid growth rates in the turtles that were studied (Reich *et al*. 2008). On the basis of a survey of the literature, Vander Zanden *et al*. (2015) formulated predictive equations for half-lives of stable isotopes in different tissues of ectothermic vertebrates of a specified body mass. These equations predict the serum half-life of a 12.8-kg juvenile ectotherm (mean mass of juvenile green turtles in our study) to be 84 days, and of a 90.5-kg adult (mean mass of adult green turtles in our study) to be 129 days. An equation for epidermis was not published, but if we use the equation for muscle as a proxy for epidermis, the half-lives are 213 days and 327 days, respectively, for juveniles and adults. On the basis of these numbers, serum $\delta^{13}C$ values reflect the turtle's diet on the order of 6–12 months, and epidermis $\delta^{13}C$ values reflect the turtle's diet on the order of 9–18 months before the tissue samples being taken.

The large proportion of turtles that showed evidence of a shift in diet was unexpected because this implies that they had shifted their feeding territory significantly (moved far enough away to encounter a substrate dominated by a different food type, which in our study area was ~10 km) and the home ranges of green turtles are generally thought to be static except when they go on breeding migrations (e.g. Bjorndal 1980; Chaloupka *et al*. 2004; Makowski *et al*. 2006), but studies that have published quantitative data on the foraging home ranges of green turtles are scarce. For juveniles, reported home ranges vary between 0.4 and 6.4 km^2 (Seminoff *et al*. 2002; Makowski *et al*. 2006; Hazel *et al*. 2013; Babcock *et al*. 2015), and for adults, reported foraging activity centres vary between 3.6 and 13.8 km^2 (Babcock *et al*. 2015). However, some juveniles observed in the gulf of California, Mexico, moved between different foraging activity centres that could be several kilometres apart (Seminoff *et al*. 2002), and six of 26 adults moved between the Pelican Banks and Wiggins Island sites in a study conducted in Port Curtis, Australia (Babcock *et al*. 2015). Hence, there remains uncertainty regarding the size of home range used by foraging green turtles – it may be larger than previously expected.

Future research

The Port Curtis population of green turtles is an ideal system to test ideas and theory associated with the SIA methodology because within a relative close area turtles are feeding on plants with very different $\delta^{13}C$ values and individual turtles appear to move between these distinctly different feeding sites. Of particular interest is the relationship between stable isotope values in the blood and epidermis and how these might change through time. It has been established that when fed a constant diet green turtle blood and epidermis $\delta^{13}C$ values are similar (Vander Zanden *et al*. 2012), and the current study has shown that in some individuals they can be very different, implying that some turtles have changed diet, presumably because they have shifted to a different feeding habitat. Our preliminary stable isotope data suggest that ~50% of the green turtles in the Port Curtis region undergo significant changes in the type of vegetation consumed and therefore that changes in diet are common. While sonic tag tracking for periods between six and 12 months indicated that six of the 26 turtles initially caught on the Pelican Banks spent some time ~10 km distant at Wiggins Island (Babcock *et al*. 2015), there remains the possibility that the turtles sampled in the present study utilised other closer or more distant sites within Port Curtis that provided similar choices in diet to our study sites. Hence, more detailed investigation of simultaneous forage distribution, turtle tracking and diet studies is warranted for elucidating the foraging behaviour of green turtles in spatially complex localities.

We recommend that in future SIA sea turtle studies both blood and epidermal tissues be sampled so that changes in diet might be detected and verified as being common, as suggested by the current study. If consistent differences in diet are detected, it would also be of interest to investigate whether different diets affect life-history characteristics of individuals such as growth rates of juveniles and reproductive output of adults. For example, SIA has been used to identify differences in reproductive output in distinctly different foraging populations of loggerhead turtles on the east coast of the United States (Vander Zanden *et al*. 2014*a*; Ceriani *et al*. 2015).

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