



Original research article

Body size distribution demonstrates flexible habitat shift of green turtle (*Chelonia mydas*)



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ABSTRACT

Green turtles (*Chelonia mydas*), listed as Endangered on the IUCN redlist, have a broad migration area and undergo a habitat shift from the pelagic (hatchling) to neritic (growth) zones. We studied habitat utilisation of the coastal feeding grounds around Okinawajima Island, Japan, in 103 green turtles. The western and eastern turtle aggregations off Okinawajima had homogeneous genetic compositions, but different body size distributions. The western coastal feeding ground supported larger individuals than the eastern coastal feeding ground. Thus, green turtles appear to prefer different feeding grounds during their growth, and have a flexible habitat shift including a secondary habitat shift from east to west around Okinawajima Island after they are recruited to the coastal habitats. This study suggests maintaining coastal habitat diversity is important for green turtle conservation.

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1. Introduction

The green turtle (*Chelonia mydas*) is a migratory reptile with a complex life cycle that includes habitat shifts. After they enter the sea as hatchlings they exist in a pelagic life stage, but then recruit to neritic feeding habitats during growth (Musick and Limpus, 1997; Bolten, 2003). When they reach maturity and reproduce, they return to the vicinity of their natal sites (Allard et al., 1994; FitzSimmons et al., 1997). Although the green turtle life cycle is roughly estimated in this way, there is a controversy about their habitat utilisation during growth. In particular, the question remains whether green turtles have a fixed life history or flexible life history. If they have a fixed life history, they are believed to enter a neritic habitat at a specific body size, and utilise one neritic habitat continuously. If they have a flexible life history, they enter a neritic habitat at various body sizes and/or shift their habitats even after they recruit to one neritic habitat. Pfaller et al. (2014) predicted that green turtles' transition from epipelagic to neritic habitats is unidirectional at a small body size, and thus they remain there throughout development. On the other hand, satellite telemetry and stable isotope analysis of western North Pacific adult green turtles indicated that they use both epipelagic and neritic habitats (Hatase et al., 2006). Moreover, green turtles appear in the coastal area of Okinawajima Island, located in the subtropical region of Japan, with a straight carapace length (SCL) greater than about 35 cm. However, the subadult stage, SCL 50–55 cm, is conspicuously absent (Hayashi and Tsuji, 2008). These results suggest a flexible life history of green turtles that may allow a shift in their habitat even after they recruit to the coastal areas, in contrast with the proposal of Pfaller et al. (2014).

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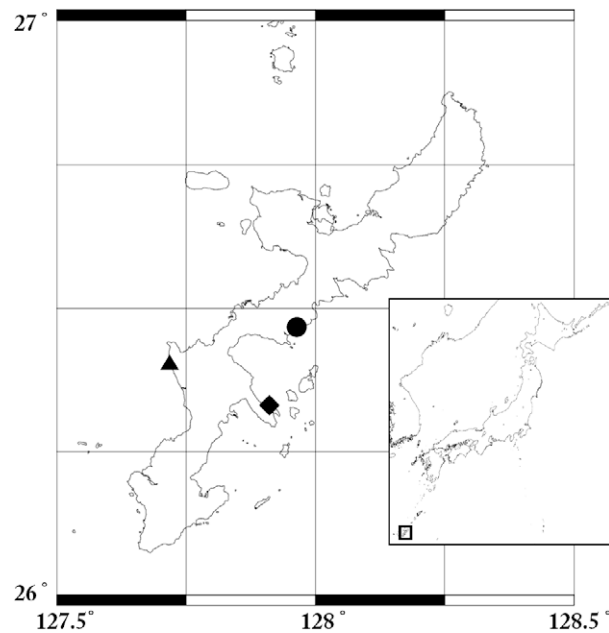


Fig. 1. Geographical locations of Yomitan (triangle), Yakena (diamond), and Ginoza (circle).

To better understand the habitat utilisation of green turtles, we compared the body size distribution and genetic composition between two green turtle aggregations around the Okinawajima Island, Japan. These data are important because (1) size distribution correlates with habitat shifts of sea turtles (Bolten et al., 1993; Musick and Limpus, 1997); and (2) genetic information provides opportunities to link nesting populations with feeding aggregations (Pella and Masuda, 2001; Bolker et al., 2003). The Japanese coastal areas represent the northern limit of green turtles' distribution in the western Pacific Ocean, and Okinawajima Island provides them both nesting beaches and feeding grounds (Kikukawa et al., 1999; Hirate, 2005; Hayashi and Tsuji, 2008). The genetic composition of northwestern Pacific green turtles has been recently investigated (Nishizawa et al., 2011, 2013, 2014; Hamabata et al., 2014), but the comparison of body size between feeding aggregations and its relationship to their habitat use have been less well studied. In this study, we investigated the bycaught individuals captured by fisheries' setnets in the western and eastern coasts of Okinawajima Island. Here we describe a flexible life history of northwestern Pacific green turtles, providing insight into how local habitat preference follows their growth stage.

2. Materials and methods

2.1. Field research

Fifty-two individual green turtles (*Chelonia mydas*) were captured by stationary setnets placed near the Toya fishery port, Yomitan, and an additional 3 individuals were captured by fishermen at Yakena, Okinawa, Japan (Fig. 1). The body size of each turtle was measured as the SCL in centimetres. Bycatches by setnet are almost nonlethal, and turtles were individually tagged and released after measurement. Minute tissue samples were collected from these turtles by punching flippers for tagging and stored in 99% ethanol (EtOH). From these, mitochondrial DNA (mtDNA) sequences of 35 individuals from Yomitan and of the 3 individuals from Yakena were determined. These individuals were bycaught in 2009–2010 (Appendix S1).

2.2. Molecular analysis

DNA extraction, polymerase chain reaction (PCR), and sequencing analysis fundamentally followed the methods of Nishizawa et al. (2014). DNA was extracted by using the Blood and Tissue Genomic DNA Extraction Miniprep System (Viogene, Sunnyvale, CA, USA). PCR with the primers LCM15382 (5'-GCTTAACCCTAAAGCATTGG-3') and H950 (5'-GTCTCGGATTTAGGGGTTTG-3') (Abreu-Grobois et al., 2006) amplified an ~820-bp segment of the mtDNA control region. A 15- μ L PCR reaction volume contained 3.0 μ L of the template, 1.5 μ L of 10 \times PCR buffer, 1.2 μ L of dNTPs (at 2 mM), 0.6 μ L each of the forward and reverse primers (at 2.0 μ M), and 0.1 μ L of *Ex Taq* polymerase (Takara, Otsu, Japan). PCR conditions were as follows: 3 min at 94 $^{\circ}$ C, followed by 35 cycles of 94 $^{\circ}$ C for 30 s, 55 $^{\circ}$ C for 60 s, and 72 $^{\circ}$ C for 70 s, and final extension at 72 $^{\circ}$ C for 3 min. Amplification was verified using electrophoresis in 1% agarose gel. The PCR products were purified using

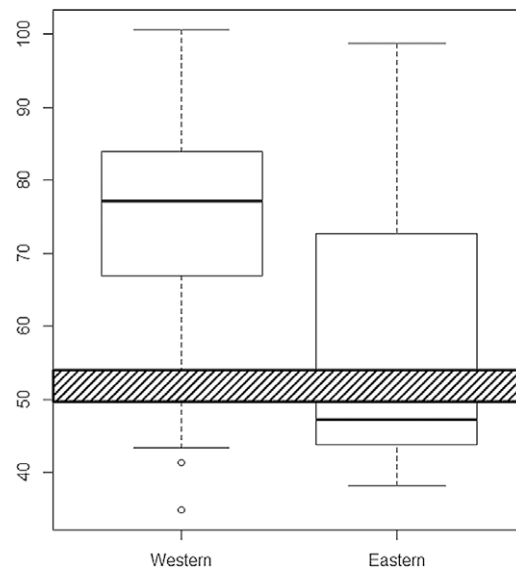


Fig. 2. Box plot of straight carapace lengths (SCLs) found in the western and eastern coast aggregations off Okinawajima Island. The shadowed box represents SCLs absent from the Okinawajima coastal area indicative of a habitat shift in their life cycle.

ExoSAP-IT (GE Healthcare Bio-Sciences K. K., Tokyo, Japan), and the sequencing reactions (forward and reverse) were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Cycle sequencing was performed in 5- μ L reaction volumes with 1.5 μ L of the PCR product, 1.0 μ L of 5 \times sequencing buffer, 1.2 μ L of 2.0 μ M primer, 0.8 μ L of sterilized water, and 0.5 μ L of dye terminator at 96 °C for 1 min, followed by 30 cycles of denaturing at 96 °C for 10 s, annealing at 56 °C for 5 s, and extension at 60 °C for 1 min. After purification using ethanol precipitation, the products were run through a 3130xl sequencing analyzer (Applied Biosystems). Sequences were aligned by using CLUSTALW in MEGA v5.1 (Tamura et al., 2011).

2.3. Data analysis

For comparison, we included the previously published size distribution of SCL ($N = 48$, Hayashi and Tsuji, 2008) and haplotype composition ($N = 20$, Nishizawa et al., 2013) observed in Ginoza. Feeding aggregations were thus classified based on the western coast (Yomitan) or the eastern coast (Yakena and Ginoza) of Okinawajima (Fig. 1).

Firstly, the Mann–Whitney U test in R v2.15.1 (R Core Team, 2012) was used to compare SCL between the western and eastern feeding aggregations. Then the haplotype frequency was compared using exact test (500,000 steps in a Markov chain with a 100,000-step dememorisation) in ARLEQUIN v3.5 (Excoffier and Lischer, 2010). For these comparisons, sequences from Yomitan and Yakena were trimmed to \sim 380-bp fragments that corresponded to the fragments amplified with primers TCR5 and TCR6 (Norman et al., 1994).

3. Results

SCL of the green turtles from Yomitan, the west coast of Okinawajima Island, ranged from 34.8 to 100.7 cm (Fig. 2, Appendix S1). In the feeding aggregation from the eastern coast that combined Yakena and Ginoza (Hayashi and Tsuji, 2008), SCL ranged from 38.1 to 98.8 cm (Fig. 2). The SCL size distribution was significantly different between the western and eastern coasts ($U = 695.5$, $p < 0.0001$).

From the genetic analysis, we identified 14 and 3 haplotypes from the Yomitan and Yakena green turtles, respectively (Table 1, Appendix S1). One green turtle from Yomitan had a CmP54 haplotype based on the \sim 380-bp fragment, but was found to be heteroplasmic at one site upon analysis using a \sim 820-bp fragment. Haplotypes observed in the Yomitan turtles included those detected in the West Central Pacific rookeries including Micronesia (CmP32 and CmP61) (Dethmers et al., 2006; Naro-Maciel et al., 2014), in addition to haplotypes previously detected only in Japanese rookeries of Yaeyama, the Ryukyus, and Ogasawara (CmP39, CmP50, CmP54, CmP121, and CmP128) (Hamabata et al., 2014; Nishizawa et al., 2011, 2013). Seven of the 14 haplotypes detected in the Yomitan group were also found in the Ginoza group, while 7 of the 9 haplotypes detected in the Ginoza group were also found in the Yomitan group (Table 1). The three haplotypes observed in Yakena were also identified in Yomitan and Ginoza (Table 1). There was no significant difference in the haplotype frequency between feeding aggregations of the western and eastern coasts of Okinawajima Island ($p = 0.96$).

Table 1
Haplotype frequency of feeding aggregations off Okinawajima Island.

Haplotype	Western coast	Eastern coast	
	Yomitan	Yakena	Ginoza ^a
CmP20	9	1	4
CmP32	1	0	0
CmP39	7	1	5
CmP49	1	0	0
CmP50	5	0	4
CmP53	1	0	1
CmP54	2	1	2
CmP61	1	0	1
CmP76	0	0	1
CmP77	2	0	0
CmP106	1	0	0
CmP121	2	0	0
CmP128	1	0	1
CmP208	1	0	0
CmP212	1	0	0
CmP209 ^b	0	0	1
Total	35	3	20

^a The genetic composition off Ginoza is derived from Nishizawa et al. (2013).

^b CmP209 is renamed from CMJ38.

4. Discussion

The lack of a significant difference in the haplotype frequency indicates that feeding aggregations off the western and eastern coasts of Okinawajima Island are genetically similar. Both contained haplotypes detected in the West Central Pacific rookeries (Dethmers et al., 2006; Naro-Maciel et al., 2014), as well as haplotypes previously detected only in Japanese rookeries (Nishizawa et al., 2011, 2013; Hamabata et al., 2014). These results indicate that both feeding aggregations contain green turtles born not only in the Japanese rookeries (e.g. Ogasawara), but also in the West Central Pacific rookeries (e.g. Micronesia). Because the contribution of remote rookeries to feeding grounds appears to be influenced by the oceanic current (Musick and Limpus, 1997), the Kuroshio Current is believed to transport green turtle juveniles born in the remote West Central Pacific rookeries to the waters around Okinawajima Island (Nishizawa et al., 2013).

Despite the similarity in genetic composition, the size distribution of green turtles off the western coast was significantly different from that off the eastern coast, indicating that the proportion of larger green turtles was relatively high in the western coast aggregation. The difference can be explained by two hypotheses that are not incompatible. One hypothesis suggests juvenile green turtles initially recruited to the eastern coast of Okinawajima Island tend to move to the western coast as a secondary habitat shift that correlates with their growth. The other suggests that juvenile green turtles are recruited to Okinawajima Island at various sizes, and more large juveniles or even adults preferentially recruit to the western coast. Notably, green turtles of 50–55 cm SCL were not found in either the western or eastern coasts. This may be a result of sampling bias caused by using only setnet bycatch, but it also supports the habit of green turtles to use flexible migratory strategies and change their habitats after they recruit to a feeding ground. The minimum size of green turtles around Okinawajima Island (34.8 cm on the western coast and 38.1 cm on the eastern coast) was similar to that of other feeding aggregations around Japan (33.0 cm in Yaeyama, 40.6 cm in Nomaike, 38.3 cm in Muroto, 37.1 cm in Kanto, and 38.9 cm in Sanriku) (Hamabata et al., 2009; Nishizawa et al., 2013, 2014). This is in agreement with the coastal habitat recruitment size of green turtle juveniles in Hawaii (Arthur and Balazs, 2008), indicating that green turtles start to recruit at about 35 cm SCL. On the other hand, utilisation of oceanic habitats by large juvenile green turtles (Parker et al., 2011) supports the variability in recruitment size of green turtles. The results of present study did not support the unidirectional habitat shift at small body size suggested by Pfaller et al. (2014). Further studies including mark-recapture or biotelemetry methodologies will be needed to confirm the secondary habitat shift hypothesis. Nevertheless, this study enhances our understanding about the utilisation of waters around Okinawajima Island by green turtles.

5. Conservation implications

For conservation of migratory marine animals, the evaluation and management of their habitats are critical. Green turtles have a broad migration range (Parker et al., 2011; Nishizawa et al., 2013, 2014; Naro-Maciel et al., 2014), and they are listed as Endangered by the International Union for Conservation of Nature (IUCN, 2014). In the whole area of Okinawajima Island, a visual count of turtles using aircraft for observation was conducted in 2008–2009 (Okinawa Defense Bureau, 2012). According to the report, sea turtles distributed all around the coastal area of Okinawajima Island, and often appeared off the eastern coast (Appendix S2). Nevertheless, some limitations are present in aircraft-based observation, including difficulty with species identification, measurement of body size, and individual specimen recognition. However, we can consider the eastern coastal area to be necessary for younger turtles. The current study, which compared the size distribution and

genetic composition of Okinawan green turtle aggregations, suggested that they utilise the locally different habitats of Okinawajima Island in accordance with their growth stages. Therefore, Okinawajima Island is confirmed as providing an important habitat for Pacific green sea turtles. These data are very important for green turtle conservation, in that if recent artificial developments and exploitations of Okinawajima Island reduce the diversity of the local habitats of its coastal areas (Okinawa Defense Bureau, 2012, 2014), the growth and survival of Pacific green turtles will be negatively impacted. Maintaining the diverse local habitats around Okinawajima Island is necessary for the conservation of endangered green turtles.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.gecco.2014.11.008>.

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