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Original Research Article

Mass sea turtle slaughter at Pulau Tiga, Malaysia: Genetic studies indicate poaching locations and its potential effects

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ABSTRACT

It is important to identify the location of illegal poaching and its effects on the conservation of endangered species. This study applied molecular techniques to estimate the origin of sea turtle carcasses (N = 53) found at Pulau Tiga, Kudat, Malaysia (Borneo) in 2014. All carcasses were of adult (77%) and large juvenile (23%) green turtles (*Chelonia mydas*). A total of 10 haplotypes of mitochondrial DNA were identified. A Bayesian mixed-stock analysis showed that the natal origin was mainly from the Sulu and Celebes Seas (uninformative prior: median = 53.0%, 95% credible interval [CI] = 34.5–76.9%; informative prior: median = 61.3%, CI = 36.9–89.4%). The estimation of source foraging grounds of the carcasses as poaching sites indicated the Brunei Bay in the South China Sea as the most probable source (median = 90.2%, CI = 11.2–99.9%), although caution is needed since there is a possibility of poaching at unsampled foraging grounds. The results indicate that such poaching has negative effects especially for the nesting populations at the Sulu and Celebes Seas. This study provides information that contributes to the development of measures against poaching activities by regional collaboration regarding sea turtle traffic and law enforcement in Southeast Asia.

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1. Introduction

Overexploitation, including illegal poaching, of bio-resources, is a challenge to wildlife conservation (Rosser and Mainka, 2002; Milner-Gulland et al., 2003). Seizing and patrolling is often difficult without information about when and where poaching occurs. Surveys on carcasses and confiscated illegal items are important for revealing the state of poaching and its effects on populations (e.g., Mancini and Koch, 2009; Senko et al., 2014; Booth and Dunham, 2016). Recently, molecular

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techniques have enabled us to identify species and geographic origins of these items (Ogden et al., 2009). This information contributes to the understanding of poaching hotspots and their geographic effects, assisting in the proposal of conservation priorities and law enforcement (e.g., Welton et al., 2013; Wasser et al., 2015; Foran and Ray, 2016).

Sea turtles are under heavy exploitation pressures since humans harvest their eggs, meat, and shells (Campbell, 2003). Although most species are listed as threatened species (“Critically Endangered”, “Endangered”, or “Vulnerable”) on the IUCN Red List (IUCN, 2018), harvesting of sea turtles, often illegally, continues in many locations (Mancini and Koch, 2009; Lam et al., 2011; Quiñones et al., 2017). In Southeast Asia, sea turtle harvesting has been a traditional activity among coastal communities, providing an important source of protein and income for local people. Harvests targeting mainly green (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) at their foraging grounds in Southeast Asia (especially within the Coral Triangle region) have been ongoing and have become more rampant in recent years (Lam et al., 2011). Evidence of seizures records highlighted a consistent illegal trade route to mainland China from the Coral Triangle region of Southeast Asia (Lam et al., 2011). Revenues generated from harvesting sea turtles on their foraging grounds, as well as other endangered marine animals, are sufficient to encourage foreign fishermen (mostly from China and Vietnam) to keep encroaching Southeast Asian waters with apprehensions concentrated in the Philippines, Malaysia, and Indonesia (van Dijk and Shepherd, 2004; Pilcher et al., 2008; Lam et al., 2011).

Poaching incidents and seizures of sea turtles have been extensively reported in Southeast Asia (e.g., 288 dead turtles aboard a Chinese fishing vessel near Mantanani Island in Malaysia; Joseph et al., 2014). However, probably only a small fraction of illegal harvesting is known. Patrolling is difficult since there are many foraging grounds of sea turtles in the South China Sea (Pilcher, 2010; Jensen et al., 2016; Joseph et al., 2014, 2016) and Celebes Sea (Nishizawa et al., 2018). The ontogenetic habitat changes of sea turtles proposed for green turtles in this region (Joseph et al., 2016; Nishizawa et al., 2018) and possible intermittent poaching activities at several locations make it difficult to specify poaching sites and its effects. Thus, even if harvested sea turtles, including dumped carcasses, are found in coastal areas in Southeast Asia, it does not always mean that (i) poaching occurs at a nearby foraging site and (ii) poached turtles were born in nearby rookeries.

In this paper, we report findings related to carcass samples found at Pulau Tiga, Kudat, Malaysia in 2014, suspected of being from a mass slaughter of sea turtles by poachers. Pulau Tiga is located in the Balabac Strait area (i.e., the strait that connects the South China Sea with the Sulu Sea). As there are no major nesting rookeries in the area, limited studies on sea turtles have been conducted. However, the strait is probably the corridor of sea turtles from rookeries in the Turtle Islands in the Sulu Sea, one of the world's few remaining major nesting rookeries in Southeast Asia for green and hawksbill turtles (Chan, 2006; Asian Development Bank, 2017), to foraging grounds in the South China Sea, and vice versa (Joseph et al., 2016; Nishizawa et al., 2018). The strait may also be utilized by poachers who are targeting source locations across the Sulu and Celebes Seas; therefore, carcasses poached at multiple foraging grounds might be dumped at Pulau Tiga. This area was promoted as a marine protected area, the Tun Mustapha Park (TMP), by the Malaysian government in 2016, but it requires protection plans and strong enforcement to ensure effective management.

This study aims to determine the species composition and size frequencies of sea turtle carcasses found on the Pulau Tiga island. Also, using genetic techniques, carcasses are assigned (i) to rookeries, to determine their natal origin, and (ii) to foraging grounds, to estimate where poaching occurred. The results provide insights regarding sea turtles migration and the potential effects of poaching on sea turtle populations.

2. Materials and methods

2.1. Study site

The turtle carcasses were found at the Pulau Tiga (7°20'27.64" N – 117°02'51.06" E; Fig. 1), which is located at the Balabac Strait, about 40 km northeast of Kudat, Sabah (Malaysia). There are no inhabitants on the island, as it was abandoned since the 1990s.

2.2. Turtle carcasses and sample collection

In a series of interviews conducted by one of the authors (James Alin) in early 2014, local fishermen in Kudat informed him about the movement of turtle poachers around the Balambangan–Banggi channel. Based on the interviews, poachers were reported to have camped out on Pulau Tiga to hunt sea turtles and sell them across the border to fishers from China and Vietnam. In March 2014, James Alin visited Pulau Tiga and found fresh and degraded sea turtle carcasses on the island (The Star Online, March 22, 2014; Daily Express, March 23, 2014). On April 2, 2014, Sabah Parks, together with the Malaysian Maritime Enforcement Agency, went to Pulau Tiga to further investigate the reported sea turtle carcasses.

A total of 53 turtles carcasses were found scattered around the island (Fig. 2). Species were identified based on their carapace and head (if any) and further confirmed genetically. Where possible, the turtles were measured for their curved carapace length (CCL) and curved carapace width (CCW) using a flexible measuring tape. Based on these measurements, the maturity stages (i.e., adults, large juveniles, and small juveniles) of specimens were estimated following Sterling et al. (2013). Also, the carcasses were checked for any tags or other indication of the origin of the turtles. All carcasses were brought back to the Sabah Parks office in Kudat. A small part of the turtle's flipper or bone was sampled for genetic analyses. Samples were cleaned, washed with ethanol, and individually packed in sealed plastic bags labelled with an identification number.

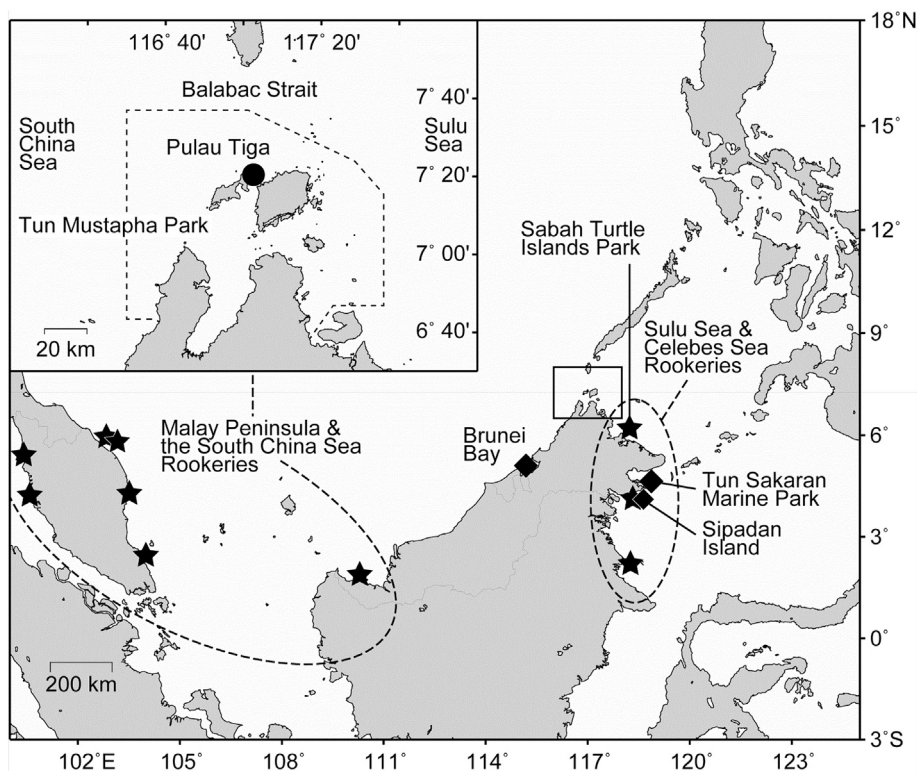


Fig. 1. Map showing the location of Pulau Tiga within the Tun Mustapha Park, Kudat, with the major sea turtle foraging grounds (diamonds; Brunei Bay, Tun Sakaran Marine Park, and Sipadan Island) and main nesting rookeries in the Malay Peninsula, the South China Sea, the Sulu Sea, and the Celebes Sea (stars).

2.3. Laboratory procedures

Genetic analyses were conducted at the Molecular laboratory of the Universiti Malaysia Terengganu. Genomic DNA was extracted using the CTAB protocol (Bruford et al., 1992). Because most of the carcass samples were badly degraded, the obtained DNA contents were very low. Hence, two-step polymerase chain reaction (PCR) protocols were used. First, a segment of the mitochondrial control region of approximately 800 bp was amplified from the extracted DNA using primers LCM15382 and H950g (Abreu-Grobois et al., 2006). Using the first PCR product as template, it was re-amplified by semi-nested PCR using primers LTEi9 and H950g (Abreu-Grobois et al., 2006). The PCR amplification was performed using an Eppendorf Mastercycler DNA Engine Thermal Cycler PCR according to protocols previously described by Joseph et al. (2016). Then, all amplified samples were verified for the targeted band size by 1% agarose gel electrophoresis. Finally, the PCR products were sent to First BASE (Kuala Lumpur, Malaysia) for purification and sequencing of both strands.

The sequences were truncated to ~770 bp region that has been widely evaluated in recent studies of sea turtles (e.g., Read et al., 2015; Jensen et al., 2016; Nishizawa et al., 2018). Haplotypes were identified by performing a search using the program BLAST in the NCBI (National Center for Biotechnology Information, USA, website <http://www.ncbi.nlm.nih.gov>). The South-west Fisheries Science Center, NOAA Fisheries Service (<https://swfsc.noaa.gov>), was also consulted for mtDNA sequences of green turtles from the Pacific and the Indian Ocean.

2.4. Molecular and mixed-stock analyses

Based on the haplotype frequencies of the carcass samples from Pulau Tiga, their natal origin was estimated by Bayesian mixed stock analysis (MSA) using the BAYES software (Pella and Masuda, 2001). Settings for the analysis followed Nishizawa et al. (2018). In short, a total of 31 management units of green turtle rookeries in Southeast Asia, the southern Pacific Ocean, and the eastern Indian Ocean, defined by Nishizawa et al. (2018) based on Dutton et al. (2014), Read et al. (2015), and Jensen et al. (2016), were used as candidate source nesting populations. These rookeries belong to five regional groups, namely (i) Southern Pacific, (ii) Micronesia and New Guinea, (iii) Malay Peninsula and the South China Sea, (iv) Sulu and Celebes Seas, and (v) the Eastern Indian Ocean, that were defined in the input file (Nishizawa et al., 2018). Contributions from these regions in the output were estimated using the regional-grouping option in BAYES. We ran five Markov Chain Monte Carlo (MCMC) chains, and each chain began with 95% of the mixed samples that were initially provided for each region. Each chain was run for 50,000 iterations, and the first 25,000 were discarded as burn-in. The convergence of the MCMC sampling was assessed



Fig. 2. Green turtle carcasses found scattered at Pulau Tiga, Kudat, Malaysia. Photo credit: Sabah Parks. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

using the Gelman–Rubin shrinkage factor (Gelman and Rubin, 1992), which indicates a lack of convergence if the value is greater than 1.2. When the resulting value was greater than 1.2, the number of iterations of each chain was increased up to 100,000 with half of them discarded as burn-in. Mixed stock analyses were conducted with (i) uninformative priors that assumed the same size for all populations and (ii) informative priors weighted by population size based on Dutton et al. (2014), Read et al. (2015), and Jensen et al. (2016).

MSA also estimated the contributions of nearby foraging grounds to the carcass samples. As the candidate sources, we used three foraging aggregations of Brunei Bay (South China Sea), Sipadan Island (Celebes Sea) and Tun Sakaran Marine Park (Celebes Sea), of which genetic compositions were reported in Nishizawa et al. (2018). We ran three MCMC chains, each of which began with the assumption that 95% of the mixed samples were contributed by each foraging ground. The regional-grouping option was not used, and only uninformative priors were used due to the lack of information about the sizes of the foraging aggregations. Other settings were identical to the ones used for the estimation of natal origin. In addition to MSA, the differences in haplotype composition of the carcass samples from foraging aggregations were also tested by exact tests (50,000 iterations in a Markov chain with a 10,000-iteration dememorization) implemented in Arlequin v 3.5 (Excoffier and Lischer, 2010).

3. Results

3.1. Species identification and size frequencies

A total of 53 sea turtle carcasses were found at Pulau Tiga, Kudat, with CCL ranging from 67.0 to 111.0 cm (Fig. 3), with a mean and standard deviation of 91.3 and 10.2 cm, respectively. All carcasses were identified on site. Some of the carcasses were still fresh, while others were badly decomposed. Based on external examinations, all carcasses were identified as green turtles. This was further supported by the mtDNA haplotypes found in the genetic analyses. Two carcasses had tags that showed they originated from the Turtle Islands of Malaysia (Tag numbers: MYS 87117 and MYS 87118) and the Philippines (Tag number: PH1526). Turtle MYS 87117/MYS 87118 (CCL = 95.0 cm) was tagged on June 25, 2013, and had laid one nest

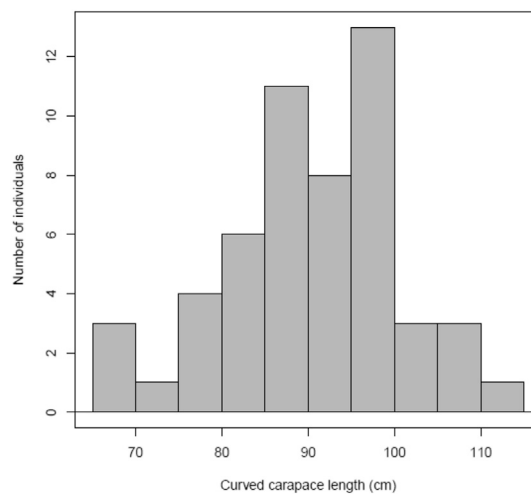


Fig. 3. Class size frequency of green turtle based on the curved carapace length of the carcasses found at Pulau Tiga, Kudat, Malaysia.

(Sabah Parks, pers. comm). However, no information was obtained from turtle PH1526. Most of the turtle carcasses were adults (77%), followed by large juveniles (23%). There were no carcasses of small juveniles.

3.2. Haplotype composition of carcass samples

Of the 53 carcass samples, 39 were successfully amplified. From these, ten haplotypes were detected (Table 1). Eight haplotypes corresponded to green turtles from nesting and foraging ground samples from Southeast Asia (Nishizawa et al., 2018). Two haplotypes had not been previously described and were registered in GenBank as CmP87.2 (MF109838) and CmP91.2 (MF109839).

Regardless of whether prior distribution was informative or not, the MSA showed that the natal origin of carcass samples was mainly from the Sulu and Celebes Seas (uninformative prior: median = 53.0%, 95% credible interval [CI] = 34.5–76.9%; informative prior: median = 61.3%, CI = 36.9–89.4%) and Malay Peninsula and South China Sea (uninformative prior: median = 36.4%, CI = 10.6–60.7%; informative prior: median = 24.4%, CI = 7.7–54.5%) (Fig. 4). Assignments to specific management units were vaguer, but the MSA results significantly specified that carcass samples originated from the Turtle Islands in the Sulu Sea (uninformative prior: median = 47.3%, CI = 26.5–65.5%; informative prior: median = 46.5%, CI = 26.9–66.2%) (Supplementary Material S1).

The estimated source foraging grounds in Southeast Asia indicated a very high contribution from the Brunei Bay foraging ground (median = 90.2%, CI = 11.2–99.9%) (Fig. 5). Contributions from the Tun Sakaran Marine Park and Sipadan foraging grounds had relatively small medians and CIs that included 0.0%, despite wide CI ranges (Tun Sakaran Marine Park: median = 4.6%, CI = 0.0–84.2%; Sipadan: median = 1.2%, CI = 0.0–25.2%). Exact tests indicated that the haplotype composition of carcass samples was significantly different from those of foraging aggregations of Sipadan ($p = 0.00006$) and Tun Sakaran Marine Park ($p = 0.00928$), but not significantly different from that of Brunei Bay ($p = 0.39148$).

4. Discussion

There are four species of sea turtles recorded nesting in Southeast Asia, the green turtle, hawksbill turtle, leatherback turtle, and olive ridley turtle (Shanker and Pilcher, 2003; Chan, 2006). Several foraging grounds for green and hawksbill turtles have been reported in the South China Sea and the Celebes Sea, but reports of hawksbill turtles are relatively sporadic (Pilcher, 2010; Joseph et al., 2016, 2017; Nishizawa et al., 2016, 2018). Probably, the reasons for this are that (i) green turtles are normally found in seagrass areas (Mortimer, 1982; Joseph et al., 2016) and (ii) green turtle nesting populations are abundant and widely distributed in Southeast Asia (Shanker and Pilcher, 2003; Chan, 2006). Thus, it is relatively easier for poachers to catch green turtles in the shallow waters of seagrass beds. This may explain why the carcasses found at Pulau Tiga were only green turtles.

All the carcasses sampled in this study consist of adults and large juveniles. Therefore, it is reasonable to assume that these turtles were poached at a foraging ground of adults and large juveniles. Green turtles generally shift their habitats ontogenetically (Hayashi and Nishizawa, 2015; Hamabata et al., 2018). In Southeast Asia, the South China Sea is utilized by green turtles born in rookeries along the South China Sea and the Sulu Sea, whereas the Celebes Sea is utilized by green turtles born in rookeries in the Sulu Sea, and Micronesia and New Guinea (Nishizawa et al., 2018). Shifts in foraging ground with growth probably occur in the South China Sea, possibly from Mantanani and Layang Layang Island to the Brunei Bay (Joseph et al., 2016). Considering that green turtles may go back and aggregate into breeding areas near their natal nesting beaches

Table 1
Haplotype composition of sea turtle carcasses found at Pulau Tiga, Kudat, Malaysia.

Haplotype	GenBank no.	N
CmP49.1	AB819808	8
CmP49.3	KJ502572	1
CmP57.1	KJ502588	12
CmP57.2	KJ502567	4
CmP82.1	KJ502584	1
CmP87.1	KJ502589	4
CmP91.1	KF311762	6
CmP40.1	KF311750	1
CmP87.2	MF109838	1
CmP91.2	MF109839	1
Total		39

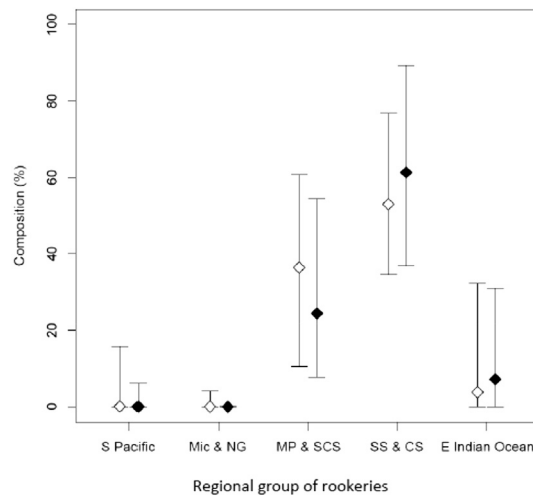


Fig. 4. Estimated natal origin of the carcasses. Median values estimated with uninformative and informative priors are illustrated by hollow and filled diamonds, respectively. Bars indicate 95% credible intervals. Abbreviations *S Pacific*: Southern Pacific, *Mic & NG*: Micronesia and New Guinea, *MP & SCS*: the Malay Peninsula and the South China Sea, *SS & CS*: the Sulu Sea and the Celebes Sea, *E Indian Ocean*: Eastern Indian Ocean.

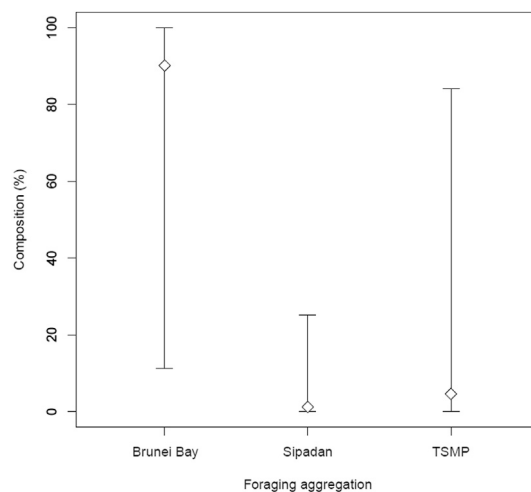


Fig. 5. Median values and 95% credible intervals of the estimated contributions from foraging aggregations to the carcasses. Abbreviation *TSMP*: Tun Sakaran Marine Park.

(FitzSimmons et al., 1997; Dethmers et al., 2006), they might be poached at breeding areas. However, this possibility is relatively low as the carcass samples in this study included not only adults but also large juveniles.

The natal origin of the green turtles slaughtered at Pulau Tiga was mainly from Southeast Asian rookeries. Significant contributions from the Turtle Islands in the Sulu Sea are not surprising because the Turtle Islands represent one of the largest rookeries of green turtles in Southeast Asia (about 30,000 green turtles nesting per year on all islands of the Sabah Turtle Islands and the Philippines Turtle Islands; Sabah Parks, pers. comm.). The estimated composition of the natal origin of Pulau Tiga carcasses was similar to that of foraging aggregations in the South China Sea rather than the Celebes Sea (Nishizawa et al., 2018). In fact, the haplotype composition of carcasses was similar to the Brunei Bay, and a high proportion was assigned to the Brunei Bay foraging aggregation in the MSA using foraging aggregations as sources. These results indicate that poaching occurred in the South China Sea, and not in distant sites in the Celebes Sea, where there are potential targeting sites for poachers (Lam et al., 2011).

Here, it is important to note that the high contribution from the Brunei Bay foraging aggregation does not necessarily indicate that the Brunei Bay is the exact poaching site. Green turtles might be poached at unreported foraging grounds in the South China Sea and the Sulu Sea near Pulau Tiga, including the Balabac Strait and TMP, where the aggregations may have similar genetic compositions to that of the Brunei Bay. Alternatively, the Balabac Strait is an important migratory corridor for green turtles from foraging grounds in the South China Sea to nesting rookeries or different foraging grounds in the Sulu Sea, and vice versa. Therefore, poachers might target migrating turtles at the Strait. In addition, high CIs assigned to foraging aggregations in MSA, probably due to shared haplotypes (e.g., Cmp49.1 and Cmp57.1), could not deny the possibility that poaching activities occur at several sites including not only around Pulau Tiga (i.e., the Balabac Strait, South China Sea, and the Sulu Sea), but also at distant sites (e.g., from Celebes Sea to South China Sea).

If mass slaughters of large-sized green turtles at Pulau Tiga commonly occur, it represents a threat to the conservation of green turtles in Southeast Asia. For slow-maturation species such as sea turtles, it is necessary to have a high number of large juveniles and adults to maintain populations (Crouse, 1999; Heppell et al., 2003). The survival probability of adult green turtles is naturally high (Chaloupka and Limpus, 2005). In this situation, harvesting adult turtles directly reduce nesting population size. In this study, the harvested turtles were estimated to have originated from Southeast Asian rookeries and are supposed to recruit to the nesting populations unless they were not poached. Also, the two harvested adults that had tags on them originated from the Sabah Turtle Islands and possibly the Philippines Turtle Islands. This information not only supports the MSA estimation of natal origin but also provides direct evidence of a mortality factor for nesting green turtles at the Turtle Islands. Rampant poaching activities represent a problem even though the number of nesting green turtles in the Turtle Islands has recovered and is currently kept high (Shanker and Pilcher, 2003).

The establishment of the Tun Mustapha Park may warn against the activities of poachers around Pulau Tiga. However, there is little information about how green turtles utilize this area. Residency of green turtles around the Balabac Strait should be confirmed by further studies for the effective management of this area. As poached sea turtles outside the protected area are possibly transported to Pulau Tiga, regional collaborations and strict enforcement are required to conserve sea turtles in Southeast Asia.

Overexploitation is one of the numerous threats for sea turtle conservation, which include poaching of eggs and meat, incidental captures, entanglements, and boat collisions (Lutcavage et al., 1997; Senko et al., 2014). In recent years, harvesting of sea turtles on their foraging grounds has become more serious, especially in Malaysia, the Philippines, and Indonesia, due to the incursion of foreign vessels (van Dijk and Shepherd, 2004; Pilcher et al., 2008; Lam et al., 2011). Identifying the location of poaching and its effects is becoming important for sea turtle conservation and law enforcement. This study applied molecular techniques that have been prevalent not only in ecological studies but also in wildlife forensics (Ogden et al., 2009). Similar techniques, in combination with the development of genetic markers for more precise geographic resolution such as mitochondrial short tandem repeats (Tikochinski et al., 2012, 2018), will further contribute to the development of measures against poaching activities.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gecco.2019.e00586>.

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