Vol. 633: 197–206, 2020
https://doi.org/10.3354/meps13179

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published January 9

When isotopes fail: importance of satellite telemetry and multi-site validation when estimating the foraging grounds of migratory species

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ABSTRACT: A combination of $\delta^{13}C$ and $\delta^{15}N$ isotope ratios, capture–mark–recapture and satellite telemetry data were used to investigate the foraging locations supporting nesting loggerhead turtles *Caretta caretta* on the Woongarra coastline, southeast Queensland, Australia. Known foraging grounds were available for a subset of these turtles and supplemented with samples taken from foraging turtles to determine whether foraging regions could be identified in untracked nesting turtles using the stable isotopic values of their sampled tissue. Despite the large distances between known foraging regions, no latitudinal gradient was observed in the isotopic values of these turtles. In addition, the isotopic values of foraging individuals from a single site encompassed the range of all loggerhead turtles sampled over the 2000 km north–south distribution along the east coast of Australia. This study demonstrates that assumptions common to $\delta^{13}C$ and $\delta^{15}N$ stable isotope inference in migratory species are not adhered to globally. Contrasting the successes of stable isotope analysis in the northern hemisphere, these results indicate that factors in the southwest Pacific such as differing prevailing oceanic currents, temperature regimes and river run-offs may prevent the establishment of region-specific unique isoscapes needed for identifying the foraging region of turtles using their isotopic values. Therefore, we caution against the use of $\delta^{13}C$ and $\delta^{15}N$ stable isotopic values as suitable indicators of foraging regions for loggerhead turtles in Australia. These findings potentially highlight the need to re-evaluate when and where the use of isotopic analysis is appropriate for identifying foraging locations in marine turtle species.

KEY WORDS: Loggerhead turtles · *Caretta caretta* · Carbon isotope · Nitrogen isotope · Migration connectivity

1. INTRODUCTION

Understanding the connectivity between breeding areas and resident foraging areas in migratory marine species is desirable for the implementation of long-term conservation management strategies. This is particularly important for species such as marine turtles that are increasingly at risk from anthropogenic threats. While marine turtles are easily surveyed at their breeding aggregations, the feeding grounds of these populations may, in some locations, be unknown or difficult to access for detailed survey. Determining the location of these feeding grounds can facilitate the management of high-usage feeding areas. Stable isotope analysis (SIA) of tissues from individuals at breeding aggregations is an increasingly used method for identifying the feeding locations of numerous migratory species (Caut et al. 2008a,b, Cherel et al. 2009, Allen et al. 2013, Lorrain et al. 2015, Pethybridge et al. 2015). In marine turtles, other information, such as differential habitat use, variation in habitat condition and consequent differences in dietary composition and migratory distances, have also been obtained from SIA (Godley et al. 1998, Hatase et al. 2010, Thomson et al. 2012, Ceriani et al. 2015, Figgener et al. 2019).

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Diet and trophic position of a sampled individual within its foraging regions can be inferred using the stable isotopic ratios of $^{12/13}C$ and $^{14/15}N$ from their body tissues (Hobson 2019). The fractionation of these isotopes occurs predictably along a trophic cascade (DeNiro & Epstein 1978, 1981, Tieszen et al. 1983, Post 2002). Additionally, the identification of oceanic latitudinal gradients in carbon stable isotopes has led to the determination of migratory routes and foraging areas in several migratory marine species, including marine turtles (Caut et al. 2008b), fur seals (Cherel et al. 2009) and blue crabs (Gelpi et al. 2013). Isotopic gradients are associated with sea surface temperature and productivity in oceanic primary producers, with enriched $\delta^{13}C$ values connected to productive regions, such as upwelling zones (Graham et al. 2010). Additionally, depletions are observed with increased latitude in the Pacific-Artic, Indian and Atlantic Oceans (Rau et al. 1982, Magozzi et al. 2017), with the lowest $\delta^{13}C$ values found in low nutrient or stratified regions (DeNiro & Epstein 1978, Peterson & Fry 1987). These systematic variations in $\delta^{13}C$ values within regions are critical for the correct identification of location, habitat use and dietary composition in integrated isotopic analyses.

Because of the insights SIA provides, it has been used in a range of marine species to investigate diet, habitat use and migration, including identifying foraging habitats and trophic positions of pelagic fish species (Lorrain et al. 2015), identifying the wintering habitats of migratory seabirds (Cherel et al. 2006) and quantifying diet of pygoscelid penguins in Antarctica (Polito et al. 2011). Marine turtle SIA studies have identified that many green turtles *Chelonia mydas*, which are primarily herbivorous as adults, continue to consume macrozooplankton prey following recruitment to benthic foraging grounds (Hatase et al. 2006, Cardona et al. 2009, Lemons et al. 2011, González Carman et al. 2014, Shimada et al. 2014, Prior et al. 2016). Additionally, SIA has confirmed that a sub-population of nesting female loggerhead turtles *Caretta caretta* in the northwest Pacific retain oceanic foraging strategies into maturity (Hatase et al. 2010). The successful prediction of foraging grounds of northwest Atlantic loggerhead turtles based on their tissue stable isotope values has also been demonstrated (Ceriani et al. 2012, 2017, Vander Zanden et al. 2015), while isotopic differences between turtles in the northeast Pacific and those from the Atlantic identified differential nitrogen cycling processes (Pajuelo et al. 2010).

Because habitat use and diet are likely to influence reproductive rates, identifying the foraging grounds of migratory species can be useful in interpreting population trends (Alerstam et al. 2003, Norris 2005, Inger et al. 2010, Authier et al. 2012). In marine turtle populations, differences in foraging strategies, habitats and migratory distances influence a range of fitness metrics for both mother and offspring (Frazer & Richardson 1985, Broderick et al. 2001, Zbinden et al. 2011). Body size of reproductive female loggerhead turtles has been linked with foraging area, with carryover effects on clutch size and time between subsequent breeding seasons from 2 nesting populations of the northern hemisphere (Hatase et al. 2013, Vander Zanden et al. 2014). Similarly, large migratory distances may be negatively correlated with reproductive output, as observed in nesting loggerheads on the east coast of North America, suggesting additional costs to breeding in individuals that have long migration pathways (Ceriani et al. 2015).

Major nesting beaches of the South Pacific genetic stock of loggerhead turtles are well known (Bustard 1972, Limpus 1985, Limpus et al. 2013, FitzSimmons & Limpus 2014, Limpus & Casale 2015) as is the distribution of foraging areas (Limpus 2008). Despite this, the relative importance of different foraging regions and the proportion of the nesting population that uses these areas remain incompletely described. Following an oceanic developmental period of more than a decade, loggerhead turtles of the South Pacific genetic stock recruit to foraging areas in the Arafura, Coral and Tasman Seas within Indonesia, Papua New Guinea, the Solomon Islands, New Caledonia and along the north and east coast of Australia (Limpus 2008, Limpus et al. 2013). After juveniles have settled to a neritic foraging area, their diet shifts from predominantly pelagic foraging, targeting macro-planktonic prey found at the surface and mid-water column (Boyle & Limpus 2008), to a diet of mostly benthic invertebrate species such as crustaceans, gastropods and bivalves (Limpus et al. 2001). Once recruited to the neritic feeding habitat, individuals remain in a localised area for the remainder of their lives, except for periodic migrations for reproduction when they are sexually mature (Limpus 2008, Limpus & Limpus 2001, 2003). Turtles intentionally displaced from their foraging areas in southeast Queensland, Australia, have been observed returning to their original foraging region, and satellite telemetry indicates that their usual home range size is 15.6–155.8 km^2, highlighting that loggerheads of the South Pacific genetic stock exhibit strong fidelity to their foraging regions (Shimada et al. 2016a, 2017). While the recruitment and fidelity to select foraging regions has been identified for loggerhead turtles of the South Pacific genetic stock (Limpus et al. 1992, Chaloupka & Limpus 2001), the

relative importance of these foraging regions is not known. SIA offers the potential to sample a large number of nesting individuals in order to identify their foraging regions and provides the potential to obtain a more comprehensive indication of the contribution of different foraging areas to the nesting population than has previously been achievable.

This study aimed to use SIA, capture–mark–recapture (CMR) and satellite telemetry data to identify the foraging regions of individual females from a major nesting aggregation of the South Pacific genetic stock of loggerhead turtles nesting along the Woongarra southeast, central Queensland, Australia. Based on prior demonstration that the foraging grounds of northern hemisphere loggerheads could be predicted from SIA (Ceriani et al. 2012, Vander Zanden et al. 2015), we hypothesised that a north to south latitudinal gradient would be observed in the $\delta^{13}C$ of our sampled nesting turtles. Such a gradient would therefore facilitate identification of foraging regions used by nesting turtles, based on their isotopic values, using predictive modelling.

2. MATERIALS AND METHODS

2.1. Study sites and sampling procedures

The Woongarra coast in southeast Queensland, including the Mon Repos rookery (24.80° S, 152.44° E), hosts the highest nesting numbers of loggerhead turtles from the South Pacific genetic stock, which is listed by the IUCN as a Critically Endangered management unit (Limpus 2008, Limpus & Casale 2015). During the nesting seasons of 2015–2016 (December and February) and 2016–2017 (December and January), females were sampled at Mon Repos and the nearby beaches. Over the sampling period, 120 and 188 nesting females were sampled from the 2015–2016 and 2016–2017 seasons, respectively. The number of turtles sampled was approximately a third of the total number of females nesting in each season. Of the 308 nesting females sampled, the foraging areas of 15 individuals were established from previous satellite telemetry or CMR studies (8 by multi-year CMR records and 7 tracked by satellite to foraging grounds where they were resident for at least 6 mo; Department of Environment and Science, Queensland Government unpubl. data). Shimada et al. (2017) previously established by satellite telemetry that foraging loggerheads of the South Pacific genetic stock exhibit strong fidelity to their foraging grounds when not undertaking reproductive migrations.

Turtles were sampled immediately following oviposition or while they were returning to the water. Each turtle had its midline curved carapace length (CCL) measured using a fiberglass measuring tape (±0.2 cm) (Table 1). All turtles were identified using titanium flipper tags and processed following Queensland Turtle Conservation Project protocol (Limpus 1985, 1992, Limpus et al. 1994). Following swabbing with 70% ethanol, a 0.5 cm^2 skin sample was taken from the trailing edge of the fore flipper between the keratinised scales using a sterile scalpel and stored in 70% ethanol until SIA. Storage in 70% ethanol has no significant effect on $\delta^{13}C$ and $\delta^{15}N$ isotopic signals (Barrow et al. 2008). Blood samples (3 ml) were taken from the external jugular vein just posterior to the head (commonly termed the dorsal cervical sinus) using a sterile single-use syringe and 18-gauge needle and stored in heparinised containers to prevent clotting (Owens & Ruiz 1980). Heparin has a negligible effect on the nitrogen isotopic value in sea turtle blood (Lemons et al. 2012). Blood samples were then separated into plasma and red blood cells by centrifuging at 5500 × *g* for 5 min and stored at −18 °C until analysed. Blood sampling was not conducted in the 2016–2017 nesting season, as skin has a slow tissue turnover rate relative to blood components and was therefore deemed adequate to reference the diet of the home foraging region of a sampled individual (Allen et al. 2013).

Table 1. Summary data of average (±SD) curved carapace length (CCL) of sampled individual loggerhead turtles identified by sampling cohort (either nesting or foraging turtles), foraging ground location, sex and maturity (SA: subadult, A: adult). STEA: sub-tropical eastern Australia; sGBR (nGBR): southern (northern) Great Barrier Reef; Unk.: unknown

Location	Season	n	Sex	Maturity	CCL (cm)	SD
Nesting						
Unk.[a]	2015–2016	120	F	A	94.7	5.2
	2016–2017	188	F	A	93.7	6.3
STEA		9	F	A	93.6	6.2
sGBR		3	F	A	95.0	4.9
nGBR		3	F	A	95.6	4.6
Foraging						
Moreton Bay		1	F	SA	77.9	–
(STEA)		7		A	92.9	2.9
		3	M	SA	87.7	6.4
		11		A	95.74	4.9
		20	Unk.	SA	79.5	4.9
		3		A	81.8	6.4

[a]Nesting individuals with unknown foraging grounds were sampled from Mon Repos turtle rookery

Additional blood and epidermal tissue samples were collected from 45 foraging turtles in Moreton Bay (southeast Queensland), one of the known major foraging areas for loggerheads of the South Pacific genetic stock (Limpus et al. 1994, Limpus & Limpus 2001), between July 2015 and October 2017. These samples were collected to identify whether baseline isotopic values were observable within one of the potential foraging regions of the sampled nesting cohort. Foraging loggerhead turtles were captured by the rodeo method (Limpus 1978). Upon capture, all turtles were processed following Queensland Turtle Conservation Project protocol (Limpus et al. 1994), and sex and maturity were defined in accordance with the key from Limpus & Limpus (2003) (Table 1).

The locations of nesting individuals with known foraging grounds spanned the east coast of Australia from northern New South Wales (28.9° S) to Torres Strait (10.4° S), covering a distance >2000 km, and was divided into the 3 broad foraging regions: northern Great Barrier Reef (nGBR), southern Great Barrier Reef (sGBR) and sub-tropical eastern Australia (STEA). This encompassed migratory distances of ~100–1200 km to the Woongarra coast nesting beaches. Regions were defined based on the GBR Marine Park Authority's reef health survey regions. The nGBR and sGBR regions are separated by a westward oceanic current from the Coral Sea that bifurcates at ~20° S, the northward branch feeding equatorial currents following the northeast Queensland coastline while the southward current forms the East Australian Current, the westward boundary of the southern Pacific Ocean (Church 1987). Consequently, it was inferred that this bifurcation would contribute to isolation of the 2 foraging regions, with an expected differentiation in isotopic baselines. The STEA region covers the sub-tropical coastline south of the GBR (Fig. 1). As this region of the coast is not influenced by the extensive coral reefs of the GBR, instead being dominated by coastal and estuarine foraging habitats, it was posited that delineation in baseline isotopic values of food items may be influenced by freshwater outflows and/or proximity to anthropogenic carbon and nitrogen sources (Garcia et al. 2007).

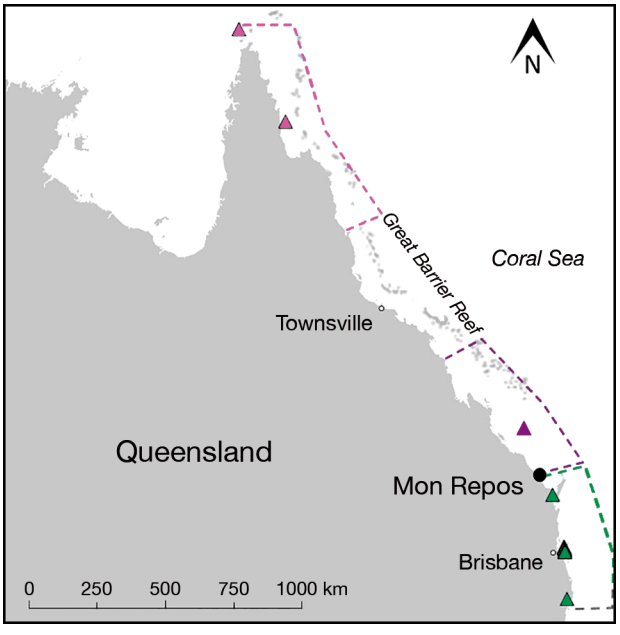

Fig. 1. East coast of Australia, detailing the end points of nesting turtles with known foraging grounds ascertained by satellite telemetry or capture–mark–recapture studies, and the 3 defined foraging regions of the study. The northern Great Barrier Reef (nGBR) region is shown by a dashed pink line (n = 3), southern Great Barrier Reef (sGBR) is purple (n = 3) and sub-tropical eastern Australia (STEA) is shown in green (n = 9). The end points of satellite-tracked nesting turtles are denoted with filled triangles, coloured according to the region within which they are located. Mon Repos, the location for sampling nesting turtles, is identified with a black circle, and Moreton Bay is located immediately adjacent to Brisbane, Queensland

2.2. Sample preparation and analysis

Tissue samples were prepared for SIA following the protocols outlined by Reich et al. (2008). Skin samples were rinsed with distilled water and cleaned with a sterile scalpel blade. Epidermis was separated from the underlying tissue, epiphytic growth was removed, and the epidermis was finely diced. Separated blood components and epidermis samples were then oven dried at 50–60°C for 48–72 h in individual sample tubes. Dried plasma and blood cells were ground with 5 mm glass beads by a Qiagen TissueLyser II. Due to the low lipid content (C:N ratio <3.5), chemical lipid extraction was not performed on the epidermis and blood samples prior to SIA (Post et al. 2007). Following drying, 1.5–2.5 mg of each tissue type were loaded into standard weight tin capsules and analysed using an Elementar Vario Cube elemental analyser coupled with an IsoPrime (Micromass) continuous flow stable isotope ratio mass spectrometer (EA-IRMS). Stable isotope values were expressed using δ notation, defined as parts per thousand (‰):

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

where R_{sample} is the ratio of heavy to light isotopes (^{13}C:^{12}C or ^{15}N:^{14}N) in the sample. $R_{standard}$ is the isotope ratio for the corresponding international stan-

dards, namely atmospheric nitrogen and Vienna PeeDee Belemnite for nitrogen and carbon isotopes, respectively (Peterson & Fry 1987, Wassenaar 2019). Throughout the analysis, technical standards were run every 20th sample with a subsequent precision of ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$.

2.3. Statistical analysis

All statistical analyses were conducted using the R statistical program (v3.4.0) and RStudio user interface (v1.1) (RStudio Team 2016, R Core Team 2017).

Welch's 2 sample *t*-tests investigated whether CCL or isotopic values differed significantly between the sampled seasons to determine whether individuals could be pooled across seasons for further analyses. Additionally, an ANOVA tested for significant differences between CCL and foraging region for individuals with known foraging grounds. Subsequent linear regression analyses considered the hypothesis that $\delta^{13}C$ and $\delta^{15}N$ exhibit latitudinal gradients using the nesting individuals with known foraging locations.

A discriminant functions analysis (DFA) was used to model the between-group differences in $\delta^{13}C$ and $\delta^{15}N$ for turtles that had previously been tracked to their foraging grounds by satellite telemetry or CMR studies. The 'lda' function in the R package 'MASS' (Venables & Ripley 2002) was used to discriminate foraging ground by $\delta^{13}C$ and $\delta^{15}N$ of tracked foragers (nGBR, n = 3; sGBR, n = 3; STEA, n = 9), with equal priors for each group; this formed the 'training' dataset. The resulting model was then used to generate linear discriminant scores for all individuals using the 'predict' function in R (R Core Team 2017). If tracked individuals had isotopic values that discriminate their foraging history, it was expected that the DFA model could separate individuals with unknown foraging grounds into clusters that reflected their predicted foraging grounds (Ceriani et al. 2012). Hence, turtles with unknown foraging grounds represented the 'test' dataset to test the predictive power of the DFA model. A leave-one-out cross validation of the model generated from tracked nesting turtles by satellite telemetry was used to determine the predictability of the subsequent model (Ceriani et al. 2012).

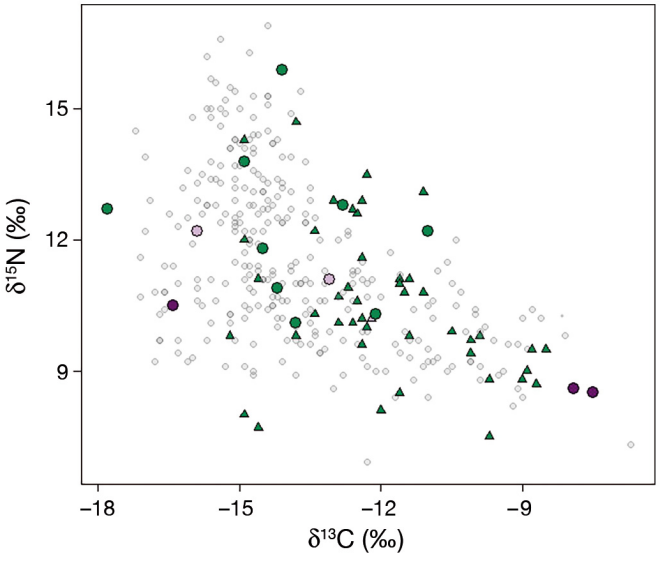

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ values in the epidermis of nesting loggerhead turtles sampled at Mon Repos and foraging loggerhead turtles sampled in southeast Queensland, Australia. Grey circles denote individuals sampled with no previously identified foraging area. Green circles represent turtles with foraging grounds in sub-tropical eastern Australia (STEA) based on satellite telemetry and capture–mark–recapture studies, while green triangles are turtles sampled foraging within Moreton Bay (STEA). Purple circles are turtles that forage in the southern Great Barrier Reef (sGBR) and pink circles represent those foraging in the northern Great Barrier Reef (nGBR) region

3. RESULTS

A Welch's 2 sample *t*-test demonstrated no difference between $\delta^{13}C$ and $\delta^{15}N$ values from epidermal tissue across sampling seasons ($\delta^{13}C$ $t_{261} = -1.09$, p = 0.28; $\delta^{15}N$ $t_{249} = -0.31$, p = 0.76). Similarly, there was no difference in CCL between 2015–2016 and 2016–2017 ($t_{240} = 0.23$, p = 0.82). Hence, samples were pooled across seasons for subsequent analyses.

An ANOVA of the CCL of turtles with known foraging regions found no difference in CCL across the 3 foraging regions ($F_{2, 25} = 0.41$, p = 0.67).

The $\delta^{13}C$ values of epidermis from nesting turtles with known foraging grounds ranged from −17.8 to −7.5‰, while $\delta^{13}C$ values from the sampled nesting cohort with unknown foraging grounds ranged from −17.2 to −6.7‰ (Fig. 2). Values of $\delta^{15}N$ from the sampled cohort with known foraging grounds ranged from 7.7 to 15.9‰, with values for nesting turtles with unknown foraging grounds ranging from 6.9 to 16.9‰ (Fig. 2). Interestingly, turtles from a single site in Moreton Bay (STEA region) encompassed the entire isotopic range of satellite-tracked turtles (−15.2 to −8.5‰ for $\delta^{13}C$ and 7.5 to 14.7‰ for $\delta^{15}N$), identifying within-site variance comparable to between-region variance.

There was no correlation between latitude and $\delta^{13}C$ ($r^2 < 0.01$, $F_{1,12} = 0.04$, p = 0.84) or $\delta^{15}N$ ($r^2 = 0.04$,

$F_{1,12}$ = 0.47, p = 0.51), indicating a lack of latitudinal-related variation in stable isotopic values. Consequently, the DFA model derived from individuals with known foraging locations could not accurately identify foraging region of the nesting cohort. Cross-validation indicated that the training dataset (turtles with known foraging grounds) were only correctly assigned to their respective foraging grounds 60% of the time, suggesting poor regional structure in foraging ground isotopic values.

The sample size used to create the DFA model was small (n = 15), which would limit statistical power. However, given the observation that region-wide satellite-tracked turtles completely overlapped with all turtles from a single site (Moreton Bay, STEA) (Fig. 2), it was concluded that predictive modelling in this system would produce biologically uninterpretable results. Hence, it was not possible to estimate foraging locations of unknown nesting turtles from the Woongarra coast due to the lack of systematic regional variation in stable isotope values, and high variance in isotopic composition of turtles within known foraging locations.

4. DISCUSSION

SIA has successfully identified migratory foraging grounds of loggerhead turtles in the northern hemisphere (Ceriani et al. 2012, Vander Zanden et al. 2015). However, our study demonstrates that $\delta^{13}C$ and $\delta^{15}N$ stable isotopes are unable to inform the location of foraging grounds of loggerhead turtles in eastern Australia. The absence of systematic regional variation and high within-region variance in isotopic values made predicting foraging grounds unreliable. These findings therefore warrant caution in the use of isotopes to infer ecological processes when assumptions of their spatial distribution have not been validated.

4.1. Isotopic values and latitudinal gradients in oceans

Previous studies conducted in the northern hemisphere have established a strong relationship between $\delta^{13}C$ and $\delta^{15}N$ isotopic values of foraging loggerhead turtles and latitude (Ceriani et al. 2012, Allen et al. 2013, Vander Zanden et al. 2015, Bradshaw et al. 2017). In the northern hemisphere, a 2–3‰ depletion in $\delta^{13}C$ is typically associated with the transition from mid- to high latitudes, while enrichment is linked to higher phytoplankton growth rates in regions of upwelling with nutrient-rich waters (Graham et al. 2010). In contrast, Magozzi et al. (2017) found no systematic variation in $\delta^{13}C$ with latitude from 10–28° S in the western Pacific Ocean, a finding consistent with our dataset.

The non-systematic variation of isotopic values in this dataset may be associated with the difference in oceanic currents and benthic topography of the Australian coastline compared to coastlines in the northwest Atlantic Ocean. The waters inhabited by the north-west Atlantic loggerhead population studied by Ceriani et al. (2012) experienced relatively frequent turnover driven by the prevailing oceanic currents of the Gulf Stream, linked to the Gulf of Mexico by the Loop Current and travelling from near the equator northward toward the Arctic (Lee & Mellor 2003, Reverdin et al. 2003). In contrast, the prevailing currents in eastern Australia originate from the Coral Sea, diverging at ~18° S into a northward current heading toward the equator and a southward current, the East Australian Current, that forms the western boundary of the South Pacific (Church 1987). Additionally, within the bounds of the GBR, where loggerhead turtles forage, the immediate coastal waters are separated from the oceanic currents by the extensive coral reef system along the eastern margin of the GBR. Waters within the GBR lagoon are subjected to east–west currents in the form of eddies, which, depending upon the size of the domain, result in turnover/residence times of oceanic water of up to 60 d, greatly influencing water residence and nutrient cycling (Graham et al. 2010, Andutta et al. 2013).

The GBR also has a reduced range of water temperatures (~21–25°C) over a comparative latitudinal range compared to the northwest Atlantic (~16–25°C) (NOAA PSD 2018), and water temperatures are known to influence latitudinal gradients in the $\delta^{13}C$ isotope signal (Graham et al. 2010). Thus, the lack of an identifiable latitudinal gradient in the $\delta^{13}C$ isotope signal along the east coast of Australia may in part be due to the absence of a strong water temperature gradient. While these factors are probably contributors to confounding the outcomes, the major reason for the poor predictability of loggerhead turtle foraging regions based on tissue stable isotope values is likely associated with the large variation in diet, and the fact that the variation in $\delta^{13}C$ and $\delta^{15}N$ from a single region can encompass almost the entire range in variation found along the east coast of Australia.

4.2. Influence of diet and foraging environment on isotopic values

The successful identification of foraging regions using SIA relies not just upon the presence of regionally systematic variation in stable isotope values, but also upon the diet of a sampled individual being reflective of a specific region and being relatively constant over time. Loggerhead turtles in eastern Australian coastal waters are known to prey upon a variety of benthic prey species, including, but not limited to, plankton-filtering bivalves, algal-grazing gastropods, scavenging crustaceans, predatory invertebrates and discarded bycatch from trawl fisheries. In contrast to green turtles, whose diets are readily determinable by distinct isotopic ranges in consumed autotrophic groups (i.e. red algae, seagrass, mangrove etc.) (Thomson et al. 2018), the prey items from which loggerheads potentially source their baseline stable isotope values come from a variety of different primary producers (algae, seagrasses and phytoplankton) (Limpus 1985, Limpus et al. 2001, 2013, Limpus & Coffee 2019). Such varied diets in east Australian loggerheads might explain the wide range in the isotopic signal within tissues from individuals sampled from the same foraging region. Variation in stable isotope signal can also be increased by inherent variation within prey items. For example, in Moreton Bay (STEA), loggerhead turtles frequently feed on crustaceans such as mud crabs *Scylla serrata*. The stable isotope values of mud crabs in Moreton Bay vary in relation to their proximity to seagrass (Connolly & Waltham 2015), highlighting the fact that within a foraging area, variation may be observed in different prey items of the same species. Variation in baseline $\delta^{13}C$ and $\delta^{15}N$ isotopic values have also been linked to anthropogenic sources, including the influence of runoff from agricultural practices (Hobson 2019). The foraging regions for nesting loggerheads on the east coast of Australia (e.g. throughout the GBR and sub-tropical southeast Queensland) are in proximity to major freshwater outflows influenced by agricultural runoff, potentially resulting in sporadic increases in baseline isotopic variation associated with carbon and nitrogen cycles. These factors, taken together, along with the observation that the range in variation found in a single foraging area, Moreton Bay, encompasses almost the entire range of values found in nesting loggerhead turtles from the Woongarra coast, means that the epidermis samples from the nesting loggerhead turtles cannot be used to differentiate between different foraging regions with any confidence.

4.3. Predictive models using isotopic values

The observed ranges of $\delta^{13}C$ and $\delta^{15}N$ in loggerhead turtles foraging within Moreton Bay covered almost the entire range of isotopic values from all sampled nesting turtles. Had a DFA been performed without extensive sampling of turtles known to forage in Moreton Bay, we might have inferred weak, but latitudinally uncorrelated, regional structuring in isotopic values. However, given that almost the entire range of stable isotope values from nesting turtles sampled throughout the 2000 km north to south distribution could be encompassed by the range of isotopic values from Moreton Bay, different regions could not be reliably assigned based on stable isotope values alone. The results of this study are therefore in contrast to previous works from the northern hemisphere (Ceriani et al. 2012, Vander Zanden et al. 2015) that identified significant among-region differences in isotopic signals, following a latitudinal gradient. Strong isotopic structuring in the west Atlantic studies provided effective training for a predictive modelling to then assign foraging regions to turtles with unknown foraging locations. These conflicting observations highlight the need to establish the presence of systematic regional variation in isotopic values before attempting to identify foraging regions with predictive modelling. The use of additional tracers, such as $\delta^{34}S$ or $\delta^{18}O$ may assist in the differentiation between foraging regions (Haywood et al. 2019). For example, in contrast to the results of this study, Pearson et al. (2019) successfully distinguished the foraging areas of nesting individuals (>400 km apart) from the South Pacific loggerhead nesting stock using $\delta^{18}O$ and $\delta^{13}C$ isotope gradients in the growth layers of commensal barnacles. Similarly, $\delta^{34}S$ isotope, which can vary with proximity to shore (Hobson 2019), when used in tandem with $\delta^{13}C$ and $\delta^{15}N$, has been used to determine the foraging regions of green turtles nesting in the Mediterranean Sea (Bradshaw et al. 2017). However, given the isotopic variation observed within a single foraging habitat in the analysed dataset, it is recommended that future studies continue to ground truth with satellite telemetry and/or CMR data until stable isoscapes have been resolved (Tucker et al. 2014, Haywood et al. 2019).

4.4. Conclusions

This study has demonstrated that SIA, using carbon and nitrogen biogeochemical tracers, cannot reliably identify foraging regions of loggerhead turtles

along the east coast of Australia. While SIA has been used successfully in the northern hemisphere, the results from the present study indicate that for reasons not completely understood, there is no systematic regional variation in stable isotope values of carbon and nitrogen. Different oceanic currents, temperature regimes and river run-off likely prevent the establishment of region-specific unique isoscapes, a condition needed to be able to identify the foraging region of turtles based on their isotopic values. As a consequence, the $\delta^{13}C$ and $\delta^{15}N$ isotopic values from sampled tissues of loggerheads from the east coast of Australia do not present predictable latitudinal variation as reported for foraging populations located in the northern hemisphere (Ceriani et al. 2012, 2017, Vander Zanden et al. 2015). Hence, caution must be exercised when conducting predictive analyses of stable isotope data with the purpose of identifying foraging grounds of migratory species, particularly when samples sizes are small in the training dataset, or the assumptions of regional structuring of isotopic values have not been verified.

Acknowledgements. Funding for this work was provided by a Sea World Research and Rescue Foundation grant. We acknowledge the traditional owners of the lands on which this research was conducted (Gurang, Gooreng Gooreng, Bailai, Bunda and Quandamooka). We thank the Queensland Government Department of Environment and Science Aquatic Threatened Species Program for logistical support in the field and the donation of samples from loggerheads from Moreton Bay. Thanks also to the volunteers and field assistants S. Chaousis, S. Doell, E. Dover, E. Duncan, K. Finlayson, D. Limpus, J. Limpus and R. Pearson for their assistance in sample collection and preparation for analysis; K. Baublys and W. Zhou of the Stable Isotope Geochemistry Laboratory, Earth and Environmental Sciences at The University of Queensland for their support in SIA; and S. Blomberg for statistical advice. Sampling and handling of loggerhead turtles was approved by the Queensland Department of Environment and Heritage Protection (Scientific Purposes Permit WITK15830215) and the University of Queensland Animal Ethics Committee (Approval No.: SBS/017/15/SRRF/URG).

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Editorial responsibility: Graeme Hays, Burwood, Victoria, Australia

Submitted: March 11, 2019; Accepted: October 29, 2019
Proofs received from author(s): November 27, 2019