

# Stock composition of green turtles *Chelonia mydas* foraging in the Ryukyu Archipelago differs with size class

Tomoko Hamabata<sup>1,8,\*</sup>, Hideaki Nishizawa<sup>2</sup>, Isao Kawazu<sup>3,4</sup>, Kazunari Kameda<sup>5</sup>, Naoki Kamezaki<sup>5,6,7</sup>, Tsutomu Hikida<sup>1</sup>

<sup>1</sup>Graduate School of Science, Kyoto University, Kitashirakawa Oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan

<sup>2</sup>Graduate School of Informatics, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto 606-8501, Japan

<sup>3</sup>Okinawa Churashima Research Center, 888 Ishikawa, Motobucho, Okinawa 905-0206, Japan

<sup>4</sup>Okinawa Churaumi Aquarium, 424 Ishikawa, Motobucho, Okinawa 905-0206, Japan

<sup>5</sup>Sea Turtle Association of Japan, 5-17-18 Nagaomotomachi Hirakata, Osaka 573-0163, Japan

<sup>6</sup>Faculty of Biosphere-Geosphere Science, Okayama University of Science, 1-1 Ridaimae Kita, Okayama 700-0005, Japan

<sup>7</sup>Suma Aqualife Park Kobe, 1-3-5 Wakamiyacho Suma-ku, Kobe, Hyogo 654-0049, Japan

<sup>8</sup>Present address: Graduate School of Life Science, Tohoku University, 6-3 Aramaki Aza Aoba, Aoba-ku, Sendai 980-8578, Japan

**ABSTRACT:** The size composition of green turtles *Chelonia mydas* in their foraging grounds (FGs) in Japanese waters of the northwestern Pacific Ocean has suggested that turtles shift their FGs according to their size. To elucidate the size-related movements among FGs, we examined whether the origin of turtles differed depending on their size at 2 FGs in the Ryukyu Archipelago by performing mixed stock analysis using a 380 bp fragment of the mitochondrial DNA. At both sites, the Japanese stock primarily contributed to the 50–70 cm straight carapace length (SCL) size class, while the contribution of the southern stock from Micronesia and the Marshall Islands was higher in turtles <50 and >70 cm SCL. Since the above size-related differences in stock contribution were maintained for at least a decade, they were not attributable to the population trends in the Japanese stock. The higher contribution of the Japanese stock to the 50–70 cm SCL class suggests the possibility of juvenile natal homing by turtles from the Japanese stock, possibly from the main islands of Japan. The shift in the contribution of different stocks of origin to turtles >70 cm SCL might have resulted from the movements of turtles to settle in their adult FGs. Additionally, the present results revealed that the Ryukyus are situated at the northern limit of the FGs for turtles from southern stocks. We hypothesize that this boundary may be created by a lack of adaptation of turtles from southern stocks to northern seasonal water temperatures.

**KEY WORDS:** Size-related movement · Mixed stock analysis · Distribution boundary · Mitochondrial DNA · Green turtle

Resale or republication not permitted without written consent of the publisher

## INTRODUCTION

Despite the fact that large marine species are important for the maintenance of marine ecosystems (Jackson 2008), current extinction risks are especially high for large marine animals (Payne et al. 2016). Information on the movements and habitat use of such species is often inferred based on inadequate

evidence (Stewart et al. 2016) due to the difficulty in tracking highly migratory species or in data collection for species with a long life cycle. Understanding the habitat utilization and scales of movement among habitats or ecosystems throughout an organism's lifetime is vital to the conservation of both its species and the marine ecosystems of which it is a part.

The green turtle *Chelonia mydas* is the largest hard-shelled sea turtle of warm tropical and sub-tropical waters worldwide. This species has an important role in promoting nutrient cycling in seagrass ecosystems (Jackson 2008). Mature female *C. mydas* lay eggs on beaches during their reproductive season. Tagging and satellite tracking studies have revealed that nesting females repeatedly make reproductive migrations between the same foraging grounds (FGs) and nesting beaches (e.g. Limpus et al. 1992). In addition, genetic studies have supported the existence in these turtles of a pattern of behavior called 'natal homing', in which females return to their area of origin to reproduce. Hatchlings emerge from nests deposited on beaches, and thereafter enter a period of developmental migration. They spend their first few years of life in an epipelagic (or oceanic) environment, living as carnivores (Reich et al. 2007). Juvenile turtles that attain straight carapace lengths (SCLs) of 25 to 30 cm in the Atlantic Ocean and 30 to 40 cm in the Pacific Ocean (Limpus et al. 2005) typically colonize seagrass/algal beds. For green turtles, which take decades to mature (Zug et al. 2002), seagrass/algal beds are an important ecosystem in which they spend the vast majority of their life after the initial epipelagic stage (reviewed in Bolten 2003). However, the habitats where they live throughout their life, especially during their immature phase, have not been completely clarified due to the difficulty of carrying out long-term tracking in this species.

Investigations of foraging green turtles in the Cedar Key Crystal River area of the Gulf coast of peninsular Florida, which commenced in the 1950s, showed that immature turtles dominated foraging aggregations there (Carr & Caldwell 1956). Similar assemblages exhibiting different size peaks have been found in several Atlantic regions (e.g. Bermuda), and immature turtles tagged in immature-dominated FGs were later recaptured in distant FGs used primarily by adult green turtles (e.g. Nicaragua), suggesting that the species inhabits multiple FGs over the course of long-distance developmental migrations (Meylan et al. 2011 and references therein). In contrast, some Pacific foraging aggregations exhibit broader size distributions, with both immature and adult turtles foraging along the coasts of eastern Australia (e.g. Jensen et al. 2016), the Palmyra Atoll of the central Pacific (Sterling et al. 2013), and Hawaii (Balazs 1980), although some differences in the habitats of immature and adult turtles are apparent within a FG (Balazs 1980). However, some studies have indicated that several foraging aggregations in the Pacific comprised or lacked tur-

bles of a specific size range (Hirth 1992, Hayashi & Tsuji 2008, Pilcher 2010, Joseph et al. 2016), suggesting that some populations of Pacific green turtles, like the Atlantic green turtle, continue to migrate after the epipelagic stage. Although sporadic reports of movements among FGs exist (e.g. Hayashi 2016), few data are available to clarify the movements of green turtles among FGs in the Pacific Ocean.

Green turtles with a wide range of body sizes are also present in the Japanese waters of the northwestern (NW) Pacific. Foraging turtles are found from the Ryukyu Archipelago, Japan (the Ryukyus), in the south to the northern coasts of the main islands of the Japanese archipelago (Fukuoka et al. 2015), which are located beyond the northernmost nesting sites in Ogasawara (Kondo et al. 2017) and the Ryukyus (Kamezaki 1989, Kikukawa et al. 1999). The size compositions of green turtles in the FGs along the coasts of the Japanese main islands show a bi-modal distribution, with peaks around 45 and 75 cm SCL, although turtles of 50 to 70 cm SCL also occur in these areas but are much less abundant (Hamabata et al. 2015). Notably, no turtles of 50 to 55 cm SCL have been caught around Okinawa, in the central Ryukyus (Hayashi & Tsuji 2008, Hayashi & Nishizawa 2015). In contrast, the size composition of turtles around the Yaeyama Islands, the southern Ryukyus, shows a unimodal distribution dominated by those of 40.0 to 49.9 cm SCL (Kameda et al. 2017). These variations in size composition among Japanese FGs strongly suggest that green turtles foraging in Japanese waters, like those in the Atlantic Ocean, also continue to shift FGs with size after the epipelagic stage.

Studies using mixed-stock analysis (MSA) (Pella & Masuda 2001) based on the mitochondrial DNA (mtDNA) haplotype frequencies in different rookeries, which differ among nesting regions because of natal homing by mature females (Bowen et al. 1992), have revealed the occurrence and nature of connectivity among rookeries and FGs of marine turtles worldwide. Such mtDNA haplotype frequencies have been determined for major green turtle rookeries in the Pacific (Jensen et al. 2016 and references therein), and assigned to baselines (i.e. candidate source rookeries) for Pacific foraging aggregations in MSA. These analyses have already shown that while both Japanese turtles and turtles that originated in some other Pacific rookeries at lower latitudes forage around the Ryukyus (Nishizawa et al. 2013), green turtles foraging along the coasts of the Japanese main islands are mainly those originating from Japanese rookeries (Nishizawa et al. 2013, 2014); further, the origins of turtles did not differ between turtles <50

and >70 cm SCL in previous studies (Hamabata et al. 2015). The low rate of occurrence of turtles with 50–70 cm SCL that originated in Japanese rookeries on the coasts of the Japanese main islands (Hamabata et al. 2015) suggests that these turtles emigrate to other FGs once they grow out of this size class.

In addition to simple connectivity between FGs and rookeries, MSA has also revealed the temporal variations of source rookeries for turtles within the same FGs (e.g. Bjorndal & Bolten 2008). For example, in the Pacific region, Jensen et al. (2016) showed that the contributions of the northern Great Barrier Reef (GBR) stock to the foraging aggregations of small immature turtles in FGs of the northern GBR decreased in the late 2000s compared to in the early 1990s, which suggested that reduced hatching success for some decades in the main rookery at Raine Island resulted in reduced recruitment into the northern GBR feeding ground from this stock. Thus, studies applying MSA can provide useful insights into the demography of foraging turtles, and application of MSA to different size classes is expected to provide better information on the movements of foraging turtles in Japanese waters. To explore the size-related shift in FGs after the initial epipelagic stage in foraging turtles in Japanese waters, we investigated the differences in the origins of turtles in 2 FGs in the Ryukyus using size-class-specific MSA. To obtain reliable results, large sample sizes are usually required—not only because foraging aggregations are commonly mixtures of turtles from various regions, but also because some breeding stocks often share widely distributed mtDNA haplotypes, reflecting historical immigration. Therefore, we increased the sample size from those in previous studies by Hamabata et al. (2009), Nishizawa et al. (2013), and Hayashi & Nishizawa (2015), and compared the origins of turtles in 2 FGs in the Ryukyus using size-specific MSA. Additionally, because the larger sample size enabled more reliable MSA, this allowed us to identify the northern boundaries of foraging distributions in turtles from southern stocks. We also discuss factors that may influence the determination of the northern foraging boundary of southern stocks in the Ryukyus.

## MATERIALS AND METHODS

### Samples

In this study, mtDNA haplotypes of 480 green turtles at 2 study sites in the Ryukyu Archipelago,

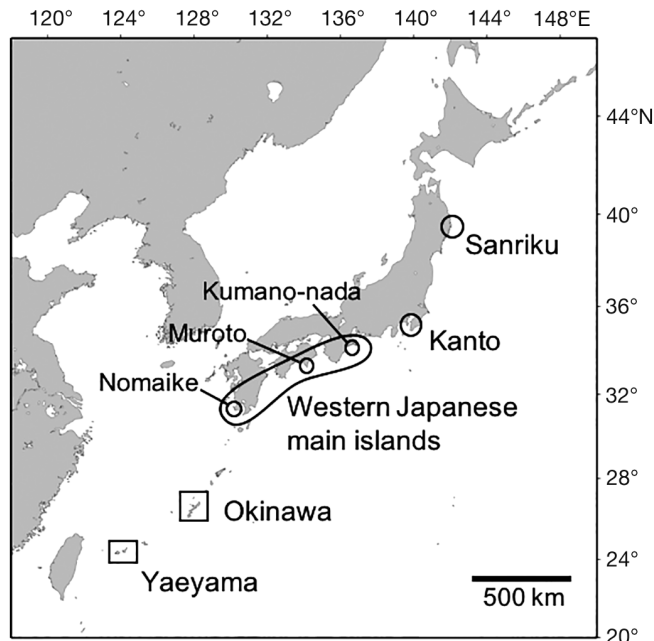


Fig. 1. Yaeyama and Okinawa green turtle foraging grounds (squares) and other foraging grounds along the coasts of the Japanese main islands (circles)

Japan, were used for analyses: 376 individuals from the Yaeyama Group (24° 18' N, 124° 1' E; Yaeyama FG) and 104 from Okinawa Island (26° 50' N, 127° 80' E; Okinawa FG) (Fig. 1). Of the 376 individuals in the Yaeyama FG, 159 and 142 individuals were the same specimens used by Hamabata et al. (2009) and Nishizawa et al. (2013), respectively, and 75 individuals sampled in 1999, 2007–2008, and 2011 were added in this study (see Table S1 in the Supplement at [www.int-res.com/articles/suppl/m600p151\\_supp.xlsx](http://www.int-res.com/articles/suppl/m600p151_supp.xlsx)). Of the 104 individuals in the Okinawa FG, 38 and 20 individuals were the same specimens used by Hayashi & Nishizawa (2015) and Nishizawa et al. (2013), respectively, and 46 individuals were added in this study (Table S1). Body sizes, measured as SCL from shell notch to tip (Bolten 1999), were available for 366 turtles in the Yaeyama FG and 84 turtles in the Okinawa FG. However, 4 turtles captured in May and July in the Yaeyama FG and 14 captured from April to July in the Okinawa FG were of the minimum adult size (SCLs of 79.4 cm in males and 82.1 cm in females; Tachikawa 1991) and thus may have been breeding migrants since breeding occurs during these months in the Ryukyus. These 18 turtles were excluded from all analyses. Size distribution histograms based on SCLs were produced for animals at both sites.

### Haplotype identification and genetic composition

Skin or muscle samples were preserved in 99% ethanol, and blood samples were mixed with heparin and frozen prior to analysis. Phenol/chloroform extraction was used to isolate DNA from skin and muscle samples, and DNeasy Blood and Tissue Kits were used to extract DNA from blood samples. Polymerase chain reaction (PCR) amplification and sequencing were performed using the primers LCM15382 and H950, which amplified an 820 bp fragment including a partial sequence of tRNA-Pro and the 5' end of the mtDNA control region (Abreu-Grobois et al. 2006). The PCR and cycle sequencing methods used were as described previously by Hamabata et al. (2015). Sequences were assembled using DNA BASER, manually checked, and aligned using the Muscle subroutine of MEGA 5 software (Tamura et al. 2011). All sequences were compared to previously described haplotypes in the Pacific of 380, 500, and 860 bp sequences of the mtDNA control region (Norman et al. 1994, Chassin-Noria et al. 2004, Dethmers et al. 2006, 2010, Dutton et al. 2008, 2014a,b, Cheng et al. 2008, Hamabata et al. 2009, 2015, Nishizawa et al. 2011, 2013, Ng et al. 2014, Naro-Maciel et al. 2014, Jensen et al. 2016).

To allow comparison with available haplotype data from breeding stocks, all sequences were truncated to 380 bp prior to further analyses. Differences in genetic compositions among the 2 FGs were further explored; we included data from the FG of the western Japanese main islands ( $n = 162$ ) reported by Hamabata et al. (2015), the Kanto FG ( $n = 47$ ) reported by Nishizawa et al. (2013), and the Sanriku FG ( $n = 39$ ) reported by Nishizawa et al. (2014), and we separately evaluated size classes within all Ryukyu foraging aggregations. Size classification followed Hamabata et al. (2015): size class I,  $SCL < 50$  cm; size class II,  $50 \text{ cm} \leq SCL < 70$  cm; and size class III,  $SCL \geq 70$  cm. The resultant sample sizes were: (I)  $n = 188$ , (II)  $n = 117$ , and (III)  $n = 59$  in the Yaeyama FG, and (I)  $n = 28$ , (II)  $n = 15$ , and (III)  $n = 27$  in the Okinawa FG (Table S2 in the Supplement). In additional sampling, relatively smaller individuals were released without tag attachments and sampling in a pound net on the western coast of Okinawa, and thus the size composition at this site was biased toward larger sizes. In addition, data on the point localities of many Yaeyama specimens were lacking. Thus, although turtles were captured over several years at different locations, we could not explore variations in size and genetic compositions on local scales. All samples were regionally pooled for evaluation only of differ-

ences among size classes. Differences in the haplotype frequencies of foraging aggregations (by region and size class) were examined using the Exact test of population differentiation (Raymond & Rousset 1995) and the pairwise  $F_{ST}$  test of haplotype frequency. All Exact tests featured a Markov chain of 500 000 steps, including 10 000 dememorization steps. Pairwise  $F_{ST}$  values were computed via 10 000 random permutations performed by ARLEQUIN version 3.5. (Excoffier & Lischer 2010). All p-values of multiple genetic composition comparisons were corrected using the sequential Bonferroni method. The haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) in each FG were estimated using the Tamura-Nei model (Tamura & Nei 1993) of nucleotide substitution contained in ARLEQUIN.

### MSA

We used MSA to estimate the contributions of green turtle breeding stocks in the Indo-Pacific region to different size classes of green turtles at the Yaeyama and Okinawa FGs separately. We also estimated the contributions to the Ryukyu foraging aggregations without reference to size. Two different Bayesian methods were used: many-to-one analysis employing BAYES software, which examined each FG size class independently (Pella & Masuda 2001), and many-to-many analysis using the MIXSTOCK package in R software, which allowed for simultaneous evaluation of multiple FGs and stocks (Bolker et al. 2007). Both methods were carried out using uninformative priors assuming that each rookery was equally able to contribute to foraging aggregations, and informative priors incorporating the relative size of each stock based on reports by Jensen et al. (2016), Abe et al. (2003), Cheng et al. (2008), Ng et al. (2014), Hamabata et al. (2014), and Kondo et al. (2017). Although more than 30 stocks divided into 6 distinct population segments (DPSs) (DPS 6 to 11 in Seminoff et al. 2015) have been identified from the East Indian Ocean to the Pacific Ocean, the posterior probability distributions of MSA based on all individual stocks did not converge. We employed the baseline narrowed down to 12 stocks selected based on 3 criteria: (1) 3 Japanese stocks were lumped together as one regional source (hereafter referred to as the Japanese stock), because they shared many unique haplotypes that seemed to make it difficult to converge; (2) Taiwan and Hong Kong were also lumped together, because they share haplotypes and the stock size in Hong Kong was too small to evaluate independently;

and (3) stocks that are geographically distant from Japan were excluded, because they often shared only haplotypes widespread in the Indo-Pacific region (such as Cmp20 or Cmp49) with the present foraging aggregations, or they shared some unique haplotypes (such as Cmp44, Cmp47, or central and eastern Pacific haplotypes) with the present aggregations, but the number of individuals was too small ( $n = 1$  to  $5$ ) to discuss the size-specific contributions from such stocks. Relatively small contributions of the removed stocks to foraging aggregations in Japan were already reported in a previous study (Nishizawa et al. 2013), and thus it was reasonable to exclude these stocks. As a result, the baseline for MSA consisted of 12 stocks: 1 lumped Japanese stock, plus 6 stocks in DPS 6 and 5 stocks in DPS 7 (sensu Seminoff et al. 2015) (Fig. 2). Eight minor haplotypes shared only with removed stocks were excluded from MSA, as were with 17 orphan haplotypes (i.e. haplotypes found only in foraging turtles) (see Tables S2 & S3 in the Supplement). We also estimated the contributions from Japan, DPS 6 and DPS7, by using the group estimates option in BAYES. We examined variations in source contributions to the Yaeyama FG for the 3 years in which sample sizes were  $>50$  ( $n = 90$  in 1997,  $n = 51$  in 1999, and  $n = 52$  in 2011), but annual variations in the Okinawa FG were not examined because of the small sample size. Temporal comparisons of size class II of the Yaeyama

FG were made between the late 1990s and 2005 to 2011. MSAs using the same baseline were carried out for 3 other FGs in Japan (the western Japanese main islands, Kanto, and Sanriku) without reference to size classes. For all MSAs, 12 chains were run that featured 60 000 to 200 000 Markov chain Monte Carlo (MCMC) steps, with burn-ins of 30 000 to 100 000 runs performed to calculate posterior distributions. Gelman and Rubin shrink factor diagnostics were applied to confirm that the posterior probability distributions of all chains had converged (shrink factor  $< 1.2$ ).

## RESULTS

### Size compositions

Both of the foraging sites contained both juvenile and adult turtles (based on SCL). The smallest turtles had an SCL of 33.0 cm in the Yaeyama FG and 31.0 cm SCL in the Okinawa FG. The size distribution of the Yaeyama sample exhibited a major peak at 40.0 to 44.9 cm and a minor peak at 70.0 to 74.9 cm (Fig. 3A), and showed similar composition to a previous observation at this site (Kameda et al. 2017). The size distribution of the Okinawa sample exhibited a clear peak at 40.0 to 44.9 cm. Turtles with 50.0 to 54.9 cm SCL were the least common, and the frequencies then increased as SCL increased to 90 cm, with small fluctuations (Fig. 3B). Since some of turtles  $<50$  cm SCL were released without sampling on the western coast of Okinawa in our additional sampling, the number of turtles  $<50$  cm SCL in this FG might have been less than that in the original distribution. However, the overall size composition of turtles in the Okinawa FG was similar to that in a previous report from the same site (Hayashi & Tsuji 2008), and the sampling bias in one locality was considered to be negligible. Thus, each sample set seemed to be sufficiently representative of each FG to conduct further analyses.

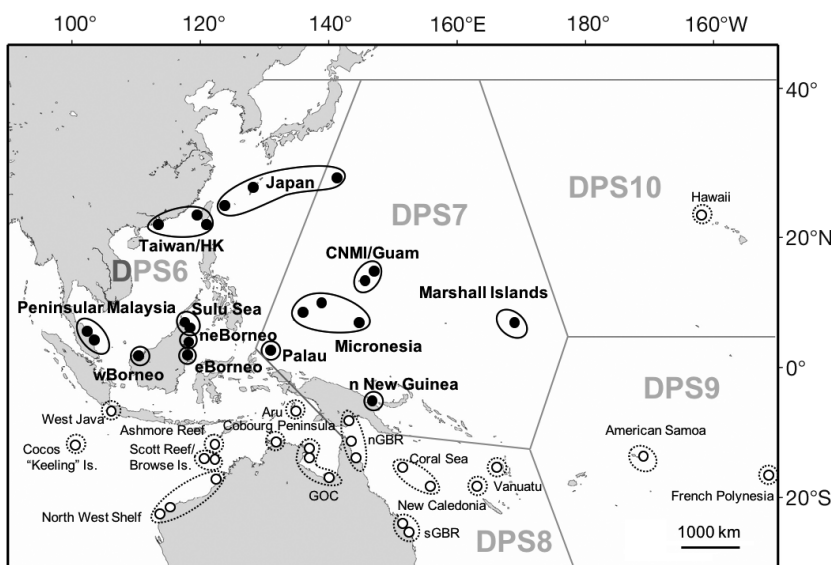


Fig. 2. Green turtle breeding stocks (black and white dots circled with lines) and distinct population segments (DPS; gray lines). Black dots circled with lines: stocks used in mixed-stock analysis (MSA); white dots circled with dashed lines: stocks excluded from MSA. Rookery data are from Dethmers et al. (2006), Cheng et al. (2008), Dutton et al. (2014a,b), Ng et al. (2014), Nishizawa et al. (2011, 2013), Hamabata et al. (2014), and Jensen et al. (2016)

### Genetic compositions

Among the 121 samples added in this study ( $n = 75$  from Yaeyama and

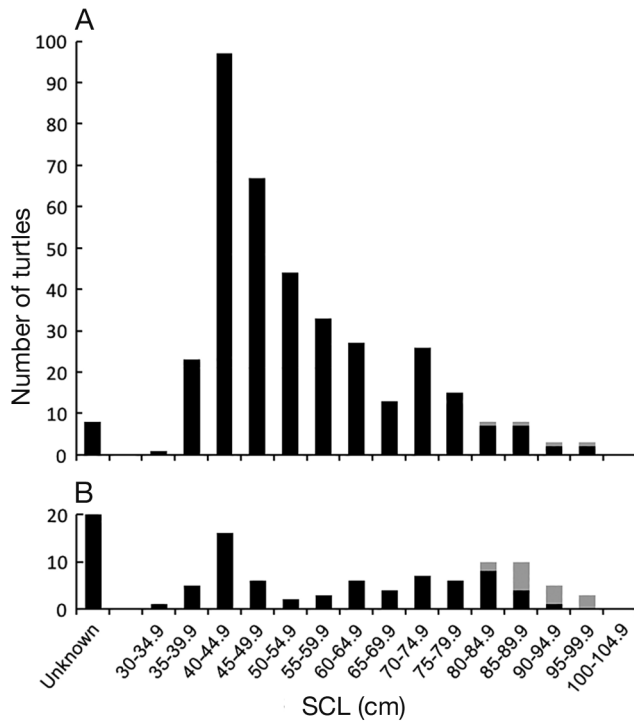


Fig. 3. Size distribution (straight carapace length; SCL) of all green turtles *Chelonia mydas* sampled at foraging grounds at (A) Yaeyama and (B) Okinawa. Gray bars: possible breeding migrants excluded from mixed-stock analysis (MSA)

$n = 46$  from Okinawa), only one from the Yaeyama FG had a novel haplotype, designated CmP214.1 (GenBank accession number LC309275). Thus, 32 haplotypes were recorded from the total sample of 376 individuals in the Yaeyama FG, and all haplotypes were represented in all size classes. A total of 21 haplotypes were identified among the 104 individuals in the Okinawa FG, 3 of which were identified only in possible breeding migrants and 2 of which were evident in turtles of unknown size (see Table S2). The number of individuals with orphan haplotypes was 10 in the Yaeyama FG (2.8% of the

total) and 4 in the Okinawa FG (5.7% of the total) (Table S2). The number of individuals with haplotypes removed from MSA, including orphan haplotypes, was 23 in the Yaeyama FGs (6.3% of the total), but there were no individuals with haplotypes removed from MSA except for orphan haplotypes in the Okinawa FG (Table S2). The  $h$  and  $\pi$  diversities in the Okinawa FGs ( $n = 70$ ,  $h = 0.849$ ,  $\pi = 0.034$ ) were almost the same as those in the Yaeyama FGs ( $n = 364$ ,  $h = 0.864$ ,  $\pi = 0.034$ ). These values for the Ryukyu FGs were all higher than those of other FGs around the Japanese main islands (Table 1). Neither the Exact nor the  $F_{ST}$  test supported a significant difference in haplotype frequency between the Yaeyama and Okinawa FGs, but they did support such a difference between the 2 Ryukyu FGs and the other 3 FGs around the Japanese main islands (Table 2A). The Exact test revealed subtle differences in genetic composition between size classes I and II of Yaeyama turtles ( $p = 0.026$ ), but this was not supported after sequential Bonferroni correction (Table 2B). No significant difference in genetic composition was observed among size classes in the Okinawa sample (Table 2C).

### MSA

The MSA using rookery data from the 12 stocks showed that the estimated contributions made by several stocks differed according to the many-to-one and many-to-many analyses (Table S4 in the Supplement). The many-to-many analysis apparently underestimated the contribution of the local Japanese stock, which shared many endemic haplotypes with the foraging aggregations in the Ryukyus, and overestimated the contributions from remote stocks (northern New Guinea). Similarly inconsistent results in many-to-many analysis were reported by Jensen

Table 1. Total sample sizes ( $n$ ) in each Japanese green turtle foraging ground studied. The number of haplotypes ( $H$ ), haplotype diversities ( $h$ , mean  $\pm$  SD), and nucleotide diversities ( $\pi$ , mean  $\pm$  SD) of each foraging aggregation were estimated based on 380 bp mtDNA marker sequences

Locality	$n$	$H$	$h$	$\pi$	Sources
Yaeyama (all)	376	32	$0.862 \pm 0.010$	$0.034 \pm 0.017$	Hamabata et al. (2009), Nishizawa et al. (2013), present study
Yaeyama (size-measured)	364	32	$0.864 \pm 0.010$	$0.034 \pm 0.017$	
Okinawa (all)	104	21	$0.854 \pm 0.019$	$0.034 \pm 0.017$	Hayashi & Nishizawa (2015), present study
Okinawa (size-measured)	70	16	$0.849 \pm 0.026$	$0.034 \pm 0.017$	
Western Japanese main islands	162	25	$0.681 \pm 0.038$	$0.023 \pm 0.012$	Hamabata et al. (2015)
Kanto	47	11	$0.744 \pm 0.045$	$0.031 \pm 0.016$	Nishizawa et al. (2013)
Sanriku	39	9	$0.648 \pm 0.075$	$0.023 \pm 0.012$	Nishizawa et al. (2014)

Table 2. Pairwise comparisons (below the diagonal: p-values of the Exact test; above the diagonal:  $F_{ST}$  values with p-values) of (A) the Japanese foraging ground (FG) aggregations; and of size-specific foraging aggregations from (B) Yaeyama and (C) Okinawa based on 380 bp marker sequences from size-measured green turtles. Data for the FG of the western Japanese main islands (Nomaie, Muroto, and Kumano-nada) are from Hamabata et al. (2015), and those for the Kanto FG and Sanriku FG are from Nishizawa et al. (2013) and Nishizawa et al. (2014), respectively. (\*) indicates that statistical significance was evident after sequential Bonferroni corrections

(A)	n	Yaeyama	Okinawa	W Japanese main islands	Kanto	Sanriku
Yaeyama	364		-0.005 (p = 0.889)	0.114 (p < 0.001*)	0.062 (p < 0.001*)	0.110 (p < 0.001*)
Okinawa	70	0.437		0.127 (p < 0.001*)	0.059 (p < 0.001*)	0.121 (p < 0.001*)
W Japanese main islands	162	<0.001*	<0.001*		0.035 (p = 0.027)	-0.005 (p = 0.598)
Kanto	47	<0.001*	0.001*	0.119		0.024 (p = 0.087)
Sanriku	39	<0.001*	<0.001*	0.331	0.058	
<b>(B)</b> Without breeding migrants						
	n	<50 cm	50–70 cm	≥70 cm		
<50 cm	188		0.006 (p = 0.062)	-0.004 (p = 0.743)		
50–70 cm	117	0.026		0.000 (p = 0.423)		
≥70 cm	59	0.578	0.502			
<b>(C)</b> Without breeding migrants						
	n	<50 cm	50–70 cm	≥70 cm		
<50 cm	28		-0.027 (p = 0.898)	-0.012 (p = 0.654)		
50–70 cm	15	0.887		0.012 (p = 0.268)		
≥70 cm	27	0.912	0.374			

et al. (2016), and many-to-one analysis provided more reliable results. Therefore, we used the results obtained by the informative many-to-one analysis to draw conclusions.

The Japanese stock made the greatest contribution to both of the foraging aggregations in all size classes (Fig. 4, Table S4), and the contribution was higher to size class II in both FGs (mean 64.2% to Yaeyama FG and 74.1% to Okinawa FG) than to size classes I and III (47.0 to 47.6% to Yaeyama FG and 46.4 to 56.3% to Okinawa FG). In classes I and III, the Micronesian and Marshallese stocks made the second-highest contributions to the Yaeyama FG and Okinawa FG, respectively, and showed similar levels of contributions to size class II in both FGs. However, since the 95% posterior probability intervals of the contributions of the Micronesian and Marshallese stocks were relatively wide, especially for size class III of Okinawa, we could not conclude unambiguously whether the contributions of these 2 stocks to FGs in the Ryukyus differed among the size classes (Table S4C,D). On a regional scale, DPS 7 consistently made the second-highest contribution to both FGs (mean 33.9 to 48.4% to the Yaeyama

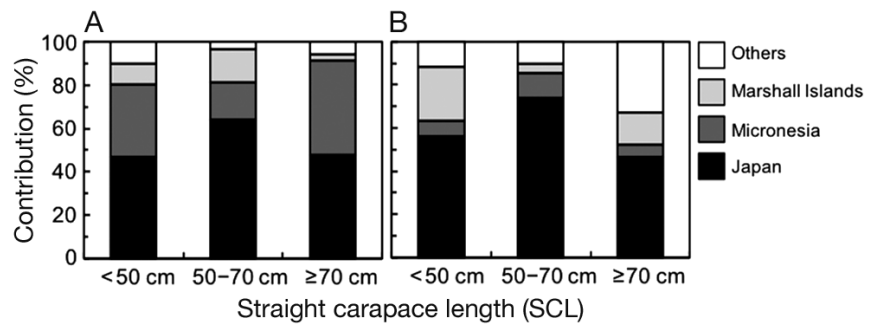


Fig. 4. Estimated contributions of the stocks from Japan, Micronesia, the Marshall Islands, and others (the remaining stocks out of a total of 9) to green turtle foraging aggregations at (A) Yaeyama and (B) Okinawa. Results are shown as mean values from size-specific mixed-stock analysis (MSA) based on informative many-to-one analysis

FG and mean 20.3 to 47.2% to the Okinawa FG; Fig. 4, Table S4B–D). The contribution of the Japanese stock to size class II of the Yaeyama FG was higher both in the late 1990s (59.8%) and from 2005 to 2011 (66.3%) (Fig. 5, Table S5A in the Supplement) than the estimates from size-pooled MSA (52.7 to 53.0% in the Yaeyama FG; Table S4A). In addition, the contribution of the Japanese stock to the Yaeyama FG (67.2%) also increased in the foraging aggregation of 2011, when there were more turtles classified as size class II than in the other years of these temporal comparisons (Fig. 6, Table S5B). The greatest contribution to the FGs

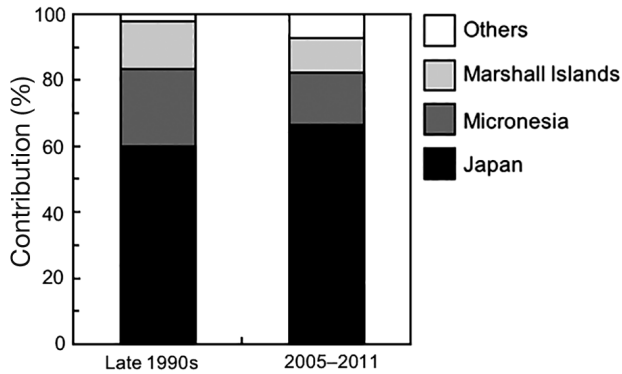


Fig. 5. Estimated contributions of the stocks from Japan, Micronesia, the Marshall Islands and others (the remaining stocks out of a total of 9) to green turtles of the  $50 \text{ cm} \leq \text{SCL} < 70 \text{ cm}$  size class (II) in the Yaeyama FG only sampled during 1997–1999 and 2005–2011. Results are shown as mean values from size-specific mixed-stock analysis (MSA) based on informative many-to-one analysis

along the coasts of the Japanese main islands (western Japanese islands, Kanto, and Sanriku) was also confirmed to come from the Japanese stock (>96.5%) in the present MSA (Table S6).

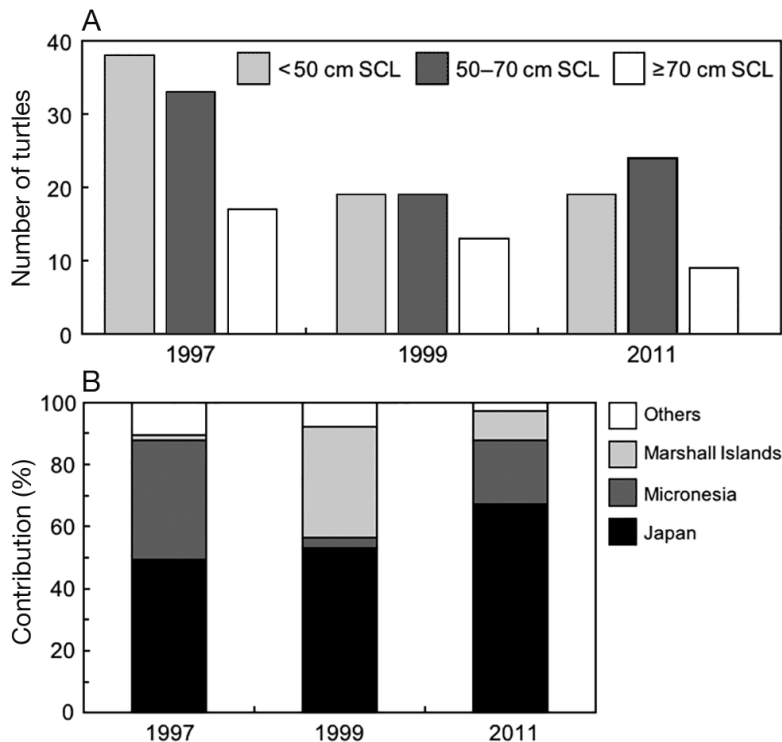


Fig. 6. (A) Frequencies of the 3 green turtle size classes (straight carapace length, SCL) in different years in Yaeyama, and (B) estimated contributions of the stocks from Japan, Micronesia, the Marshall Islands, and others (the remaining stocks out of a total of 9) to green turtle foraging aggregations at Yaeyama in 1997, 1999, and 2011. Results are shown as mean values from size-specific mixed-stock analysis (MSA) based on informative many-to-one analysis

## DISCUSSION

### Natal origin of foraging green turtles in the Ryukyus

The present study examined the genetic composition of foraging green turtles in the Ryukyus according to different size classes, and revealed that foraging aggregations in this area share haplotypes with various breeding stocks in the Indo-Pacific region, suggesting that the Ryukyu FGs are potentially connected with various breeding stocks in the Indo-Pacific regions. The contributions of stocks that shared only widespread or low-frequency haplotypes with the present foraging aggregations were not evaluated by MSA, because our purpose here was to focus on the size-related movement of turtles from major sources. However, several turtles in the Yaeyama FGs had Hawaii or eastern Pacific haplotypes, or haplotypes that were detected only in the breeding stocks of the Southern Hemisphere, such as those of the northern GBR or Coral Sea. The presence of these individuals suggests that the Ryukyu FGs have weak but non-negligible connections even with Hawaii and the eastern and southern Pacific stocks through long-distance migrations by green turtles.

### Size-related movement among foraging grounds in the NW Pacific

Our present MSA estimates, calculated based on 3 size classes, revealed that the contribution of the Japanese stock was the highest to green turtles with SCLs of 50–70 cm compared to turtles of smaller or larger sizes in the 2 FGs examined in the Ryukyus. This trend was maintained over time between the late 1990s and 2005 to 2011 (Fig. 5), and the Japanese stock also made the greatest contribution to FGs in Yaeyama in 2011, when the 50–70 cm size class was the most abundant size class (Fig. 6). Thus, the higher contribution of the Japanese stock in the 50–70 cm SCL class in both FGs seemed to be attributable to some common factors.

It is likely that demographic population shifts have affected the compositions of foraging turtles in the Pacific.



Jensen et al. (2016) suggested that the reduced hatching success evident on Raine Island in the northern GBR might explain the recent increased contributions of immature green turtles from the southern GBR and Coral Sea to FGs in the Torres Strait. In contrast, the population trends have shown steady increases of the Japanese stocks both in Ogasawara (Kondo et al. 2017) and in the Ryukyus (Ministry of the Environment 2014). In addition, the greater contribution of the Japanese stock to foraging turtles of 50–70 cm SCL was maintained for at least a decade. Hence, although we have no data on population trends of the Micronesian or Marshallese stocks, the greatest contribution of the Japanese stock to the 50–70 cm size class of the Ryukyus did not result from demographic shifts. It rather suggests that there were size-related shifts among foraging sites by turtles.

Many turtles <50 cm SCL were probably juveniles that inhabited the neritic FGs near the areas to which hatchlings were passively carried, because turtles have been reported to be recruited in the Ryukyus at around 35.0 to 44.9 cm SCL in association with life-style changes from the epipelagic to neritic stage (Kameda et al. 2017). Thus, the greater contribution of the Japanese stock and the smaller contributions of the Micronesian and Marshallese stocks to the 50–70 cm size class suggested the replacement of turtles from remote stocks with ones from domestic stocks. This migration pattern may support the ‘juvenile natal homing’ hypothesis, that juvenile turtles migrate to FGs in the vicinity of their natal beaches from the neritic FGs right after the initial epipelagic stage, as was indicated in previous studies (Bass et al. 2006, Naro-Maciel et al. 2007). Most turtles <50 cm SCL in the western Japanese islands originated in Japan (Hamabata et al. 2015), and the recruitment of turtles from the Japanese main islands may have possibly increased the contribution of the Japanese stock to the 50–70 cm size class in the Ryukyus. Shimada et al. (2014) suggested that some juveniles around Yaeyama experienced diet shifts that seemed to occur concomitantly with habitat shifts from the main islands, where algae are the principal food, to Yaeyama, where seagrass is the principal food, as shown by examinations of stable isotopic ratios, and this supports the above-stated notion of the movement of turtles from the main islands to the Ryukyus. The scarcity of turtles of 50 to 55 cm SCL, particularly in the Okinawa FG (Hayashi & Nishizawa 2015), suggests that the Micronesian and Marshallese turtles moved to somewhere outside of Japanese waters, although they inhabited the Ryukyus as initial neritic

FGs right after the epipelagic habitats, where they were transported by the Kuroshio Current.

In addition, the present results showed a decrease in the contribution of the Japanese stock and an increase in the contributions of the Micronesian and Marshallese stocks to turtles of around 70 cm. The shifts in these contributions at around 70 cm SCL suggest the occurrence of emigration by the Japanese stock from the Ryukyus and recruitment of the Micronesian and Marshallese stocks to the Ryukyus. This movement at around 70 cm SCL seems to be the opposite of juvenile natal homing. However, satellite tracking of post-nesting migrations has shown that female green turtles that nested in Micronesia moved to the Ryukyus (Kolinski et al. 2014), and turtles that nested in the central Ryukyus moved to the coasts of the Japanese main islands (Oki et al. 2015), indicating that the Ryukyus and the Japanese main islands serve as adult FGs for Micronesian and Japanese turtles, respectively. The locations of adult FGs determined by satellite tracking indicate that mature and near-mature turtles do not always forage in the vicinity of their natal beaches, and support the movement of turtles of around 70 cm SCL implied in this study. Because a few turtles tagged in Yaeyama were actually found off the coasts of the Japanese main islands (Kameda et al. 2017), the movements between the Ryukyus and the Japanese main islands may possibly occur in both directions, and seem to be complicated. The size-related movements of green turtles among FGs in the NW Pacific appear to be different from the developmental migrations observed in the Atlantic, where the earliest developmental migrations take turtles to the most distant FGs, and then later developmental migrations take them back in the direction of their natal beach. Although further research is needed to clarify whether the same turtles from the Micronesian and Marshallese stocks are recruited again to the Ryukyus once they leave there, or whether the movement among FGs occurs for turtles of various sizes, our results from size-specific MSA based on mtDNA provide evidence that supports the notion of size-related movements among FGs in the NW Pacific. Mitochondrial short tandem repeat (mtSTR) sequences, which have enabled the exploration of fine-scale population structure in Mediterranean sea turtles (Tikochinski et al. 2012), or nuclear microsatellite markers, which have been used to distinguish between Atlantic and Mediterranean stocks of loggerhead turtles sharing common mtDNA control region haplotypes (Carreras et al. 2011), may be useful to clarify further details about the movements of turtles among FGs in the NW Pacific.

### Boundaries of southern stocks

Our present genetic comparisons extended for about 2500 km across the Japanese islands and the Ryukyus, and showed that significant genetic differences in green turtle foraging aggregations were evident between the Ryukyu Archipelago and the Japanese main islands of the NW Pacific. Although differences in the genetic compositions of the turtles foraging around Yaeyama and those of other foraging aggregations along the Japanese main islands were supported by the results of previous studies (Hamabata et al. 2009, 2015, Nishizawa et al. 2013), this study is the first to show significant genetic differences between the turtles of the Okinawa FG and all foraging aggregations along the Japanese main islands.

Although a significant genetic difference was apparent even over the short distance (539 km) between the Okinawa and Nomaie FGs, the westernmost FGs of the western Japanese main islands exhibited no significant differences in genetic composition along a line >1500 km in length. Additionally, the Yaeyama and Okinawa FGs, separated by 372 km, exhibited no significant difference in genetic composition. Our present MSAs indicated that the stocks contributing to foraging assemblies in the Ryukyus were very similar (see Table S4A in the Supplement) and that Ryukyu turtles of all size classes differed from those of the main Japanese islands FGs, the turtles of which originated almost completely from the Japanese stock. Nishizawa et al. (2013) suggested that the contributions made by various southern stocks to the Ryukyus (which are located upstream of the Kuroshio Current) can be explained by hatchling transportation from southern rookeries by the current. Our results are consistent with this hypothesis. However, the movement of turtles from southern stocks ceases abruptly at the Ryukyus; thus, it does not involve continuation to the main Japanese islands. In other words, movement does not decline gradually with distance. This suggests that the Ryukyus form the northern boundary of turtles from southern tropical regions.

Marine turtles maintain a higher body temperature than the ambient water, and the difference between the 2 temperatures increases with body size (Sato 2014). Hence, tolerance of cold water might increase with growth, but differences in the distributions of the Japanese and southern stocks were maintained even in sub-adults and adults. The overwintering foraging activities of the green

turtles on coasts westward to Kanto, Japan, have been analyzed via satellite tracking (Fukuoka et al. 2015) and continuous by-catch studies (Hamabata et al. 2015). The data suggest that the Japanese and southern turtle stocks have evolved adaptive differences in response to seasonal water environments. The boundary between them corresponds to that separating the marine biogeographical realms of the Ryukyus and the seas northward of Kyushu Island. These realms are formed, and are influenced by, the oceanographic features of the Kuroshio Current (Nishimura 1992). This current enters the East China Sea and returns to the Pacific, south of Kyushu Island. Waters northward of Kyushu are seasonally temperate; thus, the sea surface temperature drops to <20°C in winter. Tropical fish are often carried to the coasts of the Japanese main islands by the Kuroshio Current and are sometimes observed westward to Kanto, but this is the case only from summer to early winter (Motomura et al. 2007a,b). Such species usually cannot tolerate low water temperatures during winter, or they fail to attain gonadal maturity if the water temperature is too low (thus, around the Japanese main islands in winter). Therefore, such dispersals constitute abortive migration (Motomura 2012). Physiological responses to varying water temperatures (tolerances and preferences) should be further investigated to determine whether biological differences between the Japanese and southern turtle stocks shape the boundary between their distributions. However, a study of the phylogeography of green turtles nesting in the NW Pacific revealed that the Japanese stock was of an endemic lineage; thus, they must have coped with historical glacial periods in the northern periphery of their range (Hamabata et al. 2014). This lineage may have undergone adaptive evolution in the northern regions, where environmental temperatures vary greatly by season, whereas the southern regions and their stocks may not have been required to adapt to such conditions.

### Conservation implications

The present study's results suggested that green turtles in the NW Pacific inhabit multiple FGs as they mature, and thus may possibly be affected by different water temperatures. Declines in the areas of seagrass meadows constitute a global problem (Waycott et al. 2009); abundances of sea algal beds along the coasts of the Japanese main islands have

plummeted, triggered (at least in part) by increases in ocean water temperatures (Fisheries Agency 2016), which are rising more rapidly along coasts than in the oceans overall (Japan Meteorological Agency: [www.data.jma.go.jp/gmd/kaiyou/english/long\\_term\\_sst\\_japan/sea\\_surface\\_temperature\\_around\\_japan.html](http://www.data.jma.go.jp/gmd/kaiyou/english/long_term_sst_japan/sea_surface_temperature_around_japan.html)). After the disappearance of native year-round sea algae beds, increased winter water temperatures have allowed southern algal species to invade and form seasonal beds around the western Japanese main islands (e.g. Tanaka et al. 2012). These phenomena may have reduced turtle food resources, although breeding stocks have recovered after conservation efforts (including fishery reductions) were instituted, and changes in seagrass patterns may affect habitat utilization and migration. Additionally, increases in sea surface temperatures may allow southern turtle stocks to expand, as has been reported in various other marine species (Fisheries Research Agency 2009). Although all extant marine turtles have weathered past climatic changes, including the Pleistocene oscillation, we know little about how marine turtle species and populations survive in, and become adapted to, environmental changes. Hence, we cannot forecast how turtles will respond to ongoing anthropogenic climate change. It is essential to monitor the biogeographical ranges and phenologies of green turtle foraging aggregations on both sides of the distributional boundary between the Ryukyus and the Japanese main islands. This will increase our understanding of biological responses to climate change. Continued efforts, including relevant research, to predict the effects of ongoing marine environmental changes on this circumglobal species are needed.

**Acknowledgements.** Sample collections were conducted under permits issued by Okinawa prefecture. We thank Motoki Wakatsuki, Kuroshima Research Station, Sea turtle Association of Japan, Kenji Kuroyanagi, Minamichita Beach Land, Meitetsu Impress Co., and Okinawa Churashima Research Center for the sampling of foraging turtles in Yaeyama. We are also grateful to Osamu Abe, Junichi Okuyama, and Ishigaki Island Sea Turtle Research Group for providing measurement data for part of the Yaeyama samples; to Ryota Hayashi for the disclosure of sampling dates in Yomitan and Yakena, Okinawa; and to the editor, Philippe Borsa and 3 anonymous reviewers for constructive suggestions which helped us improve the manuscript. This research was financially supported in part by grants for Excellent Graduate Schools, MEXT, Japan, the Sasakawa Scientific Research Grant from The Japan Science Society, and JSPS Research Fellowships for Young Scientists. We acknowledge use of the Maptool program of SEATURTLE.ORG for maps used in this paper.

## LITERATURE CITED

- Abe O, Shibuno T, Takada Y, Hashimoto K and others (2003) Nesting populations of sea turtle in Ishigaki Island, Okinawa. In: Arai N (ed) Proceedings of 4<sup>th</sup> SEASTAR2000 Workshop, 11–13 December 2003, Bangkok. Kyoto University, Kyoto, p 40–43
- Abreu-Grobois FA, Horrocks JA, Krueger B, Formia A, Beggs J (2006) New mtDNA dloop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analyses. In: Frick M, Panagopoulou A, Rees AF, Williams K (compilers) Book of abstracts. Twenty sixth annual symposium on sea turtle biology and conservation. International Sea Turtle Society, Athens, p 179
- Balazs GH (1980) Synopsis of biological data on the green turtle in the Hawaiian Island. NOAA Tech Memo NOAA-TM-NMFS-SWFC-7
- ✦ Bass AL, Epperly SP, Braun-McNeill J (2006) Green turtle (*Chelonia mydas*) foraging and nesting aggregations in the Caribbean and Atlantic: impact of currents and behavior on dispersal. *J Hered* 97:346–354
- ✦ Bjørndal KA, Bolten AB (2008) Annual variation in source contributions to a mixed stock: implications for quantifying connectivity. *Mol Ecol* 17:2185–2193
- ✦ Bolker BM, Okuyama T, Bjørndal KA, Bolten AB (2007) Incorporating multiple mixed stocks in mixed stock analysis: ‘many-to-many’ analyses. *Mol Ecol* 16:685–695
- Bolten AB (1999) Techniques for measuring sea turtles. In: Eckert KL, Bjørndal KA, Abreu-Grobois FA, Donnelly M (eds) Research and management techniques for the conservation of sea turtles. Publication 4, IUCN/SSC Marine Turtle Specialist Group, Washington, DC
- 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 2. CRC Press, Boca Raton, FL, p 243–257
- ✦ Bowen BW, Meylan AB, Ross JP, Limpus CJ, George H, Avise JC (1992) Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. *Evolution* 46:865–881
- Carr A, Caldwell DK (1956) The ecology and migrations of sea turtles, 1. Results of field work in Florida, 1955. *Am Mus Novit* 1793:1–23
- ✦ Carreras C, Pascual M, Cardona L, Marco A and others (2011) Living together but remaining apart: Atlantic and Mediterranean loggerhead sea turtles (*Caretta caretta*) in shared feeding grounds. *J Hered* 102:666–677
- ✦ Chassin-Noria O, Abreu-Grobois FA, Dutton PH, Oyama K (2004) Conservation genetics of the east Pacific green turtle (*Chelonia mydas*) in Michoacan, Mexico. *Genetica* 121:195–206
- ✦ Cheng IJ, Dutton PH, Chen CL, Chen HC, Chen YH, Shea JW (2008) Comparison of the genetics and nesting ecology of two green turtle rookeries. *J Zool* 276:375–384
- ✦ Dethmers KEM, Broderick D, Moritz C, Fitzsimmons NN and others (2006) The genetic structure of Australasian green turtles (*Chelonia mydas*): exploring the geographical scale of genetic exchange. *Mol Ecol* 15: 3931–3946
- ✦ Dethmers KEM, Jensen MP, FitzSimmons NN, Broderick D, Limpus CJ, Moritz C (2010) Migration of green turtles (*Chelonia mydas*) from Australasian feeding grounds inferred from genetic analyses. *Mar Freshw Res* 61: 1376–1387

- Dutton PH, Balazs GH, LeRoux RA, Murakawa SKK, Zarate P, Martinez LS (2008) Composition of Hawaiian green turtle foraging aggregations: mtDNA evidence for a distinct regional population. *Endang Species Res* 5:37–44
- Dutton PH, Jensen MP, Frey A, LaCasella E and others (2014a) Population structure and phylogeography reveal pathways of colonization by a migratory marine reptile (*Chelonia mydas*) in the central and eastern Pacific. *Ecol Evol* 4:4317–4331
- Dutton PH, Jensen MP, Frutchev K, Frey A and others (2014b) Genetic stock structure of green turtle (*Chelonia mydas*) nesting populations across the Pacific islands. *Pac Sci* 68:451–464
- 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
- Fisheries Agency (2016) Vision of sea grass beds and tidal-land. Ministry of Agriculture, Forestry and Fisheries, Tokyo (in Japanese)
- Fisheries Research Agency (2009) Global warming and fish. Seizando-Shoten, Kanagawa
- Fukuoka T, Narazaki T, Sato K (2015) Summer-restricted migration of green turtles *Chelonia mydas* to a temperate habitat of the northwest Pacific Ocean. *Endang Species Res* 28:1–10
- Hamabata T, Nishida S, Kamezaki N, Koike H (2009) Genetic structure of populations of the green turtle (*Chelonia mydas*) in Japan using mtDNA control region sequences. *Bull Grad Sch Soc Cult Stud Kyushu Univ* 15:35–50
- Hamabata T, Kamezaki N, Hikida T (2014) Genetic structure of green turtle (*Chelonia mydas*) peripheral populations nesting in the northwestern Pacific rookeries: evidence for northern refugia and postglacial colonization. *Mar Biol* 161:495–507
- Hamabata T, Hikida T, Okamoto K, Watanabe S, Kamezaki N (2015) Ontogenetic habitat shifts of green turtles (*Chelonia mydas*) suggested by the size modality in foraging aggregations along the coasts of the western Japanese main islands. *J Exp Mar Biol Ecol* 463:181–188
- Hayashi R (2016) A long-distance record of a recaptured green turtle (*Chelonia mydas*) from Okinawa. *Fauna Ryukyuan* 26:5–7
- Hayashi R, Nishizawa H (2015) Body size distribution demonstrates flexible habitat shift of green turtle (*Chelonia mydas*). *Glob Ecol Conserv* 3:115–120
- Hayashi R, Tsuji K (2008) Spatial distribution of turtle barnacles on the green sea turtle, *Chelonia mydas*. *Ecol Res* 23: 121–125
- Hirth HF (1992) A natural assemblage of immature green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles on the fringing reef of Wuvulu Island, Papua New Guinea. *Micronesica* 25:145–153
- Jackson JBC (2008) Ecological extinction and evolution in the brave new ocean. *Proc Natl Acad Sci USA* 105: 11458–11465
- Jensen MP, Bell I, Limpus CJ, Hamann M and others (2016) Spatial and temporal genetic variation among size classes of green turtles (*Chelonia mydas*) provides information on oceanic dispersal and population dynamics. *Mar Ecol Prog Ser* 543:241–256
- Joseph J, Nishizawa H, Arshaad WM, Kadird SAS and others (2016) Genetic stock compositions and natal origin of green turtle (*Chelonia mydas*) foraging at Brunei Bay. *Glob Ecol Conserv* 6:16–24
- Kameda K, Wakatsuki M, Kuroyanagi K, Iwase F and others (2017) Change in population structure, growth and mortality rate of juvenile green turtle (*Chelonia mydas*) after the decline of the sea turtle fishery in Yaeyama Islands, Ryukyu Archipelago. *Mar Biol* 164:143
- Kamezaki N (1989) The nesting sites of sea turtles in the Ryukyu Archipelago and Taiwan. In: Matsui M, Hikida T, Goris RC (eds) *Current herpetology in East Asia: Proceedings of the 2<sup>nd</sup> Japan–China Herpetological Symposium*. Herpetological Society of Japan, Kyoto, p 342–348
- Kikukawa A, Kamezaki N, Ota H (1999) Current status of the sea turtles nesting on Okinawajima and adjacent islands of the central Ryukyus, Japan. *Biol Conserv* 87: 149–153
- Kolinski SP, Cruce JA, Parker DM, Balazs GH, Clarke R (2014) Migrations and conservation implications of post-nesting green turtles from Gielop Island, Ulithi Atoll, Federated States of Micronesia. *Micronesica* 2014-04: 1–9
- Kondo S, Morimoto Y, Sato T, Suganuma H (2017) Factors affecting the long-term population dynamics of green turtles (*Chelonia mydas*) in Ogasawara, Japan: influence of natural and artificial production of hatchlings and harvest pressure. *Chelonian Conserv Biol* 16:83–92
- Limpus CJ, Miller JD, Paramenter C, Reimer D, McLachlan N, Webb R (1992) Migration of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles to and from eastern Australian rookeries. *Wildl Res* 19:347–357
- Limpus CJ, Limpus DJ, Arther KE, Parmenter CJ (2005) Monitoring green turtle population dynamics in Shoalwater Bay: 2000–2004. Research Publication No. 83, Queensland Environmental Protection Agency and the Great Barrier Reef Marine Park Authority. Great Barrier Reef Marine Park Authority, Townsville
- Meylan PA, Meylan AB, Grey JA (2011) The ecology and migrations of sea turtles 8. Tests of the developmental habitat hypothesis. *Bull Am Mus Nat Hist* 357:1–70
- Ministry of the Environment (2014) Threatened wildlife of Japan. Red data book 2014, 4<sup>th</sup> edn: reptiles & amphibians. Biodiversity Center of Japan, Tokyo
- Motomura H (2012) Chapter 2: Fish species diversity in Kagoshima Prefecture nurtured by the Kuroshio Current. In: Matsuura K (ed) *Fishes in the Kuroshio Current*. Tokai University Press, Tokyo, p 19–46
- Motomura H, Ito M, Ikeda H, Endo H, Matsunuma M, Hatooka K (2007a) Review of Japanese records of a grouper, *Epinephelus amblycephalus* (Perciformes, Serranidae), with new specimens from Kagoshima and Wakayama. *Biogeography* 9:49–56
- Motomura H, Kimura S, Haraguchi Y (2007b) Two carangid fishes (Actinopterygii: Perciformes), *Caranx heberi* and *Ulua mentalis*, from Kagoshima: the first records from Japan and northernmost records for the species. *Species Divers* 12:223–235
- Naro-Maciel E, Becker JH, Lima EHSM, Marcovaldi MÂ, DeSalle R (2007) Testing dispersal hypotheses in foraging green sea turtles (*Chelonia mydas*) of Brazil. *J Hered* 98:29–39
- Naro-Maciel E, Gaughran SJ, Putman NF, Amato G and others (2014) Predicting connectivity of green turtles at Palmyra Atoll, central Pacific: a focus on mtDNA and dispersal modelling. *J R Soc Interface* 11:20130888
- Ng CK, Dutton PH, Chan SK, Cheung K, Qiu J, Sun Y (2014) Characterization and conservation concerns of green turtles (*Chelonia mydas*) nesting in Hong Kong, China. *Pac*

- Sci 68:231–243
- Nishimura S (1992) Animal distribution around Japanese coastal waters. In: Guide to seashore animals of Japan with color pictures and keys, Vol 1. Hoikusha, Osaka, p xi–xix (in Japanese)
- ✦ Nishizawa H, Abe O, Okuyama J, Kobayashi M, Arai N (2011) Population genetic structure and implications for natal philopatry of nesting green turtles *Chelonia mydas* in the Yaeyama Islands, Japan. *Endang Species Res* 14: 141–148
- ✦ Nishizawa H, Naito Y, Suganuma H, Abe O and others (2013) Composition of green turtle feeding aggregations along the Japanese archipelago: implications for changes in composition with current flow. *Mar Biol* 160: 2671–2685
- ✦ Nishizawa H, Narazaki T, Fukuoka T, Sato K, Hamabata T, Kinoshita M, Arai N (2014) Juvenile green turtles on the northern edge of their range: mtDNA evidence of long-distance westward dispersals in the northern Pacific Ocean. *Endang Species Res* 24:171–179
- ✦ Norman JA, Moritz C, Limpus CJ (1994) Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. *Mol Ecol* 3:363–373
- Oki K, Arata T, Ng CK, Parker DM, Balazs GH (2015) Satellite tracking of loggerhead turtle and green turtle in Amami island. In: Proceedings 26<sup>th</sup> Japanese Sea Turtle Symposium. Sea Turtle Association of Japan, Osaka, p 63–64
- ✦ Payne JL, Bush AM, Heim NA, Knope ML, McCualey DJ (2016) Ecological selectivity of the emerging mass extinction in the oceans. *Science* 353:1284–1286
- Pella J, Masuda M (2001) Bayesian methods for analysis of stock mixtures from genetic characters. *Fish Bull* 99: 151–167
- ✦ Pilcher N (2010) Population structure and growth of immature green turtles at Matanani, Sabah, Malaysia. *J Herpetol* 44:168–171
- ✦ Raymond M, Rousset F (1995) An exact test for population. *Evolution* 49:1280–1283
- ✦ Reich KJ, Bjørndal KA, Bolten AB (2007) The ‘lost years’ of green turtles: using stable isotopes to study cryptic lifestages. *Biol Lett* 3:712–714
- ✦ Sato K (2014) Body temperature stability achieved by the large body mass of sea turtles. *J Exp Biol* 217:3607–3614
- Seminoff JA, Allen CD, Balazs GH, Dutton PH and others (2015) Status review of the green turtle (*Chelonia mydas*) under the Endangered Species Act. NOAA Tech Memo NMFS-SWFSC-539
- ✦ Shimada T, Aoki S, Kameda K, Hazel J, Reich K, Kamezaki N (2014) Site fidelity, ontogenetic shift and diet composition of green turtles *Chelonia mydas* in Japan inferred from stable isotope analysis. *Endang Species Res* 25: 151–164
- ✦ Sterling EJ, McFadden KW, Holmes KE, Vintinner EC, Arengo F, Naro-Maciel E (2013) Ecology and conservation of marine turtles in a central Pacific foraging ground. *Chelonian Conserv Biol* 12:2–16
- ✦ Stewart JD, Beale CS, Fernando D, Sianipar AB, Burton RS, Semmens BX, Aburto-Oropeza O (2016) Spatial ecology and conservation of *Manta birostris* in the Indo-Pacific. *Biol Conserv* 200:178–183
- Tachikawa H (1991) Carapace length and body weight of adult green turtle in Ogasawara. *Umigame News Lett* 8: 7–10 (in Japanese)
- ✦ 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 methods. *Mol Biol Evol* 28: 2731–2739
- ✦ Tanaka K, Taino S, Haraguchi H, Prendergast G, Hiraoka M (2012) Warming off southwestern Japan linked to distributional shifts of subtidal canopy-forming seaweeds. *Ecol Evol* 2:2854–2865
- ✦ Tikochinski Y, Bendelac R, Barash A, Daya A, Levy Y, Friedmann A (2012) Mitochondrial DNA STR analysis as a tool for studying the green sea turtle (*Chelonia mydas*) populations: the Mediterranean Sea case study. *Mar Genomics* 6:17–24
- ✦ Waycott M, Duarte CM, Carruthers TJB, Orth RJ and others (2009) Acceleration loss of seagrasses across the globe threatens coastal ecosystems. *Proc Natl Acad Sci USA* 106:12377–12381
- Zug GR, Balazs GH, Wetherall JA, Parker DM, Murakawa SKK (2002) Age and growth of Hawaiian green sea turtles (*Chelonia mydas*): an analysis based on skeletochronology. *Fish Bull* 100:117–127

Editorial responsibility: Philippe Borsa,  
Nouméa, France

Submitted: August 28, 2017; Accepted: June 4, 2018  
Proofs received from author(s): July 21, 2018