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Genetic stock compositions and natal origin of green turtle (*Chelonia mydas*) foraging at Brunei Bay



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ABSTRACT

Knowledge of genetics composition and growth stages of endangered green turtles, as well as the connectivity between nesting and foraging grounds is important for effective conservation. A total of 42 green turtles were captured at Brunei Bay with curved carapace length ranging from 43.8 to 102.0 cm, and most sampled individuals were adults and large juveniles. Twelve haplotypes were revealed in mitochondrial DNA control region sequences. Most haplotypes contained identical sequences to haplotypes previously found in rookeries in the Western Pacific, Southeast Asia, and the Indian Ocean. Haplotype and nucleotide diversity indices of the Brunei Bay were 0.8444 ± 0.0390 and 0.009350 ± 0.004964 , respectively. Mixed-stock analysis (for both uninformative and informative prior weighting by population size) estimated the main contribution from the Southeast Asian rookeries of the Sulu Sea (mean $\geq 45.31\%$), Peninsular Malaysia (mean $\geq 17.42\%$), and Sarawak (mean $\geq 12.46\%$). Particularly, contribution from the Sulu Sea rookery was estimated to be the highest and lower confidence intervals were more than zero ($\geq 24.36\%$). When estimating contributions by region rather than individual rookeries, results showed that Brunei Bay was sourced mainly from the Southeast Asian rookeries. The results suggest an ontogenetic shift in foraging grounds and provide conservation implications for Southeast Asian green turtles.

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1. Introduction

Green turtles (*Chelonia mydas*) are widely distributed in tropical regions, but are considered endangered globally because of exploitation (IUCN, 2015). The green turtle is the most abundant sea turtle species in Southeast Asia, but poaching of eggs and bycatch in fisheries are major threats to green turtle survival (Shanker and Pilcher, 2003). To prevent egg poaching,

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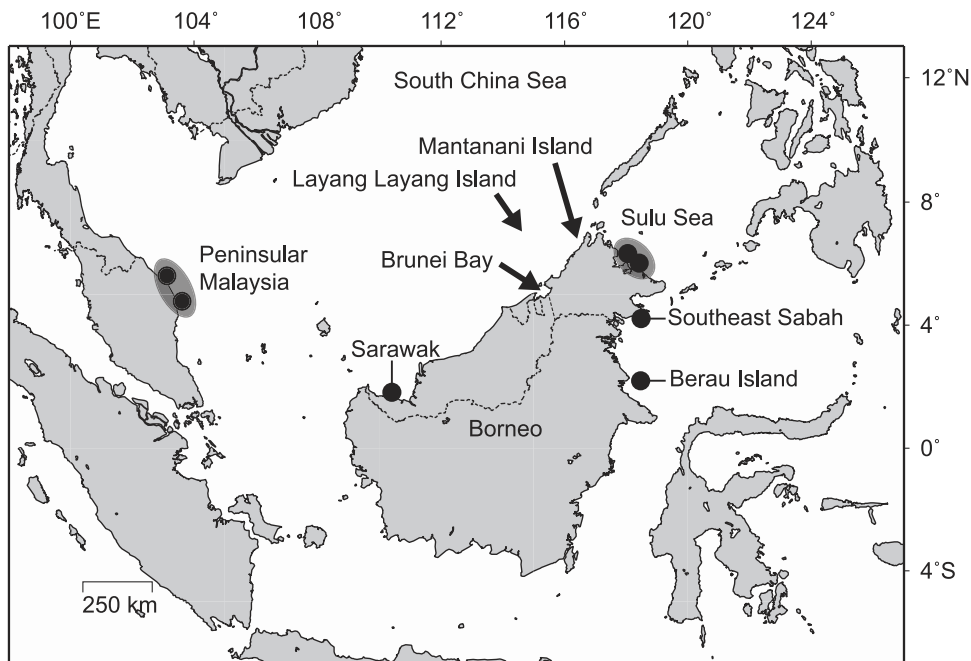


Fig. 1. Location of Brunei Bay and other foraging grounds (i.e., Mantanani Island and Layang Layang Island) and possible source rookeries (black circles) in Southeast Asia.

sanctuaries were established for example at Sabah in 1984, Sarawak in 1999, and Redang Island in 2005 (Chan, 2006, 2013). On the other hand, conservation of green turtles in the sea is difficult because of the migratory life history and wide-range dispersal of this species (Hirth, 1997). Their long-distance migrations present complex challenges for conservation because the migratory route often involves multiple countries; therefore, jurisdictions complicate legislative and regulatory conservation policies that are effective within single nations (Campbell et al., 2009). An understanding of these migrations and the establishment of international coordination according to these migrations are required for the conservation of green turtles.

Mark-recapture is a traditional approach that provides direct evidence for the movement between two capture sites. Tagging of sea turtles has been practiced globally, including in Southeast Asia (e.g. Pilcher, 2010). Satellite tracking is another method that provides direct evidence of movement and information on migratory routes. Satellite tracking research in Malaysia has shown that green turtles migrate from their nesting beaches at Redang Island in Peninsular Malaysia to foraging grounds around Borneo or Bangka Island (Luschi et al., 1996; Liew et al., 2000). Although tagging and telemetry studies provide useful information on demography, site fidelity, and migration, the available data are individual-based and biased toward intensively surveyed locations. To understand the links between foraging grounds and nesting beaches of sea turtles by tagging and telemetry, insights come mainly from individual adult females. Population-based inference based on genetic information, developed as mixed-stock analysis (MSA) (Pella and Masuda, 2001; Bolker et al., 2007), has recently been used to link genetically differentiated nesting populations to foraging grounds of sea turtles (e.g. Dutton et al., 2008; Dethmers et al., 2010; Prosdocimi et al., 2012; Nishizawa et al., 2013; Naro-Maciel et al., 2014). In Southeast Asia, Joseph et al. (2014) conducted MSA on carcass samples obtained in Mantanani Island to estimate the origins of illegally harvested turtles. In addition, Jensen et al. (in press-a) have recently estimated the origins of immature green turtles foraging at Mantanani Island and Layang Layang Island. However, there are several other foraging grounds in Southeast Asia and population-based migration between these foraging grounds and nesting rookeries are required to be determined for better understanding of green turtle migration in Southeast Asia.

In Southeast Asia, Brunei Bay ($4^{\circ}45' - 5^{\circ}02'N$, $114^{\circ}58' - 115^{\circ}10'E$) (Fig. 1) is known to be an important nursery, foraging, and transient ground for marine animals, including sea turtles, dugongs, and coastal cetaceans (Rajamani and Marsh, 2010; HICOE-UMT, unpublished data). Marine ecosystems in Brunei Bay consist of mangrove forests, seagrass beds, coral reefs, estuarine, mudflats, and continental slope (Bali, 2005; Bujang et al., 2006; Jaaman et al., 2010; Ahmad-Kamil et al., 2013), and the seagrass bed dominated by *Halophila* and *Halodule* species (Bali, 2005; Bujang et al., 2006; Ahmad-Kamil et al., 2013) attracts herbivorous marine animals such as green turtles. At the same time, Brunei Bay has high amounts of fish resources. The fishing industry is ranked second in economic importance to the petroleum and hydrocarbon industry in the area (Department of Fisheries Sabah, 2010). Because of the ecological uniqueness and economic importance, Brunei Bay is a high-priority area for research and conservation of green turtles.

To characterize the utilization of Brunei Bay by green turtles, we explored whether the seagrass bed in the geographically deeply indented Brunei Bay is utilized by green turtles originating from the proximate or distal rookeries. Previous studies



Fig. 2. Traditional fish-catching device known as 'kabat' was used to trap sea turtles at Brunei bay.

have indicated that green turtles often forage in mixed aggregations drawn from various nesting populations (Bass et al., 2006; Dethmers et al., 2010; Nishizawa et al., 2013), whereas some foraging aggregations are mainly contributed by specific populations (Dutton et al., 2008; Prosdocimi et al., 2012; Nishizawa et al., 2013; Naro-Maciel et al., 2014). Juvenile sea turtles reach their foraging grounds by transportation of oceanic current in combination with their own active swimming (Putman and Mansfield, 2015), and sea turtles in foraging grounds will return to nest at their natal regions known as natal homing, which forms genetic differences among rookeries (Allard et al., 1994; Bowen, 1995). Therefore, knowledge on the connectivity between green turtles in nesting and foraging grounds can be used to quantify the impact of threats. In addition, the size class of green turtles inhabiting Brunei Bay remains unclear. Possible ontogenetic changes in foraging grounds have been explored based on green turtles in Japan (Hayashi and Nishizawa, 2015). In fact, almost all turtles captured in Mantanani Island, Malaysia, are juveniles (Pilcher, 2010; Jensen et al., in press-a); hence, the composition of size classes is important to formulate a comprehensive management plan and policies for sea turtles in Southeast Asia.

In this study, we collected samples from green turtles foraging at Brunei Bay. The aims of this study were to (i) determine the size class distribution and genetic diversity of green turtles aggregating in Brunei Bay and (ii) estimate the contributions of different breeding stocks to aggregation in Brunei Bay using MSA based on mitochondrial DNA (mtDNA) control region sequences.

2. Materials and methods

2.1. Fieldwork and sample collection

Sampling was performed in January and March 2011 (by the Marine Fishery Resources Development and Management Department) and in December 2013 and February 2014 (by Universiti Malaysia Terengganu). Sampling was concentrated at large seagrass meadows along the 52 km coastline of Lawas, Sarawak. These seagrass meadows extended to the Brunei's district of Temburong. However, due to permit restriction, sampling was only conducted at the Malaysian bay of Brunei.

Foraging green turtles were captured ($n = 42$) by installing a net known as a *kabat*. A *kabat* is a traditional fish-catching device used by the local Malay-Brunei fishermen. It is normally installed during the highest and lowest tides of each month. It is a long net (approximately 1–2 km) installed to cover a bay area during the highest high tide (normally at night), and by the next morning during the lowest tide, the net is checked for any trapped turtles (Fig. 2). The curved carapace length (CCL), curved carapace width (CCW), and body weight of captured turtles were measured in this study. All captured turtles were double tagged at their front flippers with Inconel tags (style 681; National Band and Tag Co., Newport, KY, USA) bearing the Sarawak code MYS. Following Sterling et al. (2013), individuals with a CCL of <65.0 cm were classified as small juveniles, subadults had a CCL of 65.0 to 84.9 cm, and adults had a CCL of >85.0 cm. Blood samples were withdrawn from the dorsal cervical sinus (Dutton, 1996) and preserved in lysis buffer (100 mM Tris-HCL, 100 mM EDTA, 10 mM NaCl, 1% SDS; pH 8.0) at a 1:10 ratio of blood to buffer (Dutton, 1996). Turtles were released immediately after the measurement and sampling. Sampling of sea turtles comply the ethical guidelines and conducted under the permits NCCD.907.4.4 [Jld. 9]–67 and Export Permit No. 15017 to transport the blood samples to Universiti Malaysia Terengganu.

2.2. Laboratory procedures

Genetic analyses were conducted at the Universiti Malaysia Terengganu and MFRDMD Genetics Laboratory, Kuala Terengganu. Genomic DNA was extracted using the CTAB protocol (Bruford et al., 1992). A segment of the mitochondrial

control region of approximately 770 bp was amplified from the extracted DNA using primers LCM15382 and H950g (Abreu-Grobois et al., 2006). Polymerase chain reaction (PCR) amplification was performed using Eppendorf Mastercycler DNA Engine Thermal Cycler PCR. Template DNAs were amplified in a 50- μ l total reaction volume containing 25 to 50 ng turtle genomic DNA, 1 U/50 μ l Taq polymerase (Vivantis Technologies, Malaysia), 10 mM Tris–HCl buffer, 2.5 mM MgCl₂, 0.125 mM deoxynucleotide triphosphates (dNTPs), and 0.2 μ M of each primer. Cycling parameters consisted of initial denaturing at 94 °C for 3 min followed by 30 cycles of 30-s denaturation at 94 °C, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s, followed by a final elongation step at 72 °C for 3 min. Following PCR, all amplified samples were verified for the targeted band size by 1% agarose gel electrophoresis. The PCR products were sent to First BASE and Repfon Glamor (Kuala Lumpur, Malaysia) for purification and sequencing for both strands.

The sequences were read and checked using ABI Sequence Scanner v1.0. Multiple sequence alignments were performed using Clustal Omega Software (Sievers et al., 2011). Haplotypes were identified by performing a search against a collated database of known green turtle haplotypes. The Southwest Fisheries Science Center, NOAA Fisheries Service (<https://swfsc.noaa.gov>), was referred to for the Pacific and Indian Ocean green turtle mtDNA sequences. The GenBank database (National Center for Biotechnology Information, USA: NCBI website <http://www.ncbi.nlm.nih.gov>) was also searched for control region sequences for comparison.

2.3. Molecular and mixed-stock analysis

Nucleotide diversity (π) and haplotype diversity (h) were estimated using ARLEQUIN v3.5 (Excoffier and Lischer, 2010). To estimate nucleotide diversity, we used the Tamura–Nei model of nucleotide substitutions, which was designed for control region sequences (Tamura and Nei, 1993). Based on the exact test (50,000 steps in a Markov chain with a 10,000-step dememorization) using ARLEQUIN, haplotype frequency was compared with samples from Malaysian foraging grounds at Mantanani Island and Layang Layang Island (Jensen et al., in press-a).

The relative contribution of nesting source populations to Brunei Bay foraging aggregation was estimated based on Bayesian MSA using BAYES (Pella and Masuda, 2001). The source populations included 23 genetically separated rookeries in the regions of Southeast Asia, Australia, Micronesia, and Melanesia as revealed by Dutton et al. (2014), Jensen et al. (in press-b) (including reanalysis of samples of Dethmers et al., 2006), and Read et al. (2015). Dutton et al. (2014) reported haplotypes from rookeries in Polynesia (i.e. American Samoa and French Polynesia), but these rookeries were excluded from the analysis because of no shared haplotypes with Brunei Bay foraging aggregation. In the analyses, regional group estimation implemented in BAYES was performed based on the classification of source populations into four regions: Southeast Asia, Southwestern Pacific, Micronesia, and Eastern Indian Ocean. Taiwanese (Cheng et al., 2008) and Japanese rookeries (Nishizawa et al., 2011, 2013) were other candidate rookeries. However, because the haplotype compositions of these rookeries were investigated based on shorter sequences and analysis including these rookeries estimated only small contributions to the Brunei Bay foraging aggregation (means <0.5%; see the Supplementary Online Materials, see Appendix A), these rookeries were excluded from the final analysis.

MSAs were conducted for both uninformative Dirichlet prior to assuming the same size of all populations and informative prior weighting by the population size (based on Jensen et al., in press-b). We performed four Markov Chain Monte Carlo (MCMC) chains, and each chain was started with 95% of the mixed sample initially contributed by each region of source populations. In the analysis with informative prior, four chains were added and eight chains in total were performed for achieving convergence. Each chain contained 50,000 samples, and the first 25,000 samples were discarded as burn-in. The convergence of MCMC sampling was assessed using the Gelman–Rubin shrink factor (Gelman and Rubin, 1992), which indicates a lack of convergence if the value is greater than 1.2.

3. Results

3.1. Captured turtles and size distributions

In total, 42 green turtles were caught with a CCL ranging from 43.8 to 102.0 cm (Fig. 3) and a CCW ranging from 40.5 to 93.0 cm. Weight could not be measured in 11 turtles, but ranged from 12 to 145 kg in 31 turtles. Most turtles caught at Brunei Bay were adults (59.5%), followed by subadults (28.5%) and juveniles (12.0%). Details of samples were listed in the Supplementary Online Materials (see Appendix A).

3.2. Haplotype composition

Twelve haplotypes were detected from the 42 samples of green turtles collected from Brunei Bay foraging ground (Table 1). All haplotype sequences have already been registered to the NOAA or GenBank database. The most common haplotypes at Brunei Bay were Cmp57.1 (33.3%), followed by Cmp49.1 (16.7%) and Cmp87.1 (14.3%). All other haplotypes were relatively rare (<7% each). Ten haplotypes were previously observed in the Southeast Asian rookeries (i.e. Cmp20.1, Cmp82.1, Cmp49.1, Cmp49.3, Cmp87.1, Cmp40.1, Cmp91.1, Cmp57.1, Cmp57.2, and Cmp104.1) (Jensen et al., in press-b), and some of them were also observed in Southwestern Pacific rookeries (i.e. Cmp20.1, Cmp49.1, and Cmp91.1) (Jensen et al.,

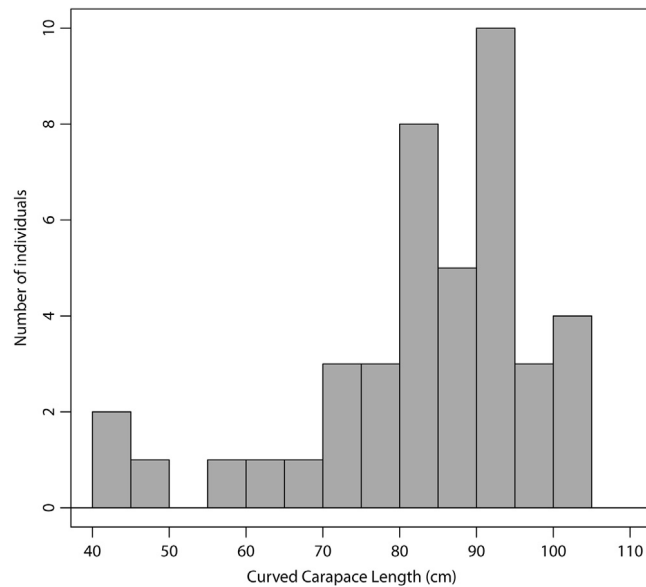


Fig. 3. Distribution of curved carapace length of sampled individuals.

Table 1

Summary of haplotypes detected from Brunei Bay foraging aggregation.

Haplotype	GenBank accession no.	Number of individuals
CmP20.1	AB819806	3
CmP40.1	KF311750	2
CmP49.1	AB819808	7
CmP49.3	KJ502572	2
CmP57.1	KJ502588	14
CmP57.2	KJ502567	1
CmP75.1	KJ502574	1
CmP82.1	KJ502584	1
CmP87.1	KJ502589	6
CmP91.1	KF311762	2
CmP104.1	KJ502569	2
CmP154.1	KM923922	1

in press-b; Read et al., 2015), Micronesian rookeries (i.e. CmP20.1, CmP49.1, and CmP91.1) (Dutton et al., 2014), Eastern Indian Ocean rookeries (i.e. CmP20.1, CmP49.1, CmP40.1, and CmP91.1) (Jensen et al., in press-b), and rookeries in Ryukyus, Japan (i.e. CmP20.1 and CmP49.1) (Hamabata et al., 2014). Two haplotypes of CmP75.1 and CmP154.1 have not previously found in nesting rookeries, but CmP75.1 contained the identical sequence to CmP75 (IND3) haplotype based on the shorter ~380-bp region that was observed in the Indian Ocean (Formia et al., 2006). The haplotype and nucleotide diversity indices of Brunei Bay were $h = 0.8444 \pm 0.0390$ and $\pi = 0.009350 \pm 0.004964$, respectively.

No significant differences between Brunei Bay foraging aggregation and foraging aggregations at Mantanani Island/Layang Layang Island (Jensen et al., in press-a) was shown by exact test ($P = 0.379$). In fact, all haplotypes observed in Brunei Bay except CmP75.1 were observed in Mantanani Island/Layang Layang Island and both contained CmP57.1, CmP87.1, and CmP49.1 in high proportion (27 of 42 samples in Brunei Bay and 47 of 90 samples in Mantanani Island/Layang Layang Island; Jensen et al., in press-a).

3.3. Mixed stock analysis

The estimated contributions from 23 rookeries and their 4 regional groups are summarized in Tables 2 and 3. The Gelman–Rubin shrink factors were ≤ 1.10 , indicative of successful convergences. The estimated means and 95% confidence intervals (CIs) indicated relatively high values of Sulu Sea (with uninformative prior: mean = 45.31%, 95% CI = 24.36%–64.32%; with informative prior: mean = 48.88%, 95% CI = 30.54%–67.64%), Peninsular Malaysia (with uninformative prior: mean = 17.42%, 95% CI = 0.0%–41.56%; with informative prior: mean = 29.67%, 95% CI = 0.00%–60.40%), and Sarawak (with uninformative prior: mean = 19.13%, 95% CI = 0.00%–37.98%; with informative prior: mean = 12.46%, 95% CI = 0.00%–46.72%) (Table 2). Particularly, contribution from the Sulu Sea rookery was estimated to be the highest and lower 95% CIs were more than zero ($\geq 24.36\%$) in both analyses with uninformative and informative

Table 2
Estimated contributions (%) from 23 rookeries to the Brunei Bay foraging aggregation.

Region	Rookery	Uninformative prior				Informative prior			
		Mean	2.50%	Median	97.50%	Mean	2.50%	Median	97.50%
Southeast Asia	Peninsular Malaysia ^a	17.42	0.00	16.60	41.56	29.67	0.00	33.44	60.40
	Sarawak, west Borneo ^a	19.13	0.00	18.63	37.98	12.46	0.00	0.00	46.72
	Sulu Sea ^a	45.31	24.36	45.64	64.32	48.88	30.54	48.82	67.64
	Berau Island, east Borneo ^a	1.84	0.00	0.00	16.56	3.26	0.00	0.00	24.44
	Southeast Sabah, northeast Borneo ^a	5.04	0.00	0.00	36.64	0.81	0.00	0.00	8.38
Southwestern Pacific	Northern Great Barrier Reef ^a	0.11	0.00	0.00	1.27	0.31	0.00	0.01	2.78
	Coral Sea/Chesterfields ^{a,b}	0.11	0.00	0.00	1.25	0.04	0.00	0.00	0.27
	Southern Great Barrier Reef ^a	0.11	0.00	0.00	1.22	0.08	0.00	0.00	0.97
	western New Caledonia ^{a,b,c}	0.11	0.00	0.00	1.21	0.03	0.00	0.00	0.12
	northern New Guinea ^a	1.61	0.00	0.00	12.26	1.03	0.00	0.00	11.20
	Vanuatu ^b	0.83	0.00	0.00	8.62	0.05	0.00	0.00	0.00
Micronesia	Yap ^{a,c}	0.42	0.00	0.00	5.37	0.15	0.00	0.00	1.17
	Marshall Islands ^c	0.82	0.00	0.00	8.59	0.19	0.00	0.00	2.56
	Palau ^c	1.07	0.00	0.00	9.90	0.21	0.00	0.00	3.05
	Guam/CNMI ^c	1.82	0.00	0.00	12.32	0.03	0.00	0.00	0.00
Eastern Indian Ocean	Aru ^a	0.90	0.00	0.00	8.28	0.21	0.00	0.00	2.95
	Gulf of Carpentaria ^a	0.16	0.00	0.00	1.93	0.14	0.00	0.00	1.54
	Ashmore Reef ^a	1.34	0.00	0.00	14.00	0.57	0.00	0.00	9.78
	Scott Reef/Browse ^a	0.28	0.00	0.00	3.32	0.01	0.00	0.00	0.00
	West Java ^a	0.82	0.00	0.00	9.41	0.04	0.00	0.00	0.00
	North West Shelf ^a	0.12	0.00	0.00	1.37	1.82	0.01	1.01	8.08
	Cobourg Peninsula ^a	0.17	0.00	0.00	1.97	0.00	0.00	0.00	0.00
	Cocos Keeling Island ^a	0.46	0.00	0.00	5.64	0.01	0.00	0.00	0.00

^a Jensen et al. (in press-b);

^b Read et al. (2015);

^c Dutton et al. (2014).

Table 3
Estimated contributions (%) from 5 groups of rookeries to the Brunei Bay foraging aggregation.

Region of rookeries	Uninformative prior				Informative prior			
	Mean	2.50%	Median	97.50%	Mean	2.50%	Median	97.50%
Southeast Asia	88.74	72.45	89.83	98.96	95.08	81.01	96.97	99.93
Southwestern Pacific	2.87	0.00	0.77	14.80	1.53	0.00	0.10	12.01
Micronesia	4.14	0.00	2.83	15.22	0.58	0.00	0.00	8.17
Eastern Indian Ocean	4.25	0.00	1.79	20.44	2.80	0.01	1.39	14.53

priors (Table 2). On the other hand, analysis with informative prior indicated contribution from the North West Shelf of western Australia (lower 95% CI > 0.00%) that was not estimated in the analysis with uninformative prior (Table 2). The regional group estimations were indicative of a contribution to Brunei Bay mainly from Southeast Asian rookeries (with uninformative prior: mean = 88.74%, 95% CI = 72.45%–98.96%; with informative prior: mean = 95.08%, 95% CI = 81.01%–99.93%) (Table 3), confirming the estimated contributions from individual rookeries.

4. Discussion

The size distribution of the sampled individuals indicated that the Brunei Bay foraging ground was utilized by relatively large green turtle juveniles or even adults (CCL \geq 65 cm), although the sampling method may have biased the size distribution of sampled individuals. Considering that green turtles recruit to foraging ground at Mantanani Island in Malaysia at 38 cm CCL (Pilcher, 2010) and to neritic foraging grounds in other regions at about a 20- to 35-cm carapace length (e.g. Colman et al., 2015; Hayashi and Nishizawa, 2015), and assuming no rapid decrease in hatchling and small juvenile survival probability, green turtles do not recruit to Brunei Bay foraging ground for the first time in their lives, but instead shift from other foraging grounds to the Brunei Bay foraging ground. Ontogenetic shifts of neritic foraging grounds were also indicated by Hayashi and Nishizawa (2015). Juvenile sea turtles may use some foraging grounds as their temporary sites where they settle after the oceanic development phase and move on to more productive foraging grounds (Pilcher, 2010).

In fact, genetic composition and estimated origins of the Brunei Bay foraging aggregation showed similarity with those of Mantanani Island/Layang Layang Island foraging aggregations (Jensen et al., in press-a), although Mantanani Island/Layang Layang Island foraging aggregation was dominated by juveniles with CCL < 65 cm (Pilcher, 2010; Jensen et al., in press-a). Both foraging aggregations were characterized by a dominance of CmP57.1, CmP49.1, and CmP87.1 haplotypes, and

main contributors were from the Southeast Asian rookeries of the Sulu Sea, Peninsular Malaysia, and Sarawak. The results indicated that Mantanani Island and Layang Layang Island are candidate foraging grounds utilized by green turtles born in Southeast Asian rookeries before they shift to the Brunei Bay foraging ground. Jensen et al. (in press-a) suggested the Balabac Straits between Borneo and Philippines as possible foraging grounds of adults and large juvenile green turtles, but this study confirmed that Brunei Bay attracts Southeast Asian adults and large juvenile turtles with the abundance of seagrasses found in Brunei Bay (Bujang et al., 2006; Ahmad-Kamil et al., 2013). Interestingly, in three female green turtles tracked from Redang Island nesting beach in Peninsular Malaysia, one reached the Brunei coast in Borneo and one reached the Balabac Straits (Luschi et al., 1996). This suggested the link between the Peninsular Malaysia rookeries and the Brunei Bay foraging aggregation, and that both Brunei Bay and Balabac Straits are utilized by adult green turtles.

The reason why small juveniles do not utilize Brunei Bay is still unclear, but a possible reason is a surface oceanic current. Southward flow from Peninsular Malaysia and anticlockwise flow in the South China Sea indicated by Lagrangian drifter buoy data (Nishizawa et al., 2016) suggest that it is difficult for hatchlings born in rookeries at Peninsular Malaysia and Sulu Sea to reach Brunei Bay by passive drifting. Large juveniles and adults may arrive at Brunei Bay by active swimming.

The utilization of Brunei Bay foraging ground mainly by green turtles born in the Southeast Asian rookeries, relatively proximate to this foraging ground, was confirmed by the regional group estimations. This is in contrast to the estimations in southern Japanese foraging aggregations contributed from Japanese rookeries and Micronesian rookeries (Nishizawa et al., 2013; Hayashi and Nishizawa, 2015) and in Australian foraging aggregations contributed from Eastern Indian Ocean rookeries (Dethmers et al., 2010). The foraging ground of Brunei Bay is utilized by different source populations from those in the northwestern Pacific and northern Australia, and green turtles born in Southeast Asia are suggested to utilize foraging grounds in Southeast Asia.

In addition to main contribution from Southeast Asian rookeries, estimations with informative prior weighted by population size indicated that contribution from the North West Shelf of Western Australia was small but its lower 95% CI was more than 0.00%. Because only Cmp49.1 was shared with Brunei Bay foraging aggregation and the North West Shelf rookery, the result is considered to be derived from largest population size of North West Shelf. Validity of this estimation depends on the assumption that contribution to the foraging aggregation is proportional to the population size of rookeries, and conclusion for the estimate from the North West Shelf rookery needs to be interpreted with caution.

By using haplotypes based on longer ~770-bp regions than ~380-bp regions in previous studies (Dethmers et al., 2006, 2010; Cheng et al., 2008; Nishizawa et al., 2011, 2013), MSA in this study achieved higher resolution. MSA based on short regions indicated that 95% CIs of all nesting rookeries, except for the North West Shelf weighted by population size, included zero (Supplementary Online Materials, see Appendix A), but MSA based on longer regions confirmed the contribution from the Sulu Sea rookery with lower 95% CIs more than zero ($\geq 24.36\%$). This is attributed to the fact that longer regions identified Cmp49.3 haplotype that is specific to the Sulu Sea and the Southeast Sabah rookeries and that could not be identified based on the shorter regions from Cmp49.1 widely observed in the other rookeries.

Despite estimated contribution with high resolution, two haplotypes not previously found in nesting rookeries indicate the existence of unidentified rookeries or under-sampling in some rookeries. The presence of Cmp75.1 that contains the identical sequence to Cmp75 (IND3) based on ~380-bp regions observed in the western Indian Pacific rookery (Formia et al., 2006) may indicate a contribution from the western Indian Ocean rookeries. Analyzing longer ~770-bp sequences of more samples from rookeries will increase our understanding of green turtle foraging aggregations in Southeast Asia.

5. Conclusions

This study increases our understanding of the conservation of green turtles in Southeast Asia. Brunei Bay is confirmed to be an important foraging ground for large juveniles and adults that nest in Southeast Asian rookeries, and an ontogenetic shift in foraging grounds is supported by the difference in size class distribution but similarity in haplotype composition and estimated origins between Brunei Bay and Mantanani Island/Layang-Layang Island foraging aggregations. Because green turtles born in Southeast Asia are suggested to utilize foraging grounds in Southeast Asia, conservation efforts in Southeast Asia are required for protection of regional populations. Conversely, considering that the green turtle is a member of the unique marine ecosystem of Brunei Bay, conservation of Southeast Asian rookeries of the green turtle may be important for the Brunei Bay ecosystem. Even within Southeast Asia, international or interstate collaboration will be required because Brunei Bay and assumed migratory routes are shared between Brunei Darussalam and the east Malaysian States of Sabah, Sarawak, and Federal Territory of Labuan.

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Appendix A. Supplementary material

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.gecco.2016.01.003>.

References

- Abreu-Grobois, F.A., Horrocks, J., Formia, A., Leroux, R., Velez-Zuazo, X., Dutton, P., Soares, L., Meylan, P., Browne, D., 2006. New d-loop primers which work for a variety of marine turtle species may increase the resolution capacity of mixed stock analyses. In: Presentation at the 26th Annual Symposium on Sea Turtle Biology and Conservation, Crete, Greece, 2–8 April.
- Ahmad-Kamil, E.I., Ramli, R., Jaaman, S.A., Bali, J., Al-Obaidi, J.R., 2013. The effects of water parameters on monthly seagrass percentage cover in Lawas, East Malaysia. *Sci. World J.* <http://dx.doi.org/10.1155/2013/892746>.
- Allard, M.W., Miyamoto, M.M., Bjorndal, K.A., Bowen, B.W., 1994. Support for natal homing in Green turtles from mitochondrial DNA sequences. *Copeia* 1994, 34–41.
- Bali, J., 2005. Marine resources of Kuala Lawas and their utilisation. In: Hornbill Vol. 7: Proceedings of the Fourth Annual Workshop of the National Parks and Wildlife Division. Sarawak Forestry Department, Kuching, pp. 148–162.
- Bass, A.L., Epperly, S.P., 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.
- Bolker, B., Okuyama, T., Bjorndal, K., Boltzen, A., 2007. Incorporating multiple mixed stocks in mixed stock analysis: ‘many-to-many’ analysis. *Mol. Ecol.* 16, 685–695.
- Bowen, B.W., 1995. Tracking marine turtles with genetic markers. *Bioscience* 45, 528–534.
- Bruford, M.V., Hanotte, O., Brookfield, J.F.Y., Bruke, T., 1992. Single-locus and multilocus DNA fingerprinting. In: Hoelzel, A.R. (Ed.), *Molecular Genetic Analysis of Population*. IRL Press, Oxford, pp. 225–269.
- Bujang, J.S., Zakaria, M.H., Arshad, A., 2006. Distribution and significance of seagrass ecosystem in Malaysia. *Aquat. Ecosyst. Health Manag.* 9 (2), 203–214.
- Campbell, L.M., Silver, J.J., Gray, N.J., Ranger, S., Broderick, A., Fisher, T., Godfrey, M.H., Gore, S., Jeffers, J., Martin, C., McGowan, A., Richardson, P., Sasso, C., Slade, L., Godley, B., 2009. Co-management of sea turtle fisheries: Biogeography versus geopolitics. *Mar. Policy* 33, 137–145.
- Chan, E.H., 2006. Marine turtles in Malaysia: On the verge of extinction? *Aquat. Ecosyst. Health Manag.* 9, 175–184.
- Chan, E.H., 2013. A report on the first 16 years of a long-term marine turtle conservation project in Malaysia. *Asian J. Conserv. Biol.* 2, 129–135.
- Cheng, I.-J., Dutton, P.H., Chen, C.-L., Chen, H.-C., Chen, Y.-H., Shea, J.-W., 2008. Comparisons of the genetics and nesting ecology of two green turtle rookeries. *J. Zool.* 267, 375–384.
- Colman, L.P., Patrício, A.R.C., McGowan, A., Santos, A.J.B., Marcovaldi, M.Â., Bellini, C., Godley, B.J., 2015. Long-term growth and survival dynamics of green turtles (*Chelonia mydas*) at an isolated tropical archipelago in Brazil. *Mar. Biol.* 162, 111–122.
- Department of Fisheries Sabah. 2010. Summary of Annual Fisheries Statistics Sabah 2008. Kota Kinabalu, Malaysia.
- Dethmers, K.E.M., Broderick, D., Moritz, C., Fitzsimmons, N.N., Limpus, C.J., Lavery, S., Whiting, S., Guinea, M., Prince, R.I.T., Kennett, R., 2006. The genetic structure of Australasian green turtles (*Chelonia mydas*): exploring the geographical scale of genetic exchange. *Mol. Ecol.* 15, 3931–3946.
- Dethmers, K.E.M., Jensen, M.P., Fitzsimmons, N.N., Broderick, D., Limpus, C.J., Moritz, C., 2010. Migration of green turtles (*Chelonia mydas*) from Australasian feeding grounds inferred from genetic analyses. *Mar. Freshw. Res.* 61, 1376–1387.
- Dutton, P.H., 1996. Methods for collection and preservation of samples for sea turtle genetic studies. In: Bowen, B.W., Witzell, W.N., (Eds.), *Proceedings of the International Symposium on Sea Turtle Genetics*. NOAA Technical Memorandum, NMFS-SEFSC-396, Miami, Florida, pp. 17–24.
- Dutton, P.H., Balazs, G.H., LeRoux, R.A., Murukawa, S.K.K., Zarate, P., Martinez, L.S., 2008. Compositions of Hawaiian green turtle foraging aggregations: mtDNA evidence for a distinct regional population. *Endanger. Species Res.* 5, 37–44.
- Dutton, P.H., Jensen, M.P., Frutchey, K., Frey, A., LaCasella, E., Balazs, G.H., Cruce, J., Tagarino, A., Farman, R., Tatarata, M., 2014. Genetic stock structure of green turtle (*Chelonia mydas*) nesting populations across the Pacific Islands. *Pac. Sci.* 68, 451–464.
- Excoffier, L., Lischer, H.E.L., 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.
- Formia, A., Godley, B.J., Dontaïne, J.-F., Bruford, M.W., 2006. Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. *Conserv. Genet.* 7, 353–369.
- Gelman, A., Rubin, D.B., 1992. Inference from iterative simulation using multiple sequences. *Statist. Sci.* 7, 457–511.
- 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.
- Hayashi, R., Nishizawa, H., 2015. Body size distribution demonstrates flexible habitat shift of green turtle (*Chelonia mydas*). *Glob. Ecol. Conserv.* 3, 115–120.
- Hirth, H.F., 1997. *Synopsis of the Biological Data on the Green Turtle Chelonia mydas* (Linnaeus 1758). Fish and Wildlife Service, US, Washington, DC.
- IUCN. 2015. IUCN Red List of Threatened Species version 2015.1. <http://www.iucnredlist.org> (accessed on 1.05.2015).
- Jaaman, S.A., Bali, J., Hussain-Lee, A., Mohamad-Isa, S., Tisen, O.B., 2010. A preliminary sighting record of shorebirds in Sundar Estuary, Lawas, Sarawak. In: Paper presented in the Sarawak Forestry Corporation (SFC) 10th. Hornbill Workshop: Managing Ecosystem for Sustainability, Mega Hotel, Miri, 15–17 December.
- Jensen, M.P., Pilcher, N., Fitzsimmons, N.N., 2016. Genetic markers provide insight on origins of immature green turtles (*Chelonia mydas*) with biased sex ratios at two foraging grounds in Sabah, Malaysia. *Endanger. Species Res.* (in press-a).
- Jensen, M.P., Bell, I., Limpus, C.J., Hamann, M., Ambar, S., Whap, T., David, C., Fitzsimmons, N.N., 2016. Spatial and temporal genetic variation among size classes of green turtles (*Chelonia mydas*): provide information on oceanic dispersal and population dynamics. *Mar. Ecol. Prog. Ser.* (in press-b).
- Joseph, J., Chong, Y.K., Palaniappan, P., Liew, H.C., 2014. Genetic investigation of green turtles (*Chelonia mydas*) harvested from a foraging ground at Mantanani, Sabah, Malaysia. *Herpetol. Conserv. Biol.* 9 (3), 516–523.
- Liew, H.C., Bali, J., Chan, E.H., Braken, O., 2000. Satellite tracking of Green Turtles from the Sarawak Turtle Islands, Malaysia. *Mar. Turtle Newslett.* 87, 20.
- Luschi, P., Papi, F., Liew, H.C., Chan, E.H., Bonadonna, F., 1996. Long-distance migration and homing after displacement in the green turtle (*Chelonia mydas*): a satellite tracking study. *J. Comp. Physiol.* 178, 447–452.
- Naro-Maciel, E., Gaughran, S.J., Putman, N.F., Amato, G., Arengo, F., Dutton, P.H., McFadden, K.W., Vintinner, E.C., Sterling, E.J., 2014. Predicting connectivity of green turtles at Palmyra Atoll, central Pacific: a focus on mtDNA and dispersal modelling. *J. R. Soc. Interface* 11, 08888. 2013.
- 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. *Endanger. Species Res.* 14, 141–148.
- Nishizawa, H., Joseph, J., Chong, Y.K., 2016. Spatio-temporal patterns of mitochondrial DNA variation in hawksbill turtles (*Eretmochelys imbricata*) in Southeast Asia. *J. Exp. Mar. Biol. Ecol.* 474, 164–170.
- Nishizawa, H., Naito, Y., Suganuma, H., Abe, O., Okuyama, J., Hirate, K., Tanaka, S., Inoguchi, E., Narushima, K., Kobayashi, K., Ishii, H., Tanizaki, S., Kobayashi, M., Goto, A., Arai, N., 2013. Composition of green turtle feeding aggregations along the Japanese Archipelago: implications for changes in composition with current flow. *Mar. Biol.* 160, 2671–2685.
- Pella, J., Masuda, M., 2001. Bayesian methods for analysis of stock mixtures from genetic characters. *Fish. Bull.* 99, 151–167.

- Pilcher, N.J., 2010. Population structure and growth of immature green turtles at Mantanani, Sabah, Malaysia. *J. Herpetol.* 44 (1), 168–171.
- Prosdocimi, L., Carman, V.G., Albareda, D.A., Remis, M.I., 2012. Genetic composition of green turtle feeding grounds in coastal waters of Argentina based on mitochondrial DNA. *J. Exp. Mar. Biol. Ecol.* 412, 37–45.
- Putman, N.F., Mansfield, K.L., 2015. Direct evidence of swimming demonstrates active dispersal in the sea turtle lost years. *Curr. Biol.* 25, 1221–1227.
- Rajamani, L., Marsh, H., 2010. Using parallel regional- and local-scale initiatives to inform conservation management of rare wildlife: a case study of the dugong *Dugong dugon* in Sabah, Malaysia. *Endanger. Species Res.* 13, 17–23.
- Read, T.C., FitzSimmons, N.N., Wantiez, L., Jensen, M.P., Keller, F., Chateau, O., Farman, R., Werry, J., MacKay, K.T., Petro, G., Limpus, C.J., 2015. Mixed stock analysis of a resident green turtle, *Chelonia mydas*, population in New Caledonia links rookeries in the South Pacific. *Wildl. Res.* <http://dx.doi.org/10.1071/WR15064>.
- Shanker, K., Pilcher, N.J., 2003. Marine turtle conservation in South and Southeast Asia: hopeless cause or cause for hope? *Mar. Turtle Newslett.* 100, 43–51.
- Sievers, F., Wilm, A., Dineen, D.G., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D., Higgins, D.G., 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* 7, 539.
- Sterling, E.J., Mcfadden, K.W., Holmes, K.E., Vintinner, E.C., Arengo, F., Naro-Maciel, E., 2013. Ecology and conservation of marine turtles in a central Pacific foraging ground. *Chelonian Conserv. Biol.* 12, 2–16.
- 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.