Vol. 24: 85–93, 2014
doi: 10.3354/esr00588

ENDANGERED SPECIES RESEARCH
Endang Species Res

Published online April 24

Genetic composition of loggerhead turtle feeding aggregations: migration patterns in the North Pacific

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ABSTRACT: The loggerhead turtle *Caretta caretta* is highly migratory and undertakes trans-oceanic migrations. In the North Pacific, loggerhead turtles that hatch on Japanese beaches reach the vicinity of Baja California in the eastern Pacific. As they grow, they return and recruit to the feeding areas around Japan. By using mtDNA control-region sequences, we identified the genetic composition of the feeding aggregation around the Sanriku coastal area (n = 107), >500 km north of the main nesting beaches of Japan and located on the north of the Japanese mainland. Performing a mixed-stock analysis using the published data from 5 Japanese nesting rookeries (n = 279 in total) as sources, the origin of the feeding aggregation was estimated with Bayesian statistics. The results indicated a high contribution from the southern rookeries (mean ≥ 82.10%), mainly the Yakushima rookery (mean ≥ 51.45%), to the Sanriku feeding aggregation, whether the number of nests was considered as an informative prior or not. Therefore, the Sanriku coastal area is estimated to be utilized by loggerhead turtles born in the southern nesting rookeries relatively far from Sanriku. The strong connectivity between loggerheads from the Sanriku feeding aggregation and the southern Japanese rookeries suggests that loggerhead turtles in the North Pacific generally settle in the Japanese coastal areas in the large juvenile stage, but not in the direct vicinity of their natal sites, and some juveniles that use an oceanic feeding strategy are recruited to the Sanriku coastal area.

KEY WORDS: mtDNA · Haplotype · Mixed-stock analysis · Japan · Juvenile · Recruitment

INTRODUCTION

The highly migratory loggerhead sea turtle *Caretta caretta* is a globally Endangered species (IUCN 2013), and its nesting grounds are found in temperate and tropical regions worldwide. After hatching on the nesting beaches, the turtles enter the ocean. Relatively little is known about the location of the post-hatchlings and small pelagic juveniles during the subsequent 'lost years,' where they may circulate in oceanic gyres and drift with the currents, but active swimming may play an important role in forming the settlement patterns (Musick & Limpus 1997, Mansfield & Putman 2013). Subsequent to the oceanic stage, which may span a decade, large juveniles enter a neritic (benthic feeding) stage, during which they consume hard-shelled invertebrates in shallow habitats (Bolten 2003). However, recent reports have

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indicated that oceanic habitats are also utilized by large juveniles (McClellan & Read 2007, Mansfield et al. 2009) or even adults (Hatase et al. 2010, Eder et al. 2012). Similar to other species of sea turtles, after reaching sexual maturity, the adult female loggerheads undertake seasonal breeding migrations between the feeding areas and the nesting beaches at intervals of 1 to 6 yr (Bolten 2003, Hatase et al. 2004).

In the Pacific, nesting beaches of loggerhead turtles are restricted to the western side of the ocean basin in the Japanese Archipelago in the Northern Hemisphere (Bowen et al. 1995, Kamezaki et al. 2003), and in eastern Australia and New Caledonia in the Southern Hemisphere (Limpus & Limpus 2003, Limpus 2004). Loggerhead turtles hatching on Japanese beaches undertake developmental migrations by using the Kuroshio Current, and some turtles reach the vicinity of Baja California in the eastern Pacific (Bowen et al. 1995), whereas turtles born in eastern Australia and New Caledonia migrate to the coasts of Chile and Peru in the southeastern Pacific (Boyle et al. 2009). Once the loggerhead turtles near Baja California grow to 50–75 cm in straight carapace length (SCL), they return and recruit to the feeding areas around Japan as large juveniles (Nichols et al. 2000, Polovina et al. 2004, Ishihara et al. 2011). When female turtles mature, they are thought to nest in their natal regions, resulting in significant genetic differentiation among loggerhead sea turtles in the 5 Japanese rookeries (Hatase et al. 2002a, Watanabe et al. 2011). Rookeries in Japan and those in the Southern Hemisphere do not share common haplotypes (Bowen et al. 1995, Hatase et al. 2002a, Boyle et al. 2009), indicating that there is little or no gene flow between them.

Although there are several studies on the post-nesting migration and feeding areas of female loggerhead turtles nesting in Japan (Kamezaki et al. 1997, Sakamoto et al. 1997, Hatase et al. 2002b, 2007), there is little knowledge about how large juvenile loggerhead turtles use the available feeding areas around Japan after returning from Baja California and how these turtles migrate to the nesting beaches when they mature. While sea turtles nest only in tropical or subtropical regions, some individuals may at least seasonally migrate to feeding areas in higher latitudes (Hawkes et al. 2007, Mansfield et al. 2009). Around the Japanese Archipelago, large juvenile or mature loggerhead turtles (56 to 105 cm SCL) appear in the coastal areas of Muroto (Kochi prefecture), and Shimakatsu (Mie prefecture) (Ishihara & Kamezaki 2011, Ishihara et al. 2011), located relatively close to their main nesting beaches in Japan (Fig. 1). On the other hand, the northern Sanriku coastal area, located more than 500 km away from the main nesting beaches in Japan, also provides feeding areas for large juvenile loggerhead turtles (Fig. 1; Narazaki et al. 2009, 2013).

When loggerhead turtles recruit to the feeding areas at the end of their developmental migration as large juveniles, they are hypothesized to settle in suitable feeding areas they have encountered, possibly in oceanic habitats (Hatase et al. 2010, Eder et al. 2012). On the other hand, loggerhead turtles in the large juvenile stage are known to settle in feeding areas in the vicinity of their natal beaches (Bowen et al. 2004), and once sexually mature, the female adults appear to have high site fidelity to feeding areas (Broderick et al. 2007). The Sanriku coastal area is one of the most productive areas because the Kuroshio Current from the south and the Oyashio Current from the north interact and enhance primary production (Sugimoto & Tameishi 1992), attracting juveniles that encounter this area to settle despite the distance from the rookeries. Therefore, the composition and origin of the northern feeding aggregation in the Sanriku coastal area is important for estimating how the large juvenile turtles recruit to the feeding area and how these turtles migrate to the nesting beaches when they mature.

The origins of juvenile loggerhead sea turtles have been difficult to elucidate. However, recently, the examination of differences in mitochondrial DNA (mtDNA) haplotype frequency that are caused by

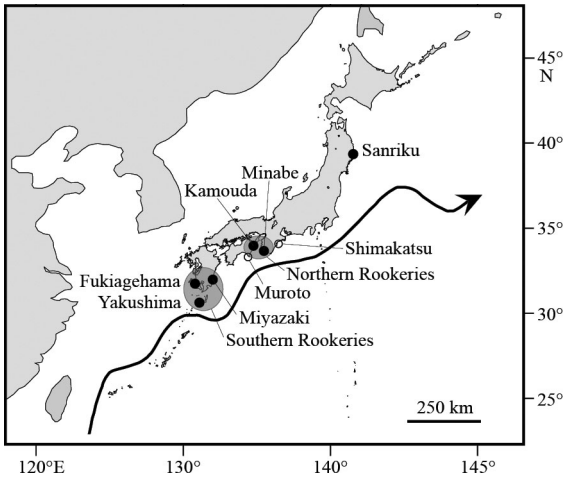

Fig. 1. *Caretta caretta*. Geographical locations of the Sanriku coastal area and major nesting rookeries for loggerhead turtles in Japan (●). Shimakatsu and Muroto, where other feeding aggregations of loggerheads are observed, are also shown (○). Larger grey circles indicate the northern and southern rookeries. The arrow indicates the direction of the Kuroshio Current

genetic isolation among nesting rookeries (e.g. Bowen et al. 1994, Encalada et al. 1998, Hatase et al. 2002a) has afforded an opportunity to link feeding aggregations back to their nesting region of origin and to estimate the contribution of genetically differentiated nesting rookeries to feeding aggregations by using mixed-stock analysis (MSA; Pella & Masuda 2001, Bolker et al. 2003). The significant genetic differentiation of loggerhead turtles in Japanese rookeries (Hatase et al. 2002a, Watanabe et al. 2011) presents an opportunity to link the feeding aggregation to specific Japanese rookeries by using mtDNA haplotypes and MSA.

In this study, we identified mtDNA control-region sequences of turtles from the feeding aggregation around the Sanriku coastal area and estimated the contribution of each Japanese rookery to the feeding aggregation. The specific objectives were (1) to estimate whether the Sanriku coastal area is utilized by turtles originating from rookeries near Sanriku, and (2) to hypothesize how loggerhead turtles in the North Pacific migrate in the large juvenile and adult-sized stages.

MATERIALS AND METHODS

Sample collection

Samples were collected from a total of 107 loggerhead turtles (bycaught in set nets) during June to September at the Sanriku coast in Japan (Otsuchi Bay between Miyako and Ofunato: 38° 55′ to 39° 40′ N, 141° 40′ to 142° 05′ E, Fig. 1). Small pieces of tissue (ca. 5 mm in diameter) were collected while punching the flippers for tagging and were stored in 99% ethanol. All sampled turtles were identified by the attached tags, avoiding the risk of pseudoreplication. Samples were collected in 2005 (n = 1), 2006 (n = 1), 2007 (n = 9), 2008 (n = 27), 2009 (n = 43), 2010 (n = 21), and 2012 (n = 5). The SCL of the turtles ranged from 49.5 to 88.4 cm.

DNA analysis

DNA was extracted from a small amount of tissue and prepared for polymerase chain reaction (PCR) analysis by using the Blood and Tissue Genomic DNA Extraction Miniprep System (Viogene). An 817 bp segment of the mtDNA control region was amplified using PCR with the primers LCM15382 (5′-GCT TAA CCC TAA AGC ATT GG-3′) and H950 (5′-GTC TCG GAT TTA GGG GTT TG-3′) (Abreu-Grobois et al. 2006). Typically, 3.0 µl of template was used in a 20 µl PCR reaction volume containing 2.0 µl of 10× PCR buffer, 2.0 µl of deoxynucleoside triphosphates (dNTPs) (at 2 mM), 0.8 µl of $MgSO_4$ (at 25 mM), 1.5 µl each of the forward and reverse primers (at 2.0 µM), and 0.4 µl of KOD-Plus (Toyobo). PCR conditions were as follows: 2 min at 94°C, followed by 35 cycles of 15 s at 94°C, 30 s at 58°C, and 20 s at 68°C. Alternatively, 1.0 to 2.5 µl of the template was used in a 15 µl PCR reaction volume containing 1.5 µl of 10× PCR buffer, 1.2 µl of dNTPs (at 2 mM), 0.6 µl each of the forward and reverse primers (at 2.0 µM), 0.2 µl of bovine serum albumin, and 0.1 µl of *Ex Taq* polymerase (Takara) under the following conditions: 3 min at 94°C, followed by 35 cycles of 94°C for 30 s, 55°C for 60 s, and 72°C for 70 s, and a final extension at 72°C for 3 min. The amplification was verified using electrophoresis in 1% agarose gel, and a second round of PCR was conducted when the bands indicated a low yield. The PCR products were purified using ExoSAP-IT (GE Healthcare Bio-Sciences K. K.). The sequencing reactions (forward and reverse) were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Cycle sequencing was performed in 5 µl reaction volumes with 0.5 to 2.3 µl of the PCR product (diluted up to 1:5 depending on the results of the electrophoresis), 1.0 µl of 5× sequencing buffer, 1.2 µl of 2.0 µM primer, 0.8 µl of sterilized water, and 0.5 µl 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. The products were purified using ethanol precipitation and run through a 3130xl sequencing analyzer (Applied Biosystems).

An 817 bp fragment was used in the analyses of the Sanriku samples, but a shorter 350 bp fragment, trimmed from the 817 bp fragment, was used for all other analyses, including published data from the nesting rookeries (Bowen et al. 1995, Hatase et al. 2002a, Watanabe et al. 2011). Sequence alignments were performed using CLUSTALW in MEGA v5.1 (Tamura et al. 2011). An unrooted haplotype network was created using TCS v1.21 (Clement et al. 2000), and nucleotide diversity (π) and haplotype diversity (h) were estimated. For examining temporal variation, differences in haplotype frequencies among the years were examined by using the exact test (Raymond & Rousset 1995) with 817 bp fragments. Since only 1 sample was collected each year in 2005 and 2006, these years were excluded from the comparisons. A total of 42 and 80 of the 107 loggerhead turtles were smaller than the minimum size for nesting

loggerhead turtles in Japan reported by Kamezaki et al. (1995) (SCL ≤ 69.1 cm) and Hatase et al. (2002b, 2004) (SCL ≤ 74.0 cm), respectively. In order to assess these reported values for the minimum size at maturity during nesting, we tested differences in haplotype frequencies among the following 3 size classes by using the exact test (Raymond & Rousset 1995) with 817 bp fragments: (1) SCL ≤ 69.1 cm (defined as 'juveniles'; n = 42), (2) 69.2 cm ≤ SCL ≤ 74.0 cm (defined as 'recruits'; n = 38), and (3) 74.1 cm ≤ SCL (defined as 'adults'; n = 27). For the above estimates and tests, we used Arlequin v3.5 (Excoffier & Lischer 2010) and set 500 000 steps in the Markov chain with a 100 000-step dememorization for exact tests. For all tests that required estimates of sequence divergence, we used the Tamura-Nei model of nucleotide substitutions, which was designed for control region sequences (Tamura & Nei 1993).

Mixed-stock analysis

Bayesian computation coupled with the Markov chain Monte Carlo (MCMC) estimation procedure was used for estimating the relative contributions to the Sanriku feeding aggregation of (1) each of 5 Japanese nesting rookeries reported in previous studies (Fig. 1; Hatase et al. 2002a, Watanabe et al. 2011), and (2) 2 geographic groups of these rookeries, the northern rookeries, i.e. Minabe and Kamouda, and the southern rookeries, i.e. Miyazaki, Yakushima, and Fukiagehama. In this study, we used the program BAYES (Pella & Masuda 2001) for analyzing the stock mixtures, and used the regional grouping option implemented in BAYES for the contribution to the Sanriku feeding aggregation from the northern and southern groups, respectively. Haplotypes not observed in the rookeries were removed from this analysis, discarding 2 of the 107 samples. Only Japanese nesting rookeries were used as sources of the feeding aggregation because the contribution of the rookeries of eastern Australia and New Caledonia (Boyle et al. 2009) was extremely low (mean < 0.8%) when they were included as sources. For each potentially contributing rookery, we ran the MCMC with 5 chains, each with 50 000 iterations, of which the first 25 000 were regarded as burn-in and discarded. The convergence of MCMC sampling was assessed using the Gelman-Rubin shrink factor (Gelman & Rubin 1992). This shrink factor provides an indication of convergence by comparing the variation within a single chain to the total variation among all the chains. Shrink-factor values greater than 1.2 indicate lack of convergence (Pella & Masuda 2001). Initially, individual chains were started with 95% of the mixed sample contributed by each of the source rookeries, and the rest were divided equally among the remaining rookeries. The Dirichlet prior distribution was set in 2 ways: a noninformative prior, in which the proportions of each rookery in the mixture are assumed to be equal; and an informative prior, in which the number of nests in each rookery (Watanabe et al. 2011) is taken into consideration. To estimate the possible factors relating to the contribution to the feeding aggregation, we tested the correlation between the contributions estimated with the noninformative prior and (1) the number of nests in each rookery or (2) the distance between each nesting rookery and the Sanriku coastal area, by using R v2.12.2 (R Development Core Team 2011).

RESULTS

We found 10 haplotypes by screening for polymorphisms within the 817 bp mtDNA control-region fragment of the 107 turtles (Fig. 2, Table 1). In the 817 bp fragment, 6 polymorphic sites outside the 350 bp fragment (Bowen et al. 1995) were detected. These polymorphic sites divided haplotypes B and C (Bowen et al. 1995) into 5 haplotypes (CcP-2.1, CcP-2.2, CcP-2.3, CcP-2.6, and CcP-2.7) and 2 haplotypes

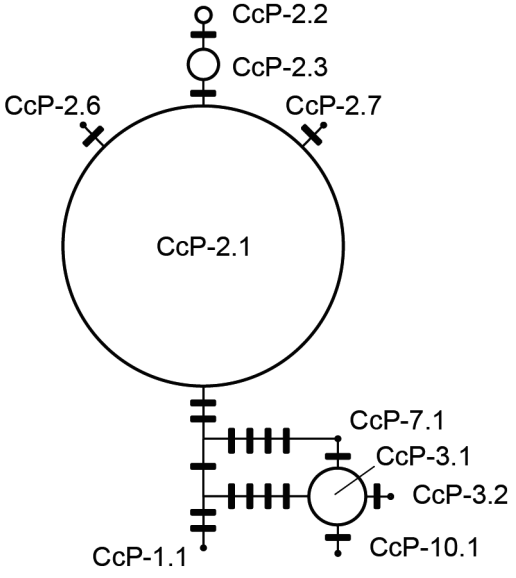

Fig. 2. *Caretta caretta.* The haplotype network for the Sanriku feeding aggregation based on an 817 bp fragment. The diameter of each circle is proportional to the number of individuals found for each haplotype. The solid bars indicate single nucleotide substitutions

Table 1. *Caretta caretta*. Frequencies of mtDNA haplotypes (GenBank accession numbers are shown in parentheses) in the Sanriku feeding aggregation and in the nesting grounds (Minabe, Kamouda, Miyazaki, Yakushima, Fukiagehama), Japan. Haplotype names for Sanriku (CcP system) are based on an 817 bp fragment and those for the nesting grounds (A, B, and C) are based on a 350 bp fragment. The haplotypes from the nesting grounds were obtained from Hatase et al. (2002a) (Minabe, Miyazaki, Yakushima, and Fukiagehama) and Watanabe et al. (2011) (Kamouda). Nests per season are the medians of the values from Watanabe et al. (2011) and were used as a prior for the mixed-stock analysis

Haplotype		(GenBank no.)	Feeding site	Nesting ground				
			Sanriku	Minabe	Kamouda	Miyazaki	Yakushima	Fukiagehama
A	CcP-1.1	(AB830477)	1	0	0	0	1	0
B	CcP-2.1	(AB830478)	74					
	CcP-2.2	(AB830479)	4					
	CcP-2.3	(AB830480)	8	99	19	33	77	21
	CcP-2.6	(AB831106)	1					
	CcP-2.7	(AB831107)	1					
C	CcP-3.1	(AB830481)	15					
	CcP-3.2	(AB830482)	1	3	1	13	11	1
CcP-7.1		(AB831108)	1	0	0	0	0	0
CcP-10.1		(AB831109)	1	0	0	0	0	0
Total			107	102	20	46	89	22
Nests per season				189	18	550	1100	72

(CcP-3.1 and CcP-3.2), respectively. One haplotype, CcP-1.1, contained the same 350 bp region as haplotype A (Bowen et al. 1995). We detected 2 additional polymorphic sites in the 350 bp fragment that differentiated CcP-7.1 or Cc-P10.1 from CcP-3.1. These haplotypes were deposited in GenBank (accession numbers: AB830477 to AB830482 and AB831106 to AB831109).

Haplotype diversity (h) and nucleotide diversity (π) estimates for the Sanriku feeding aggregation were $h = 0.4992 \pm 0.0543$ and $\pi = 0.002992 \pm 0.001812$. There were no significant differences in haplotype frequency among the years (exact test, $p > 0.05$ in all comparisons) or among size classes (exact test, $p > 0.05$ in all comparisons). Therefore, all samples were pooled in the subsequent mixed-stock analysis.

Bayesian estimates of the nesting colony origins of the Sanriku feeding aggregation are provided in Fig. 3. The Gelman-Rubin shrink factors were 1.03 or less, indicating convergence among the MCMC estimates. This result demonstrates that the 95% credible interval (CI) of the contribution from the Yakushima rookery alone to the Sanriku feeding aggregation was more than 0.00%, whether the informative prior was considered (mean = 72.93%, 95% CI = 13.43 to 99.98%) or not (mean = 51.45%, 95% CI = 0.05 to 98.61%) (Fig. 3a), although the lower limit of contribution from the noninformative prior (which assumed the same nesting population size) was 0.05%. In the group estimation, the 95% CI of the contribution from the southern rookeries to the Sanriku feeding aggregation was more than 0.00%, and the mean values of the contribution were 82.10% (95% CI = 26.57 to 99.99%) with the noninformative prior, and 95.69% (95% CI = 62.82 to 100.00%) with the informative prior (which considered the nesting population size) (Fig. 3b). There was a significant correlation between the estimated mean contribution with the noninformative prior and the number of nests in the rookery ($r = 0.955$, $p = 0.0114$), but not between the

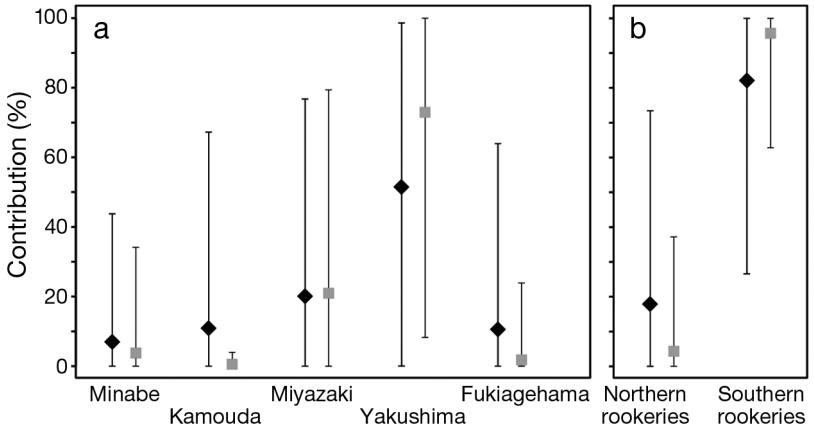

Fig. 3. *Caretta caretta*. Estimated contributions (%) of (a) each nesting rookery and (b) groups of rookeries to loggerhead turtles in the Sanriku feeding area. Results obtained using (◆) uninformative priors and (■) informative priors are represented. Bars indicate the 95% credible interval

estimated mean contribution and the geographic distance between Sanriku and each rookery ($r = 0.674$, $p > 0.05$).

DISCUSSION

In the Atlantic, the waters of Virginia and North Carolina in the USA are major habitats of large juvenile loggerhead turtles, and the juveniles seasonally migrate between them (Mansfield et al. 2009). Some post-nesting adults are also reported to migrate during summer to forage at the higher latitudes in Virginia and North Carolina (Hawkes et al. 2007). The Sanriku coastal area is located at a latitude similar to that of the Virginia coast (Chesapeake Bay, 38° 35′ N) and is considered to be utilized by large juveniles during the summer (Narazaki 2009). In the present study, 39.3% of the turtles had an SCL shorter than 69.2 cm, which is the minimum SCL of Japanese nesting loggerhead turtles reported by Kamezaki et al. (1995); 74.8% had an SCL shorter than 74.1 cm, which is the minimum SCL reported by Hatase et al. (2002b, 2004); and 98.1% had an SCL shorter than 82.1 cm, which is the mean SCL at maturity (Ishihara & Kamezaki 2011). Although the size of sea turtles at maturity is variable among individuals (Musick & Limpus 1997, Bjorndal et al. 2013), the wide size range of the individuals indicates that the feeding area in Sanriku may include both juveniles and adults.

The age of juvenile loggerhead turtles at recruitment to the coastal habitats is estimated to be 6 to 12 yr (Bjorndal et al. 2000, Scott et al. 2012). In addition, because the SCLs of the individuals in this study were longer than 49 cm, the loggerhead turtles in the Sanriku coastal area were estimated to be older than 10 yr, based on the age estimates outlined by Zug et al. (1995). These individuals are thought to probably migrate to the Sanriku coastal area after the transoceanic migration from Baja California. The population genetic structure in the Japanese rookeries was determined from the samples collected in 1994 to 1999, except those from Kamouda (Hatase et al. 2002a, Watanabe et al. 2011). Most of the samples in the present study were collected in 2007 to 2012, 8 to 18 yr after the sampling at the rookeries. Therefore, the data from these Japanese rookeries (Hatase et al. 2002a, Watanabe et al. 2011) provide temporally adequate sources for estimating the stock composition of the feeding aggregation, despite shared haplotypes. The number of nests per season in Japan varies among years (Kamezaki et al. 2003), possibly leading to the fluctuations in the results. However, no significant differences in haplotype frequencies were detected among the sampling years in the rookeries (Hatase et al. 2002a) and those in this study, supporting the stability of stock composition.

Most haplotypes detected in the Sanriku coastal area were consistent with haplotypes B and C (Bowen et al. 1995), dominant in the Japanese rookeries. Only 1 sample contained haplotype A, which has mainly been observed in the southwestern Pacific (Bowen et al. 1995, Boyle et al. 2009), but has also been detected in the Yakushima rookery (Hatase et al. 2002a). Two haplotypes, CcP-7.1 and CcP-10.1, have not been detected in previous studies (Hatase et al. 2002a, Boyle et al. 2009), indicating the existence of undersampled or unsampled rookeries. However, the present study estimated that there was little or no contribution from the rookeries in the Southern Hemisphere (data not shown), supporting the argument that all of the loggerheads inhabiting the North Pacific are derived from Japanese rookeries (Hatase et al. 2002a). The MSA estimated a high contribution from the southern rookeries of Japan, mainly from the Yakushima rookery, to the Sanriku feeding aggregation, although the distance between the southern Japanese rookeries and Sanriku is greater than the distance between the northern rookeries and Sanriku. The estimation of a high contribution from the Yakushima rookery might have been driven by haplotype A being detected in 1 sample from Sanriku. When haplotype A was removed from Sanriku, the MSA assigned a higher contribution from Miyazaki and a lower contribution from Yakushima, but the high contribution from the southern rookeries was valid (data not shown). Therefore, the Sanriku coastal area appears to be utilized by loggerhead turtles born in the southern Japanese rookeries relatively far from Sanriku. Both the MSAs using the informative prior (considering the number of nests) and the noninformative prior (assuming the same number of nests among the rookeries) showed a high contribution from the southern rookeries, supporting the validity of the results.

The MSA results highlight the contribution from the southern rookeries to Sanriku, but the contribution from the northern rookeries is not rejected. The higher contribution from the southern rookeries reflects the larger number of nests there, particularly in Yakushima, where the largest number of loggerhead turtles nest in Japan (Kamezaki et al. 2003, Watanabe et al. 2011), which might mask the contribution from the northern rookeries. In fact, the contribution from the Yakushima rookery was high when the number of nesting turtles was considered as a weighting factor.

In addition, the significant correlation between the contribution estimated with the noninformative prior and the number of nesting females supports this idea.

From the MSA results, 2 hypotheses can be proposed about the settlement and migration of large juvenile loggerheads to Japanese coastal areas. The first is that loggerhead turtles originating from the southern rookeries settle widely around Japanese coastal areas, including Sanriku, and move to the southern feeding areas near their natal beaches as they grow up. In the Atlantic, loggerhead turtles in the large juvenile stage are known to settle in feeding areas in the vicinity of their natal beaches, but the contribution of the southern nesting rookeries of loggerheads (South Florida) to the northern feeding aggregation (Virginia coastal area) has also been estimated (Bowen et al. 2004). The degree of philopatry in loggerhead turtles may increase with age (Bowen et al. 2005). This hypothesis is supported by the fact that the SCL of loggerhead turtles in Sanriku (mean ± SD: 70.9 ± 6.4 cm, range: 49.5 to 88.4 cm) is smaller than that in southern Muroto (mean ± SD: 75.7 ± 6.7 cm, range: 56.3 to 105.0 cm; Ishihara et al. 2011).

The second hypothesis is that the utilization of the Sanriku coastal area by individuals born in the southern rookeries reflects the feeding strategies of loggerhead turtles. When loggerhead turtles recruit to coastal feeding areas at the end of their developmental migration as large juveniles, they are hypothesized to settle in suitable feeding areas that they have encountered, possibly in oceanic habitats (Hatase et al. 2010, Eder et al. 2012). Some individuals may shift from oceanic to neritic habitats with age when they encounter better feeding areas (Eder et al. 2012). As there are no genetic differences between neritic foragers and oceanic foragers within Minabe and Yakushima rookeries, the difference in feeding strategy is assumed to indicate phenotypic plasticity (Watanabe et al. 2011). Juveniles that use the oceanic feeding strategy after the transoceanic migration, originating from the southern rookeries and possibly from the northern rookeries, are considered to migrate along the Kuroshio Current, as do some oceanic post-nesting adults nesting in Japan (Hatase et al. 2002b, 2007), analogous to the juveniles in the Atlantic migrating along the Gulf Stream (Mansfield et al. 2009). When juveniles that are transported to the north in the Kuroshio Current encounter the Sanriku coastal area, they settle or at least opportunistically forage in this area because of its high productivity. The smaller size of individuals in Sanriku may indicate that they have used the oceanic feeding strategy (Hatase et al. 2002b). These 2 hypotheses are not mutually exclusive. An analysis of the southern feeding aggregations of loggerhead juveniles in Japan (e.g. Muroto) will facilitate better understanding of their migration and provide validation for these hypotheses.

As there are some mature-sized loggerhead turtles in the Sanriku coastal area, the results might provide insight into the migration of adults. The absence of significant differences in haplotype frequencies among the size classes indicates that the composition of the aggregation does not change with size. As the females appear to have high site fidelity to feeding areas (Broderick et al. 2007), it may indicate that adults go back to the Sanriku coastal area after their breeding migration. According to tag-recapture data (Kamezaki et al. 1997), the post-nesting females that nest in Yakushima migrate mainly to Japanese coastal areas and the East China Sea, whereas, according to satellite telemetry (Hatase et al. 2007), some small adults migrate following the Kuroshio Current to the pelagic Pacific after nesting. These adult-sized loggerhead turtles migrating along the Kuroshio Current after their breeding migration may recruit to the Sanriku coastal area.

The shared haplotypes among the Japanese rookeries probably result from the bottlenecks (Hatase et al. 2002a) limiting the resolution of the MSA, especially because the 350 bp fragment data were used for the MSA. However, by using the 817 bp fragment, additional polymorphic sites have been detected in Atlantic (Monzón-Argüello et al. 2010, Shamblin et al. 2012) and Mediterranean loggerhead turtles (Yilmaz et al. 2011, Saied et al. 2012, Clusa et al. 2013). Although the haplotypes CcP-2.1 and CcP-3.1 were observed in most samples in Sanriku (69.2 and 14.0%, respectively), there were some variations in the traditional haplotypes B and C (e.g. CcP-2.2, CcP-2.3) that may characterize the Japanese nesting rookeries. Therefore, reanalysis of the population structure of nesting rookeries in Japan using the 817 bp fragment may enhance the resolution of the MSA.

Although the shared haplotypes limit the resolution, there was a strong contribution from the southern nesting rookeries of Japan to the northern Sanriku coastal area. The strong connectivity between loggerheads from the Sanriku feeding aggregation and the southern Japanese rookeries suggests that loggerhead turtles in the North Pacific generally settle in the Japanese coastal areas at the large juvenile stage, but not in the direct vicinity of their natal sites, and some juveniles that use an oceanic feeding strategy are recruited to the Sanriku coastal area.

Acknowledgements. This study could not have been achieved without great volunteers from the Fisheries Cooperative Association of Funakoshi Bay, Hirota Bay, Kamaishi Bay, Kamaishi-Tobu, Miyako, Ofunato, Okirai, Omoe, Otsuchi, Ryori, Sanriku-Yamada, Sasaki, Toni and Yoshihama, who provided assistance in collecting turtles. We thank Y. Fujiwara, S. Takuma, H. Murasaki and K. Onitsuka for their assistance in the field. We thank the 3 anonymous reviewers for their valuable comments on this manuscript. Field sampling was financially supported by a Grant-in-Aid for Science Research from JSPS and a grant from Bio-Logging Science, University of Tokyo (UTBLS). This study was partly supported by a Grant-in-Aid for JSPS Fellows to H.N. (25-1999).

LITERATURE CITED

Abreu-Grobois FA, Horrocks J, Formia A, Leroux R and others (2006) New d-loop primers which work for a variety of marine turtle species may increase the resolution capacity of mixed stock analyses. Presentation at the 26th Ann Symp Sea Turtle Biol Conserv, Crete, Greece, 2–8 April

Bjorndal KA, Bolten AB, Martins HR (2000) Somatic growth model of juvenile loggerhead sea turtles *Caretta caretta*: duration of pelagic stage. Mar Ecol Prog Ser 202:265–272

Bjorndal KA, Parsons J, Mustin W, Bolten AB (2013) Threshold to maturity in a long-lived reptile: interactions of age, size, and growth. Mar Biol 160:607–616

Bolker B, Okuyama T, Bjorndal K, Bolten A (2003) Sea turtle stock estimation using genetic markers: accounting for sampling error of rare genotypes. Ecol Appl 13:763–775

Bolten AB (2003) Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. In: Lutz PL, Musick JA, Wyneken J (eds) The biology of sea turtles, Vol II. CRC Press, Boca Raton, FL, p 243–257

Bowen BW, Kamezaki N, Limpus CJ, Hughes GH, Myelan AB, Avise JC (1994) Global phylogeography of the loggerhead turtle (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. Evolution 48:1820–1828

Bowen BW, Abreu-Grobois FA, Balazs GH, Kamezaki N, Limpus CJ, Ferl RJ (1995) Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. Proc Natl Acad Sci USA 92: 3731–3734

Bowen BW, Bass AL, Chow SM, Bostrom M and others (2004) Natal homing in juvenile loggerhead turtles (*Caretta caretta*). Mol Ecol 13:3797–3808

Bowen BW, Bass AL, Soares L, Toonen RJ (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). Mol Ecol 14:2389–2402

Boyle MC, FitzSimmons NN, Limpus CJ, Kelez S, Velez-Zuazo X, Waycott M (2009) Evidence for transoceanic migrations by loggerhead sea turtles in the southern Pacific Ocean. Proc R Soc Lond B Biol Sci 276:1993–1999

Broderick AC, Coyne MS, Fuller WJ, Glen F, Godley BJ (2007) Fidelity and over-wintering of sea turtles. Proc R Soc Lond B Biol Sci 274:1533–1538

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9: 1657–1659

Clusa M, Carreras C, Pascual M, Demetropoulos A and others (2013) Mitochondrial DNA reveals Pleistocenic colonisation of the Mediterranean by loggerhead turtles (*Caretta caretta*). J Exp Mar Biol Ecol 439:15–24

Eder E, Ceballos A, Martins S, Pérez-García H, Marín I, Marco A, Cardona L (2012) Foraging dichotomy in loggerhead sea turtles *Caretta caretta* off northwestern Africa. Mar Ecol Prog Ser 470:113–122

Encalada SE, Bjorndal KA, Bolten AB, Zurita JC and others (1998) Population structure of loggerhead turtle (*Caretta caretta*) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences. Mar Biol 130:567–575

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564–567

Gelman A, Rubin DB (1992) Inference from iterative simulation using multiple sequences. Stat Sci 7:457–511

Hatase H, Kinoshita M, Bando T, Kamezaki N and others (2002a) Population structure of loggerhead turtles, *Caretta caretta*, nesting in Japan: bottlenecks on the Pacific population. Mar Biol 141:299–305

Hatase H, Takai N, Matsuzawa Y, Sakamoto W and others (2002b) Size-related differences in feeding habitat use of adult female loggerhead turtles *Caretta caretta* around Japan determined by stable isotope analyses and satellite telemetry. Mar Ecol Prog Ser 233:273–281

Hatase H, Matsuzawa Y, Sato K, Bando T, Goto K (2004) Remigration and growth of loggerhead turtles (*Caretta caretta*) nesting on Senri Beach in Minabe, Japan: life-history polymorphism in a sea turtle population. Mar Biol 144:807–811

Hatase H, Omuta K, Tsukamoto K (2007) Bottom or mid-water: alternative foraging behaviours in adult female loggerhead sea turtles. J Zool (Lond) 273:46–55

Hatase H, Omuta K, Tsukamoto K (2010) Oceanic residents, neritic migrants: a possible mechanism underlying foraging dichotomy in adult female loggerhead turtles (*Caretta caretta*). Mar Biol 157:1337–1342

Hawkes LA, Broderick AC, Coyne MS, Godfrey MH, Godley BJ (2007) Only some like it hot — quantifying the environmental niche of the loggerhead sea turtle. Divers Distrib 13:447–457

Ishihara T, Kamezaki N (2011) Size at maturity and tail elongation of loggerhead turtles (*Caretta caretta*) in the North Pacific. Chelonian Conserv Biol 10:281–287

Ishihara T, Kamezaki N, Matsuzawa Y, Iwamoto F and others (2011) Reentry of juvenile and subadult loggerhead turtles into natal waters of Japan. Curr Herpetol 30: 63–68

IUCN (International Union for Conservation of Nature) (2013) IUCN Red List of Threatened Species version 2013.2. www.iucnredlist.org (accessed on 16 January 2014)

Kamezaki N, Goto K, Matsuzawa Y, Nakajima Y, Oomuta K, Sato K (1995) Carapace length and width of the loggerhead turtle, *Caretta caretta* nested in the coast of Japan. Umigame Newsl Jpn 26:12–13 (in Japanese)

Kamezaki N, Miyawaki I, Suganuma H, Omuta K and others (1997) Post-nesting migration of Japanese loggerhead turtle, *Caretta caretta.* Wildl Conserv Jpn 3:29–39 (in Japanese with English Abstract)

Kamezaki N, Matsuzawa Y, Abe O, Asakawa H and others (2003) Loggerhead turtles nesting in Japan. In: Bolten AB, Witherington BE (eds) Loggerhead sea turtles. Smithsonian Institution Press, Washington, DC, p 210–217

Limpus CJ (2004) A biological review of Australian marine turtles I: Loggerhead turtle, *Caretta caretta* (Linnaeus). Queensland Environmental Protection Agency, Brisbane

Limpus CJ, Limpus DJ (2003) Loggerhead turtles in the equatorial and southern Pacific Ocean: a species in decline. In: Bolten AB, Witherington BE (eds) Loggerhead sea turtles. Smithsonian Institution Press, Washington, DC, p 199–209

Mansfield KL, Putman NF (2013) Oceanic habits and habitats: *Caretta caretta*. In: Wyneken J, Lohmann KJ, Musick JA (eds) The biology of sea turtles, Vol III. CRC Press, Boca Raton, FL, p 189–210

Mansfield KL, Saba VS, Keinath JA, Musick JA (2009) Satellite tracking reveals a dichotomy in migration strategies among juvenile loggerhead turtles in the Northwest Atlantic. Mar Biol 156:2555–2570

McClellan CM, Read AJ (2007) Complexity and variation in loggerhead sea turtle life history. Biol Lett 3:592–594

Monzón-Argüello C, Rico C, Naro-Maciel E, Varo-Cruz N, López P, Marco A, López-Jurado LF (2010) Population structure and conservation implications for the loggerhead sea turtle of the Cape Verde Islands. Conserv Genet 11:1871–1884

Musick JA, Limpus CJ (1997) Habitat utilization and migration in juvenile sea turtles. In: Lutz PL, Musick JA (eds) The biology of sea turtles. CRC Press, Boca Raton, FL, p 137–163

Narazaki T (2009) Diving behaviour of loggerhead turtles, *Caretta caretta*, migrating to the northern Pacific coast of Japan. PhD dissertation, University of Tokyo

Narazaki T, Sato K, Abernathy KJ, Marshall GJ, Miyazaki N (2009) Sea turtles compensate deflection of heading at the sea surface during directional travel. J Exp Biol 212: 4019–4026

Narazaki T, Sato K, Anernathy KJ, Marshall GJ, Miyazaki N (2013) Loggerhead turtles (*Caretta caretta*) use visions to forage on gelatinous prey in mid-water. PLoS ONE 8: e66043

Nichols W, Resendiz A, Seminoff JA, Resendiz B (2000) Transpacific migration of a loggerhead turtle monitored by satellite telemetry. Bull Mar Sci 67:937–947

Pella J, Masuda M (2001) Bayesian methods for analysis of stock mixtures from genetic characters. Fish Bull 99: 151–167

Polovina JJ, Balazs GH, Howell EA, Parker DM, Seki MP, Dutton PH (2004) Forage and migration habitat of loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*) sea turtles in the central North Pacific Ocean. Fish Oceanogr 13:36–51

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Saied A, Maffucci F, Hochscheid S, Dryag S and others (2012) Loggerhead turtles nesting in Libya: an important management unit for the Mediterranean stock. Mar Ecol Prog Ser 450:207–218

Sakamoto W, Bando T, Arai N, Baba N (1997) Migration paths of the adult female and male loggerhead turtles *Caretta caretta* determined through satellite telemetry. Fish Sci 63:547–552

Scott R, Marsh R, Hays GC (2012) Life in the really slow lane: loggerhead sea turtles mature late relative to other reptiles. Funct Ecol 26:227–235

Shamblin BM, Bolten AB, Bjorndal KA, Dutton PH and others (2012) Expanded mitochondrial control region sequences increase resolution of stock structure among North Atlantic loggerhead turtle rookeries. Mar Ecol Prog Ser 469:145–160

Sugimoto T, Tameishi H (1992) Warm-core rings, streamers and their role on the fishing ground formation around Japan. Deep-Sea Res A 39:S183–S201

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10: 512–526

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method. Mol Biol Evol 28: 2731–2739

Watanabe KK, Hatase H, Kinoshita M, Omuta K and others (2011) Population structure of the loggerhead turtle *Caretta caretta*, a large marine carnivore that exhibits alternative foraging behaviors. Mar Ecol Prog Ser 424:273–283

Yilmaz C, Turkozan O, Bardakci F (2011) Genetic structure of loggerhead turtle (*Caretta caretta*) populations in Turkey. Biochem Syst Ecol 39:266–276

Zug GR, Balazs GH, Wetherall JA (1995) Growth in juvenile loggerhead sea turtles (*Caretta caretta*) in the North Pacific pelagic habitat. Copeia 1995:484–487

Editorial responsibility: Jeffrey Seminoff, La Jolla, California, USA

Submitted: August 20, 2013; Accepted: January 24, 2014
Proofs received from author(s): April 4, 2014