Site fidelity, ontogenetic shift and diet composition of green turtles *Chelonia mydas* in Japan inferred from stable isotope analysis

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ABSTRACT: Incomplete knowledge about local foraging ecology of green turtles hampers their conservation management in Japan, where stocks have only partially recovered from heavy exploitation in previous centuries. We used stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ for turtle carapace scutes, where successive layers contain a chronological record of diet assimilated over a period of years. Turtles were sampled at 2 geographically separate foraging grounds in Japan: the temperate Main Islands (n = 32) and the sub-tropical Nansei Islands (n = 42). Site fidelity was inferred for the majority of turtles at each site (81 and 64% resident turtles) because isotope data indicated diets consistent with food taxa at the respective sites. Immigrant turtles (previous diet outside their current site) were few (n = 4) at the Main Islands site but numerous (n = 14) at the Nansei Islands site, where they were significantly smaller than residents. An ontogenetic shift (Main Islands to Nansei Islands) was inferred for many of the immigrants on the basis of isotope evidence and body size. These immigrants corresponded to a size cohort that was relatively scarce in Main Islands foraging grounds according to previous studies. Bayesian mixing models, used to estimate proportional components of diet, showed varying degrees of imbalance between seagrass and algae and indicated that hypothetical consumption of non-trivial amounts of animal matter was plausible. The latter represented a hypothetical diet component for study turtles since animal matter was rarely found in stomach contents. Potential ambiguity and other issues that constrained inference from mixing models are discussed.

KEY WORDS: Green Turtle · Foraging site fidelity · Ontogenetic shift · Diet composition · Stable isotope analysis · $\delta^{13}C$ · $\delta^{15}N$ · *Chelonia mydas*

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INTRODUCTION

Green turtles *Chelonia mydas* are vulnerable or endangered throughout their range (Seminoff 2004, Wallace et al. 2011), and their global decline is largely due to anthropogenic activities (Jackson et al. 2001, Frazier 2003). In Japan, the Ministry of Environment

(MOE) lists green turtles as a vulnerable species (MOE 2012), but stocks were heavily exploited in previous centuries, particularly in Ogasawara during the 1800s. Up to 3000 turtles were harvested per year in peak periods, but the annual catch had declined to approximately 100 individuals in 1930 (Sato 1989). Despite recent increases in the Ogasawara nesting

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population, the Japanese stock remains below historical abundance and requires continuous conservation management, as is the case for depleted stocks in other parts of the world (Suganuma 1998, Chaloupka et al. 2008, Kittinger et al. 2013).

Understanding the foraging ecology of green turtles is essential for the development of successful conservation and management programmes (Bjorndal 1999, Martin et al. 2007, Hamann et al. 2010), but the task is challenging because both movement and diet of the species vary between ontogenetic stages. Initially, green turtle hatchlings migrate from their natal beaches to oceanic habitats where evidence suggests they drift passively with ocean currents and consume an omnivorous diet (Balazs 1976, Wyneken & Salmon 1992, Boyle & Limpus 2008, Mansfield et al. 2014). After several years, small juveniles enter neritic habitats (Bjorndal 1997, Reich et al. 2007, Arthur et al. 2008) where, in general, green turtles spend the major part of their lives and consume a diet that is predominantly, but not exclusively, herbivorous (Bjorndal 1997). After reaching maturity, most green turtles migrate at irregular intervals between their foraging grounds and distant nesting sites, with female turtles exhibiting high site fidelity over many years (Limpus et al. 1992, Balazs 1994, Miller 1997).

The post-oceanic ecology of green turtles appears to vary widely among geographic regions. For example, in the western North Atlantic, juvenile green turtles are understood to shift from temperate and subtropical habitats to the tropical Caribbean (the natal region for many of them) based on studies of size distribution (e.g. Mendonca & Ehrhart 1982, Bjorndal & Bolten 1988, Epperly et al. 1995, Meylan et al. 2011), mark-recapture (Bjorndal & Bolten 1995, Moncada et al. 2006, Meylan et al. 2011) and genetic analysis (Lahanas et al. 1998, Bass & Witzell 2000). In the western South Atlantic, some juvenile green turtles make seasonal shifts between neritic and oceanic habitats, as revealed by satellite telemetry (González Carman et al. 2012, 2014). However, similar shifts have not been reported for green turtles in the western Pacific, and long-term fidelity to neritic foraging sites has been confirmed in eastern Australia by mark-recapture studies (Limpus et al. 1994, Chaloupka et al. 2004).

In Japan, green turtle breeding sites are restricted to the Ogasawara and Nansei Islands (Kamezaki 1989, Yamaguchi et al. 2005), while foraging grounds exist both in these remote island groups and along the coast of the Main Islands (Uchida & Nishiwaki 1982, Ogasawara Marine Center 1994, Shimada 2008) (Fig. 1). Natal origins of adult and juvenile green tur-

tles in Japan have been elucidated through mark-recapture, satellite telemetry and genetic analysis. Turtles foraging at Main Islands sites originated predominantly from the Ogasawara Islands, with limited records from Taiwan and Micronesia (Tachikawa & Sasaki 1990, Ogasawara Marine Center 1994, Cheng 2000, Miyawaki et al. 2000, Hatase et al. 2006, Nishizawa et al. 2013). Turtles foraging in the Nansei Islands had diverse origins in the western Pacific and Indian Oceans (Ogasawara Marine Center 1994, Cheng 2000, Song et al. 2002, Hamabata et al. 2009, Nishizawa et al. 2013). In contrast to detailed scientific insight regarding natal origins, fidelity to foraging sites, possible ontogenetic shifts and variation in diet remain obscure for Japanese green turtles. Our study addresses this knowledge gap.

Green turtle foraging aggregations in the Main Islands and the Nansei Islands encompass the size range from small juveniles (straight carapace length [SCL] ~40 cm) to adults (SCL > 82 cm) (e.g. Okamoto et al. 2011 and references therein), but measurements reported in earlier studies imply a disparity in size distribution. Juvenile turtles of intermediate size (approximate SCL range: 50 to 70 cm) were notably under-represented at Main Islands sites (e.g. Okamoto et al. 2011 and references therein), whereas turtles in this size range were relatively abundant at southern Nansei Islands sites (Kameda et al. 2013).
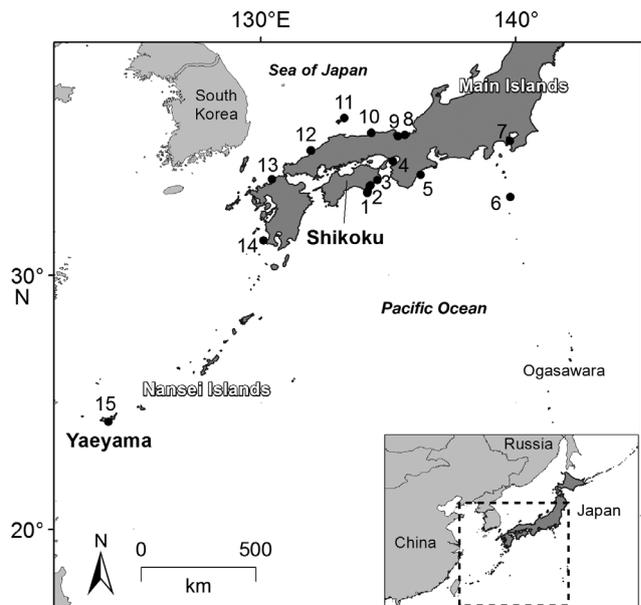

Fig. 1. *Chelonia mydas.* Green turtles were sampled at 2 geographically separate foraging grounds in Japan: southeastern Shikoku and Yaeyama. Turtle food sources were sampled at 15 locations (●). Dashed-line rectangle in the regional map (inset, lower right) shows the geographic extent of the detailed map

We surmised that the under-represented size cohort at Main Islands foraging grounds could indicate an ontogenetic shift by juvenile turtles of intermediate size moving away from the Main Islands, with the southern Nansei Islands a plausible successive foraging habitat.

As a tool for investigating residency at these 2 geographically separate foraging grounds and possible movement between them, we turned to stable isotope analysis. The rationale underlying this method is well established: stable isotope ratios in animal tissue reflect the stable isotope ratios of their food sources (Fry 2006). Correspondence of isotope ratios between animal tissue and known food sources at a locality can be used to infer residence. Similarly, movement can be inferred when tissue isotopes differ from those of local food sources. In addition, mathematical mixing models can be used to estimate the proportions of various food sources that have contributed to tissue formation (Fry 2006).

In our study, mixing models would allow us to estimate major components of green turtle diets from the isotope ratios of their tissues. Prior studies have found gut contents of Main Islands turtles were dominated by macroalgae, whereas both macroalgae and seagrass were found in the gut contents of turtles at the Nansei Islands (Kameda & Ishihara 2009, Suganuma et al. 2010). It has been inferred that each individual turtle forages preferentially either on macroalgae or on seagrass (Sea Turtle Association of Japan, STAJ, unpubl. data), but this understanding is based on a 'snapshot' view of diet provided by gut contents. Insight regarding the relative importance of assimilated macroalgae and seagrass might be gained from stable isotopes that reflect diet over a longer period.

In addition, we wanted to investigate the possible consumption of animal matter by green turtles in both foraging regions of Japan. Prior studies using gut contents analysis have reported only a minor component of animal matter, e.g. <5.5% animal matter by volume (Kameda & Ishihara 2009). However, inference from the stable isotope ratios of green turtle eggs suggested that some nesting females in the Ogasawara Islands consumed a substantial proportion of invertebrates prior to breeding migration (Hatase et al. 2006). Consumption of animal matter by green turtles in other parts of the world has been confirmed by direct observation of episodic ingestion or inferred from stable isotope data or both (Heithaus et al. 2002, Seminoff et al. 2006b, Amorocho & Reina 2007, Arthur et al. 2007, Cardona et al. 2010, Burkholder et al. 2011, Lemons et al. 2011).

In summary, our primary objective was to measure stable isotope ratios of green turtle scute tissue, compare the tissue data with stable isotope ratios of local food sources and, hence, infer either residence in the capture area or immigration from a different area. In addition, we wanted to use mixing models to estimate (a) the relative contributions of seagrass and macroalgae and (b) the hypothetical inclusion of animal matter in the diets of study turtles.

MATERIALS AND METHODS

To allow comparison between 2 geographically separate green turtle foraging regions in Japan, our study sites were located (1) at south-eastern Shikoku (Shikoku hereafter) in the Main Islands and (2) at Yaeyama in the Nansei Islands group (Fig. 1). Green turtles were sampled between May 2010 and November 2011.

Tissue and food samples

Study turtles comprised stranded animals, turtles accidentally caught in coastal fisheries (5 to 78 m depth), and turtles caught for research using entanglement nets (stretched mesh size = 45 cm, water depth ≤ 2 m). SCL was measured between the nuchal notch and posterior tip of the carapace (Uchida 1967). Turtles with SCL > 82 cm were considered to be adult size, following data for nesting turtles (Tachikawa 1991).

Turtle tissue was sampled from the carapace because chronological changes in diet can be detected by measuring the isotope ratios of successive layers of carapace scutes (Reich et al. 2007). We sampled carapace tissue using a sterile cutter to remove approximately 1 cm^2 from anterior and posterior sites on the second costal scute (Fig. 2) according to the method of Reich et al. (2007) and based on the pattern of scute formation (Kobayashi 2001). For a small subset of turtles we also obtained skin tissue samples (approximately 1 cm^2) from the shoulder area. The latter were used to derive an adjustment to discrimination values as described in the Supplement at www.int-res.com/articles/suppl/n025p151_supp.pdf.

We assembled food samples from multiple sources. Field samples were collected at coastal sites up to 3 m depth (Fig. 1: Locations 1–3, 7, 9, 15). We collected only those taxa known to be eaten by green turtles in Japan, based on identification of stomach contents in
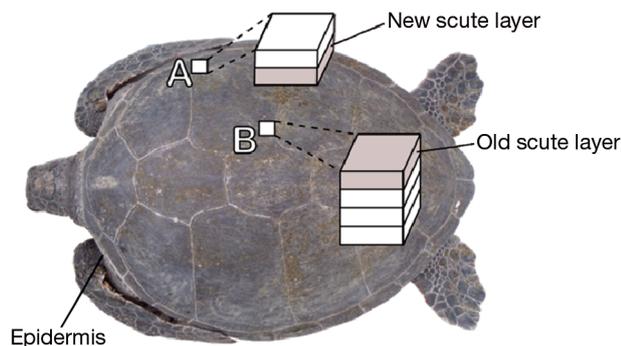
Fig. 2. *Chelonia mydas*. Carapace tissue was sampled at anterior (A) and posterior (B) positions on the second costal scute. Stable isotope ratios were measured for newly synthesized tissue from the basal layer at A and the oldest retained tissue from the surface layer at B, in order to infer the recent and previous diet of the turtle. Skin tissue (epidermis) was sampled at the shoulder area. Figure adapted from Reich et al. (2007)

prior research (Kameda & Ishihara 2009). Ingested food samples were obtained from live turtles by stomach lavage (Forbes & Limpus 1993) and from freshly dead turtles by dissection. Ingested items were identified to the lowest possible taxonomic level using standard keys (Yoshida 1998). Macroalgal and seagrass samples were subsequently prepared for stable isotope analysis as detailed in the next section. Animal matter in stomach contents was not used in analysis because it was found only in rare instances, and because animal matter may be altered in the stomachs of some animals (Hwang et al. 2007). Digestion of plant cell walls occurs in the hindgut of green turtles (Bjorndal et al. 1991). Therefore, isotope ratios of macroalgae and seagrass were not expected to be altered in turtle stomachs. To validate this expectation, stable isotope ratios of stomach samples and field samples were compared statistically. Finally, we obtained data from prior studies for potential food sources (Takai et al. 2001, Hatase et al. 2002, 2006).

Stable isotope samples

All samples were stored frozen (−18°C) prior to preparation. Surface contamination was removed by careful scrubbing and rinsing in distilled water. A portion of each sample was tested in hydrochloric acid solution (1 N HCL; Longin 1971). Absence of bubbles confirmed absence of calcium carbonates in every case. Therefore, the remaining portions were deemed suitable for stable isotope analysis without

pre-treatment. Acid-treated portions were not used in analysis.

Scute material was mounted horizontally on a wooden block with epoxy glue to facilitate the removal of very thin layers (50 μm) with a microtome. From each scute we obtained separate samples of (1) recently synthesized tissue from the basal layer of the anterior site (A in Fig. 2), hereafter termed 'new' scute tissue, from which 'recent' diet was inferred, and (2) 'old' tissue from the surface layer of the posterior site (B in Fig. 2), from which 'previous' diet was inferred. All samples were pulverized in a mill homogenizer, treated for lipid extraction with a 2:1 mixture of chloroform and methanol (Folch et al. 1957) and dried at 60°C for 24 h.

Elemental analysis provided carbon and nitrogen isotope ratios $\delta^{13}C$ and $\delta^{15}N$, reported as relative deviation (‰) from the conventional standards Pee-Dee Belemnite and atmospheric nitrogen, respectively (Fry 2006). Laboratory-calibrated alanine samples were inserted after every 10 research samples for control. Standard deviation of the alanine samples was below ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, confirming the precision of analysis. For all analyses the $\delta^{13}C$ and $\delta^{15}N$ data were corrected for diet–tissue discrimination. We estimated discrimination values for scute tissue by applying an empirically derived adjustment to published discrimination values for skin tissue (Seminoff et al. 2006a). Details of the adjustment are available in (Table S1 in the Supplement at www.int-res.com/articles/suppl/n025p151_supp.pdf).

Stable isotope analysis

The first stage of our analysis considered only confirmed food sources (taxa identified in samples of ingested material from green turtles in Japan). These were represented by samples collected at our study sites, Shikoku and Yaeyama. Hereafter we refer to these samples collectively as 'known food'. The isotopic boundary of known food was defined for each study site by a minimum convex polygon enclosing the isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of food samples with a buffer of width equal to the precision of our elemental analysis (0.2‰). We then evaluated scute data for each turtle in relation to the boundary at the relevant capture site. When a scute sample had isotope ratios within the boundary, a diet consistent with known food at the site was inferred. Conversely, when a scute sample had isotope ratios beyond the boundary, a diet sourced outside the site was inferred.

Study turtles were then classified according to their inferred diet as follows: (1) If both new and old scute samples indicated a diet consistent with known food at the site, the turtle was classified as 'resident'. (2) If the old scute sample indicated a diet obtained outside the site, the turtle was classified as 'immigrant'. For the latter, the new scute could fall inside or outside the boundary, allowing for diverse durations since recruitment to the study site. (3) If the old scute indicated diet within the site and the new scute indicated diet outside the site, the turtle was classified as 'nomad'.

In the next stage of analysis, we evaluated the relative importance of macroalgae and seagrass for Yaeyama resident turtles through the use of mixing models as detailed below. Food sources used in this analysis were the known food for Yaeyama. This analysis excluded other turtles because seagrass was rare at Shikoku and food availability was unknown for immigrants and nomads.

Finally, we considered the potential dietary contribution of animal food such as gelatinous and soft-bodied invertebrates, although such items were rarely identified in ingested samples. In addition to the data for known food, we needed proxy data to represent this hypothetical diet component. We used isotope ratios for macroplanktonic invertebrates from prior studies in which these invertebrates represented potential prey of chelonians in Japanese waters (Hatase et al. 2002, 2006). These data for invertebrates were the most appropriate proxy data available for our purpose, although the samples had been obtained at a location distant from our study sites. The sampling area was off the Sanriku coast of the Main Islands of Japan (Hatase et al. 2002, 2006).

We ran Bayesian mixing models for diet source contribution in SIAR (Stable Isotope Analysis in R; Parnell & Jackson 2013). For each stage of our mixing analysis, the SIAR model, fit via Markov Chain Monte Carlo, was run for 500 000 iterations with a burn-in of 50 000. The model produced probability density distributions of potential dietary solutions (Parnell et al. 2010). We confirmed uni-modality of the distributions and thus used the median values to estimate the most probable contribution of each food source to the diet of each turtle (Moore & Semmens 2008). The 2-sample t-test was used for parametric data, and the Wilcoxon rank-sum test was used for non-parametric data. Shapiro-Wilk test and Levene's test were used to examine normality and homogeneity of variance. All statistical analyses were conducted using R software (R Core Team 2013).

RESULTS

We sampled a total of 74 green turtles comprising 32 turtles at Shikoku (SCL = 37.6 to 91.3 cm, median = 45.5 cm) and 42 turtles at Yaeyama (SCL = 42.5 to 83.0 cm, median = 53.5 cm). For each individual we obtained $\delta^{13}C$ and $\delta^{15}N$ values for new and old scute tissue (Table 1). We compiled $\delta^{13}C$ and $\delta^{15}N$ values of green turtle food sources sampled at our 2 study sites and at other Main Island sites (Tables 2 & 3). Use of stable isotope values from ingested samples (from stomach contents) was validated by t-tests that showed no significant difference between stomach and field samples of the dominant species *Gelidium elegans* ($\delta^{13}C$ values $t_{(2)10} = -1.23$, p = 0.25; $\delta^{15}N$ values $t_{(2)10} = 1.04$, p = 0.32).

New scute layers of Shikoku turtles were more depleted in ^{13}C and enriched in ^{15}N as compared with the corresponding measures for turtles at Yaeyama (Fig. 3, Table 1). Differences were statistically significant for $\delta^{13}C$ values ($W = 19$, p < 0.0001) and for $\delta^{15}N$ values ($t_{(2)72} = 9.90$, p < 0.0001), indicating diets differentiated by site. The majority of turtles were classified as resident at their respective sites, by inference from stable isotope ratios indicating previous and recent diet consistent with known food for the site: Shikoku, n = 26 (81%); Yaeyama, n = 27 (64%) (Fig. 3, Table 1).

Immigrants (previous diet outside the site) were numerous at Yaeyama (n = 14, 33%) and were significantly smaller than residents ($W = 290.5$, p < 0.01). In contrast, Shikoku had few immigrants (n = 4, 13%), and these were not significantly different in size to residents ($W = 55.5$, p = 0.85). Nomads (Shikoku, n = 2; Yaeyama, n = 1) were too few for statistical evaluation and were excluded from further analysis (Fig. 3, Table 1).

The relative importance of seagrass and macroalgae was assessed for Yaeyama residents, the only study turtles for which both food sources could be assumed continuously available. Mixing model results are summarized here as old%|new% (based on isotope data for old and new scutes) to indicate estimates for the proportion of a food source in the previous and recent diet, respectively. Macroalgae appeared to be more important for 5 turtles (85%|87%; 80%|81%; 80%|71%; 93%|76%; 83%|62%), while seagrass appeared more important for 3 turtles (88%|90%; 86%|82%: 76%|81%). For the remainder, estimates were lower and varied. For 14 turtles both estimates (previous and recent) were >50% macroalgae, while for 3 turtles both estimates were >50% seagrass. Two turtles had an estimated previous diet

Table 1. *Chelonia mydas*. Stable isotope ratios (δ^{13}C, δ^{15}N) of green turtles sampled at 2 study sites, Shikoku and Yaeyama. SCL: straight carapace length. Status was inferred from stable isotope data — R: resident; I: immigrant; N: nomad

Turtle ID	SCL (cm)	New scute layer δ^{13}C (‰)	New scute layer δ^{15}N (‰)	Old scute layer δ^{13}C (‰)	Old scute layer δ^{15}N (‰)	Inferred status	Turtle ID	SCL (cm)	New scute layer δ^{13}C (‰)	New scute layer δ^{15}N (‰)	Old scute layer δ^{13}C (‰)	Old scute layer δ^{15}N (‰)	Inferred status
Shikoku							Y05	43.3	−14.8	7.4	−15.3	6.1	R
S01	37.6	−17.6	9.1	−18.1	6.9	R	Y06	43.7	−15.3	7.4	−18.5	6.0	R
S02	37.7	−17.5	9.8	−18.3	5.0	I	Y07	44.6	−16.0	8.8	−18.7	6.1	N
S03	39.3	−17.7	9.2	−20.0	7.9	R	Y08	44.8	−15.3	7.3	−17.6	7.2	I
S04	39.3	−18.5	7.1	−19.8	7.4	R	Y09	45.6	−12.7	7.1	−16.4	11.0	I
S05	39.4	−18.6	9.5	−19.4	9.1	R	Y10	46.4	−9.9	4.1	−9.1	4.4	R
S06	40.3	−19.1	9.0	−19.3	8.5	R	Y11	47.8	−14.1	7.0	−18.9	10.0	I
S07	40.6	−18.6	9.3	−18.7	8.0	R	Y12	48.1	−14.2	7.8	−15.4	7.7	R
S08	40.6	−17.8	8.4	−17.6	9.3	R	Y13	49.0	−14.4	6.6	−15.0	6.2	R
S09	40.9	−18.4	8.7	−19.6	7.8	R	Y14	49.3	−14.0	7.5	−14.2	8.3	I
S10	41.1	−18.5	7.8	−19.3	8.6	R	Y15	50.2	−13.2	6.2	−16.6	13.9	I
S11	41.6	−19.0	8.5	−19.3	7.7	R	Y16	50.6	−13.4	5.4	−13.2	6.1	R
S12	42.0	−18.7	9.4	−18.1	5.8	I	Y17	51.3	−14.3	6.1	−15.5	9.2	I
S13	42.0	−18.5	8.0	−18.3	6.2	R	Y18	51.6	−11.4	6.3	−13.1	6.7	R
S14	42.4	−18.4	8.6	−19.3	8.4	R	Y19	52.0	−14.1	6.4	−19.3	8.4	I
S15	42.5	−19.1	8.1	−19.7	9.6	R	Y20	52.6	−13.5	5.5	−15.7	6.4	R
S16	43.6	−19.1	8.4	−18.9	7.8	R	Y21	52.6	−16.5	6.9	−16.3	7.4	R
S17	47.4	−19.3	9.4	−19.4	8.6	R	Y22	54.3	−14.7	7.3	−14.1	6.5	R
S18	50.8	−18.9	9.2	−19.1	10.0	R	Y23	54.4	−10.7	5.1	−12.4	5.2	R
S19	59.1	−17.8	10.9	−18.4	10.6	R	Y24	55.5	−13.3	5.9	−13.5	5.9	R
S20	63.3	−18.3	10.5	−17.6	12.1	I	Y25	55.8	−14.4	7.0	−13.8	7.1	R
S21	64.3	−18.2	9.2	−17.5	10.0	R	Y26	56.1	−15.0	6.9	−15.6	8.9	I
S22	68.6	−16.3	8.3	−16.0	8.5	R	Y27	56.5	−15.5	6.7	−15.7	7.3	R
S23	74.7	−17.1	7.7	−16.9	7.5	R	Y28	57.2	−9.8	4.1	−13.8	6.1	R
S24	75.9	−22.2	7.7	−23.5	8.0	I	Y29	57.4	−13.5	5.8	−14.7	5.2	R
S25	77.6	−19.0	9.5	−18.6	10.2	R	Y30	57.7	−8.4	4.4	−8.9	4.4	R
S26	77.7	−18.4	7.7	−17.7	9.1	R	Y31	58.6	−18.3	5.0	−25.8	5.1	I
S27	78.7	−19.3	8.9	−18.0	9.0	R	Y32	59.9	−12.8	6.7	−13.8	6.7	R
S28	81.7	−17.4	11.7	−16.3	10.4	N	Y33	61.3	−14.5	7.2	−13.5	6.8	R
S29	88.0	−17.8	8.8	−17.6	9.2	R	Y34	62.7	−12.1	6.1	−13.0	6.6	R
S30	89.1	−16.1	9.2	−16.5	8.6	R	Y35	62.9	−9.5	5.3	−11.2	5.8	R
S31	90.3	−18.3	12.2	−19.0	11.2	N	Y36	63.9	−16.5	5.8	−13.3	4.8	R
S32	91.3	−17.1	10.4	−17.5	9.6	R	Y37	67.0	−9.1	5.5	−9.7	5.2	R
Yaeyama							Y38	70.5	−8.4	6.1	−11.1	7.0	R
Y01	42.5	−13.9	8.7	−19.8	8.1	I	Y39	71.3	−15.0	7.5	−13.2	6.5	R
Y02	42.7	−14.5	7.0	−18.7	7.6	I	Y40	72.5	−15.1	7.7	−13.4	7.8	R
Y03	42.9	−16.4	6.1	−18.3	10.6	I	Y41	73.2	−13.3	7.0	−15.2	7.8	R
Y04	43.3	−15.3	7.2	−18.9	6.9	I	Y42	83.0	−7.6	6.8	−6.3	5.8	I

Table 2. Stable isotope ratios (δ^{13}C, δ^{15}N) of food source taxa sampled from field sites and turtle stomachs. Means (±SD) provided where multiple samples were available. n: number of samples; SCL: straight carapace length. Location numbers correspond to sampling sites marked in Fig. 1

Taxon	n	δ^{13}C (‰)	δ^{15}N (‰)	Location	Sampling method	SCL (cm)
Shikoku macroalgae[a]						
Gelidium elegans	1	−16.9	6.4	1. Muroto	Stomach	43.3
Gelidium elegans	1	−16.9	6.5	1. Muroto	Stomach	54.3
Gelidium elegans	1	−14.5	5.7	1. Muroto	Stomach	39.3
Unidentified species	1	−22.0	7.8	1. Muroto	Stomach	43.6
Gelidium elegans	1	−15.4	5.5	1. Muroto	Field	–
Codium sp.	1	−17.6	4.0	1. Muroto	Stomach	74.7
Halymeniaceae	1	−17.6	4.2	1. Muroto	Stomach	74.7
Gelidium elegans	1	−17.5	6.0	1. Muroto	Stomach	74.7
Gelidium elegans	1	−17.4	6.0	1. Muroto	Stomach	47.4

Table 2 (continued)

Taxon	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Location	Sampling method	SCL (cm)
Halymeniaceae	1	−13.5	8.1	1. Muroto	Stomach	47.4
Prionitis angusta	1	−13.0	8.3	1. Muroto	Stomach	47.4
Meristotheca sp.	1	−21.6	4.5	1. Muroto	Field	−
Gelidium elegans	1	−15.3	5.5	1. Muroto	Field	−
Codium intricatum	1	−12.9	5.6	1. Muroto	Field	−
Gigartinales	1	−17.2	7.4	1. Muroto	Stomach	40.9
Gelidium elegans	1	−16.5	6.5	1. Muroto	Stomach	40.9
Codium sp.	1	−15.7	7.5	1. Muroto	Stomach	40.9
Gelidium sp.	1	−14.6	6.2	1. Muroto	Stomach	40.9
Gelidium sp.	1	−18.5	5.5	1. Muroto	Stomach	68.6
Halymeniaceae	1	−17.1	4.2	1. Muroto	Stomach	68.6
Unidentified species	1	−16.0	8.0	1. Muroto	Stomach	81.7
Unidentified species	1	−15.2	7.2	1. Muroto	Stomach	81.7
Gelidium elegans	1	−16.8	7.2	1. Muroto	Field	−
Gelidium elegans	1	−16.1	6.9	1. Muroto	Field	−
Gelidium sp.	1	−18.8	6.0	2. Shishikui−ikumi	Stomach	37.3
Gelidium elegans	1	−17.4	6.2	2. Shishikui−ikumi	Field	−
Chondrus ocellatus	1	−17.0	8.5	3. Yuki	Field	−
Gelidium elegans	1	−13.5	9.2	3. Yuki	Field	−
Main Islands 'city-bay' macroalgae						
Gelidium elegans	1	−14.5	9.6	4. Osaka	Stomach	76.5
Chondrus giganteus	1	−16.6	9.3	7. Futtsu	Field	−
Sargassum fusiforme	1	−14.0	9.7	7. Futtsu	Field	−
Multiple species[c]	43	−16.0 ± 2.3	9.0 ± 1.3	Hiroshima	Field/trawl	−
Main Islands macroalgae (excluding Shikoku and 'city-bay' macroalgae)						
Gelidium elegans	1	−17.2	5.3	5. Shimakatsu	Stomach	40.2
Chondrus giganteus	1	−16.2	5.9	5. Shimakatsu	Stomach	45.6
Codium latum	1	−15.1	5.9	5. Shimakatsu	Stomach	45.6
Gelidium elegans	1	−15.9	6.7	5. Shimakatsu	Stomach	45.6
Chondrus ocellatus	1	−19.9	5.0	5. Shimakatsu	Stomach	46.4
Gelidium elegans	1	−16.1	4.4	6. Hachijo	Stomach	99.0
Gelidium elegans	1	−21.1	5.4	8. Ohi	Stomach	49.7
Gelidium elegans	5	−16.5 ± 1.2	4.6 ± 0.3	9. Maizuru	Field	−
Gelidium elegans	5	−16.8 ± 1.3	5.6 ± 1.6	9. Maizuru	Field	−
Gelidium linoides	1	−19.4	5.2	10. Tottori	Stomach	43.7
Coccophora langsdorfii	1	−17.5	6.8	11. Okinoshima	Stomach	71.6
Codium fragile	1	−15.8	5.0	11. Okinoshima	Stomach	71.6
Prionitis angusta	1	−16.4	5.6	11. Okinoshima	Stomach	71.6
Sargassum macrocarpum	1	−15.1	6.3	11. Okinoshima	Stomach	71.6
Gelidium elegans	1	−16.3	7.1	12. Shimane	Stomach	−
Gelidium elegans	1	−18.9	7.9	13. Tsuyazaki	Stomach	−
Gelidium elegans	1	−15.7	5.3	14. Nomaike	Stomach	78.7
Yaeyama macroalgae[a]						
Gelidiella acerosa	1	−13.9	4.6	15. Kuroshima	Field	−
Gelidiella acerosa	10	−15.3 ± 1.8	3.6 ± 0.8	15. Kuroshima	Field	−
Codium sp.	5	−12.4 ± 2.2	3.6 ± 0.6	15. Kuroshima	Field	−
Codium sp.	10	−12.0 ± 1.9	3.6 ± 0.4	15. Kuroshima	Field	−
Gelidiella acerosa	4	−14.3 ± 0.1	4.3 ± 0.2	15. Kuroshima	Field	−
Betaphycus gelatinum	4	−13.0 ± 1.0	3.7 ± 0.1	15. Kuroshima	Field	−
Yaeyama seagrass[a]						
Thalassia hemprichii	5	−7.8 ± 0.5	3.3 ± 1.5	15. Kuroshima	Field	−
Cymodocea serrulata	7	−8.9 ± 0.8	5.1 ± 0.7	15. Kuroshima	Field	−
Thalassia hemprichii	5	−6.3 ± 0.7	2.7 ± 0.9	15. Kuroshima	Field	−
Planktonic invertebrates						
Lepas anatifera	1	−	−	1. Muroto	Stomach	47.4
Chrysaora melanaster	1	−	−	5. Shimakatsu	Stomach	46.4
Multiple species[b,d]	11	−18.5 ± 1.6	9.4 ± 2.2	NW Pacific	Trawl	−

[a]Known food (defined in 'Materials and methods'); [b]Hypothetical food (defined in 'Materials and methods'); [c]Data from Takai et al. (2001); [d]Data from Hatase et al. (2002, 2006)

Table 3. Stable isotope ratios (δ^{13}C, δ^{15}N) of food sources listed in Table 2 were represented in mixing models by means (±SD) for each component food group. n: number of samples; Y: Yaeyama; S: Shikoku

Model components (sites)	Food taxa	δ^{13}C (‰)	δ^{15}N (‰)	n
Macroalgae / seagrass (Y only)	Macroalgae at Y[a,c]	−13.5 ± 2.1	3.8 ± 0.6	34
	Seagrass at Y[a,c]	−7.8 ± 1.3	3.9 ± 1.5	17
Known food / hypothetical animal	Macroalgae at S[a,c]	−16.5 ± 2.2	6.5 ± 1.4	28
food (S & Y)	Macroalgae & seagrass at Y[a,c]	−11.6 ± 3.3	3.8 ± 1.0	51
	Planktonic invertebrates at remote site[b,e]	−18.5 ± 1.6	9.4 ± 2.2	11
Known food / hypothetical 'city-bay'	Macroalgae at S[a,c]	−16.5 ± 2.2	6.5 ± 1.4	28
food (S only)	Macroalgae at 'city-bay' sites[b,c,d]	−15.9 ± 2.2	9.1 ± 1.3	46

[a]Known food (as defined in 'Materials and methods'); [b]Hypothetical food (as defined in 'Materials and methods'); [c]Present study; [d]Takai et al. (2001); [e]Hatase et al. (2002, 2006)
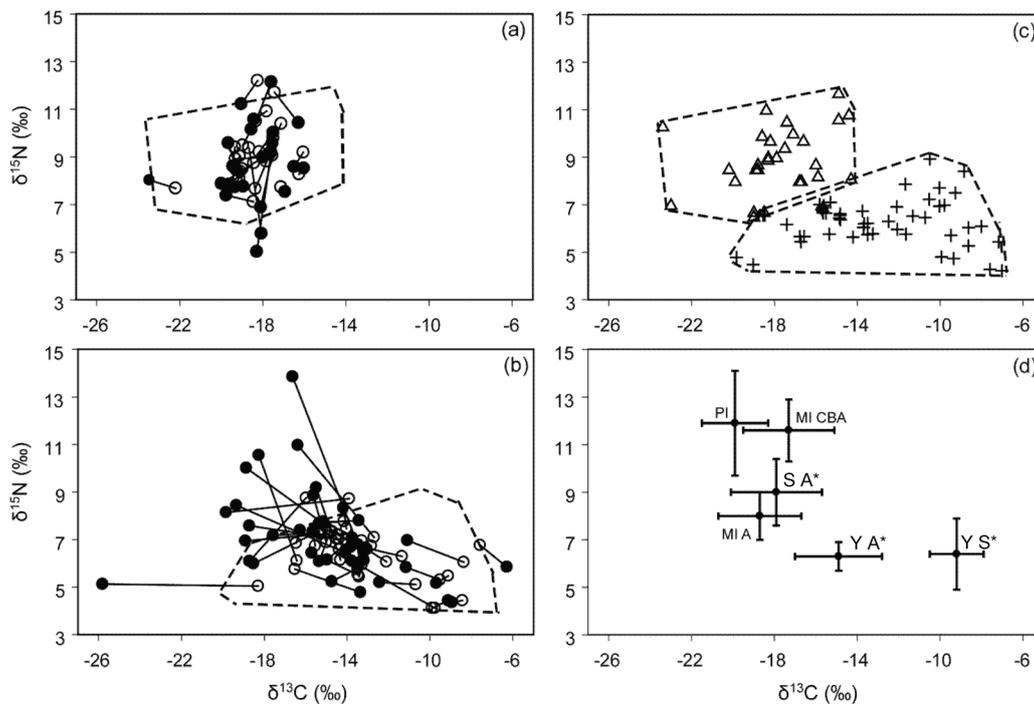
Fig. 3. *Chelonia mydas.* Diet history was inferred from stable isotope ratios of old scute tissue (●) and new scute tissue (○) of green turtles at (a) Shikoku (n = 32) and (b) Yaeyama (n = 42). Polygons (broken line) depict isotopic boundaries for the known food (defined in 'Materials and methods') at each site. (c) Data for known food at Shikoku (△) and Yaeyama (+) in relation to isotopic boundaries. (d) Mean (±SD) values for food sources. PI: planktonic invertebrates (hypothetical food source); MI CBA: Main Islands 'city-bay' macroalgae (hypothetical food source); S A: Shikoku macroalgae; MI A: Main Islands macroalgae, excluding Shikoku and Main Islands 'city-bay' macroalgae; Y A: Yaeyama macroalgae; Y S: Yaeyama seagrass; asterisk: 'known food' as defined in 'Materials and methods'. Food source data have been adjusted for diet–scute discrimination

of >50% macroalgae and an estimated recent diet of >50% seagrass. There was no apparent relationship to turtle size. Details of these model estimates can be found in Table S2 in the Supplement at www.int-res.com/articles/suppl/n025p151_supp.pdf. We caution that the biological relevance of these estimates was uncertain, as explained in the 'Discussion'.

Mixing models that included a hypothetical animal food source as well as known foods (Tables 2 & 3)

indicated that diets of Shikoku resident turtles could have included a non-trivial proportion of animal matter (mean ± SD = 39 ± 7%), if this was the only source additional to their known food and if the turtles had access to invertebrates with similar stable isotope values to our proxy data. The latter were sampled at a distant location as explained in 'Materials and methods'. For all Shikoku residents, the known food (macroalgae) was estimated to be the larger compo-

nent of the diet. Similarly, for Yaeyama, the known food (macroalgae and seagrass) of all residents was estimated to be the larger component of the diet. Their estimated proportion of hypothetical animal matter (mean ± SD = 18 ± 10%) was smaller than that of Shikoku turtles. Details of all model estimates are available in Table S2.

As a post hoc addition explained in the 'Discussion', we ran mixing models (as detailed in 'Materials and methods') for a different hypothetical food source, together with the known food for Shikoku residents. The hypothetical food source was a subset of food source data for Main Islands sites, namely macroalgae growing in enclosed bays near large cities (Tables 2 & 3). The model estimates indicated diets of Shikoku resident turtles could have included a non-trivial proportion of macroalgae that had stable isotope values corresponding to those of samples sourced from bays near large cities (mean ± SD = 33 ± 11%). Details of these model estimates are available in Table S2.

DISCUSSION

We confirmed that the known food of green turtles, as depicted by stable isotope ratios, differed significantly between our 2 study sites. Therefore, any turtles foraging at either site for extended periods could be expected to have distinctive scute isotope values that corresponded to their site. Our results indicated the majority of study turtles (81% at Shikoku, 64% at Yaeyama) could be considered long-term residents, having previous diets (incorporated in old scute tissue) and recent diets (incorporated in new scute tissue) corresponding to the known food at their respective sites.

Our finding of site fidelity for the majority of study turtles (those classified as resident) was consistent with evidence from a 3 yr tagging study at Yaeyama (Kameda et al. 2013) in which green turtles were recaptured at the site up to 744 d after marking. Mark-recapture data were not available for Shikoku.

At Yaeyama, a substantial minority of turtles (n = 14, 33%) were apparent immigrants, having previous diets beyond the range of isotope ratios for known food at this site. Collectively these apparent immigrants were significantly smaller in size than residents at Yaeyama. Isotope ratios indicated that the previous diets of immigrants were broadly similar to Shikoku diets, apart from 2 exceptions discussed below. Shikoku diets could be generalised, with caution, to other Main Islands foraging sites based on similar isotopic signatures of food sources (Fig. 3, Table 2).

On the basis of isotope ratios alone, previous diets similar to Shikoku diets did not exclude the possibility of an oceanic origin for some Yaeyama immigrants. Direct comparison of isotope ratios was not feasible because data were not available for juvenile green turtles inhabiting ocean waters near Japan. However, the change in isotope ratios shown by Yaeyama immigrants matched the direction of change found at a neritic site in Australia for new recruits from the Pacific Ocean (Arthur et al. 2008), where the latter new recruits corresponded to the smallest size cohort at the neritic site. Similarly, only the smallest Yaeyama immigrants could have been new recruits of oceanic origin, although new recruit status could not be confirmed. A white plastron with prominent ridges can assist in identifying new recruits, e.g. Limpus et al. (2005), but we were unable to assess such evidence because the plastron morphology of our study turtles had not been examined in detail.

We concluded that the majority of the Yaeyama immigrants had diets consistent with a Main Islands origin and had body sizes broadly consistent with the under-represented size cohort previously reported for Main Islands sites (e.g. Okamoto et al. 2011 and references therein), and thus supported the proposition that Yaeyama immigrants could have made an ontogenetic shift from Main Islands foraging grounds. (The 'majority of immigrants' cannot be exactly enumerated because the under-represented size cohort is not bounded by exact limits, nor is there an exact size limit for potential new recruits from oceanic habitat.)

Two Yaeyama immigrants had previous diets very different from other immigrants. The first of these (SCL = 58.6 cm) had old scute data falling far to the left of the Yaeyama food boundary (Fig. 3). Erroneous data for this turtle cannot be ruled out, but the authors believe error is unlikely. The δ^{13}C value (−25.6‰) suggests a diet of terrestrial plant matter. Broadly similar δ^{13}C values have been found for leaves and fruits of mangroves, and these items have been confirmed as forming a part, albeit minor, of the diet eaten by green turtles in some other parts of the world (e.g. Arthur et al. 2009). We found no records of mangrove consumption by green turtles in Japan. The origins of this Yaeyama immigrant remain a mystery. The second case was a turtle of adult size (SCL = 83 cm) with its old scute data point to the right of the Yaeyama food boundary. From its isotope ratios, we surmise this turtle might have migrated from another sub-tropical or tropical area where seagrass was available.

At Shikoku, the apparent immigrants were a small minority (n = 4). Stable isotope ratios (old scute) for the 2 larger individuals (SCL = 75.9 and 63.3 cm) were only slightly different to Shikoku residents, suggesting these turtles might not be immigrants and might simply have consumed other food (in modest proportion), as well as known food for Shikoku. However, the 2 smaller individuals (SCL = 37.7 and 42.0 cm) had stable isotope ratios (old scute) consistent with Yaeyama residents. The old-to-new-scute change in isotope ratios for these 2 small turtles was contrary in direction to that identified in new recruits from oceanic habitats (Arthur et al. 2008), and showed these 2 turtles had not recruited to Shikoku directly from the ocean. We surmised the 2 small immigrants at Shikoku might previously have (1) recruited from the ocean to Yaeyama (or similar Nansei Islands sites), (2) remained long enough (years) for old scute tissue to become consistent with Yaeyama food, then (3) made an ontogenetic shift to Shikoku. The latter shift would have occurred at a relatively early stage, i.e. while these 2 immigrants were still among the smallest size cohort at neritic foraging grounds in Japan (e.g. Okamoto et al. 2011 and references therein, Kameda et al. 2013).

Taking a 'big picture' view, the apparent ontogenetic movement by some Japanese green turtles from Main Islands sites towards the Nansei Islands may be in the direction of their natal origin, as has been reported for the western North Atlantic (e.g. Moncada et al. 2006, Meylan et al. 2011). The Kuroshio Current flows north-eastward (Henry & Yoshida 1972) and may assist hatchling green turtles originating from rookeries in the Nansei Islands during their passive migration towards oceanic habitat. After their oceanic phase, if some of these turtles recruit to the Main Islands of Japan and remain at Main Islands sites during their early neritic stage, their subsequent ontogenetic shift (consistent with our original proposition and our study findings) to the southern Nansei Islands including Yaeyama would represent movement closer to their natal region. However, we remain cautious about such broad inference and suspect more complex and diverse patterns may exist. Greater insight could be gained through future use of satellite tracking, particularly for juvenile green turtles of known genetic origin.

Our mixing model estimates suggested that individual study turtles each consumed an unequal mix of macroalgae and seagrass, based on scute tissue representing food assimilated over a period of years, a duration estimated to span about 1.4 to 6.5 yr (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/ n025p151_supp.pdf and Vander Zanden et al. 2013). Interpretation was relatively clear for 8 turtles (macroalgae more important in 5 instances, seagrass more important in 3 cases) and less clear for others. We were unable to define a numeric threshold to distinguish between biologically relevant imbalance and trivial imbalance because there was no identifiable null point for comparison. Numerically equal proportions would not be expected, even if a turtle always consumed food at random. A study-relevant random selection of the 2 taxa could not be defined in the absence of detailed data for fine-scale distribution, nutritional value and abundance of these 2 taxa at the study site over the years represented by scute tissue.

Relative importance of macroalgae and seagrass in green turtle diets holds particular interest for some researchers. Digestion appears to be more efficient if a turtle consumes one or the other rather than both taxa (Bjorndal 1979, Bjorndal et al. 1991). This difference in digestive efficiency has been suggested as a possible explanation for selective foraging, the latter observed over short temporal duration at diverse locations (Bjorndal 1997 and references therein) and inferred for green turtles in Japan, similarly referring to a relatively short time span (gut content analysis by the Sea Turtle Association of Japan unpubl. data). However, assessing this taxonomic dichotomy in diet over a much longer time span through application of Bayesian mixing models proved problematic. For our mixing models, the 2 food sources were treated as equally likely to be consumed (i.e. uniform priors were specified) although it was implausible that study turtles always had equal opportunity to eat both foods. As noted above, there were no data available to support informative priors. We therefore caution that biological relevance of our proportional estimates for macroalgae and seagrass remains uncertain. The topic might be more effectively addressed for a shorter time span (provided corresponding data for food availability could be obtained) by measuring isotope ratios of tissue with relatively rapid turnover such as blood or plasma (e.g. Reich et al. 2008).

The inclusion of animal matter in our mixing models confirmed that this could be a cryptic component of green turtle diets at our study sites if the turtles had access to invertebrates with isotope values similar to the proxy data we used. The estimates indicated a greater proportion of animal matter for Shikoku diets than for Yaeyama diets. Our modelling used stable isotope ratios for known food (macroalgae and seagrass) sampled at our study sites, together with proxy values for the hypothetical animal food. The proxy data came from samples col-

lected in Japanese waters distant from our sites (Hatase et al. 2002, 2006) and comprised salps, jellyfish and floating gastropods, all biologically plausible prey for green turtles (Kameda & Ishihara 2009, present study). However, our direct evidence for animal consumption was extremely weak (in the stomach contents of 22 turtles we found a total of 2 small invertebrates). Therefore, we emphasise the hypothetical nature of our model estimates for animal consumption. We note also that uniform priors were problematic (as discussed above) for these models.

Mixing models are mathematically rigorous, but diets inferred from these models have limited biological relevance unless food sources are appropriately represented (Parnell et al. 2010). It is challenging to appropriately represent a hypothetical food source. In addition, the conundrum regarding uniform or informative priors (considered above) applies. Furthermore, inference may be confounded by the existence of more than one plausible diet component with coincidentally similar isotope ratios but disparate biological relevance. We discovered such a constraint on our inference.

It emerged that data we used to represent animal matter were relatively close to the isotope ratios for macroalgal samples from enclosed bays near large cities in the Main Islands. The latter samples ('city-bay' macroalgae hereafter) were notably enriched in ^{15}N relative to macroalgae from open areas of Main Islands, including Shikoku. Reasons for the difference in city-bay samples lay beyond the scope of our study, but differences in water quality associated with intensive human activity were plausible (Umezawa et al. 2002). According to mixing model estimates, if city-bay macroalgae had been consumed by Shikoku residents, then city-bay macroalgae could have comprised a slightly lower but broadly similar proportion of the diet to that estimated for animal matter.

Thus from stable isotope data we infer that diets of turtles considered resident at Shikoku could have included non-trivial quantities of animal matter, city-bay macroalgae, or both. (For clarity we note that biologically relevant estimates could not be obtained from a mixing model that included 2 sources with similar stable isotope ratios.) Both hypothetical sources were plausible for Shikoku turtles. Shikoku turtles could have spent periods in offshore waters where macroplankton might be episodically abundant. Distances of 60 to 140 km between Shikoku and sites where city-bay macroalgae could be consumed did not preclude movement between these locations. However, no satellite tracking data were available to confirm such movement scenarios, and no compelling supportive evidence could be found in stomach samples. We recognise also that animal matter and city-bay macroalgae are not implausible in the previous diets of Yaeyama immigrants, except for the 2 individuals with exceptional diets discussed earlier. Yet the hypothetical inclusion of those sources does not contradict our original proposition regarding ontogenetic shifts from the Main Islands to the Nansei Islands.

For the green turtle foraging aggregations under consideration, known food sources and plausible hypothetical foods (when considered in biologically meaningful categories) proved to be weakly differentiated in their stable isotope values. Therefore, inference from stable isotopes must necessarily remain constrained by ambiguity, even if turtles and foods can be sampled more comprehensively in future studies. Nevertheless we conclude that the combination of stable isotope methods and SCL data have provided important support for the proposition that many juvenile green turtles make an ontogenetic shift to Yaeyama and similar Nansei Islands sites from Shikoku and similar Main Islands foraging grounds. For the majority of study turtles, stable isotope methods also indicated fidelity to Japanese foraging grounds over years. Both findings are important for future conservation management of green turtles in Japan.

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