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Low genetic diversity after a bottleneck in a population of a critically endangered migratory marine turtle species

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ABSTRACT

Hawksbill turtles (*Eretmochelys imbricata*), which are distributed throughout the world's oceans, have undergone drastic declines across their range, largely due to anthropogenic factors. Assessing sizes, genetic variability and structure of their populations at global and regional levels is critical to the development of conservation management strategies. Here, nuclear and mitochondrial markers were used to analyse patterns of parentage and population structure in hawksbill turtles in United Arab Emirates (UAE) waters, utilizing samples from two life stages (hatchlings and juveniles), and to compare the UAE population with neighboring populations. Weak genetic differentiation was detected between juveniles and hatchlings and between the nesting sites of Dubai and Sir Bu Nair. Parentage analysis suggested that only 53 females and 74–80 males contributed to the hatchlings from 67 nests across three nesting sites in UAE (Dubai, Sir Bu Nair, Abu Dhabi). No females were identified as nesting in more than one location. In Dubai and Abu Dhabi, single paternity was the norm (75%), whereas on Sir Bu Nair, multiple paternity was detected in the majority of nests (67%). Polygyny was also frequently detected on Sir Bu Nair (15% of the overall number of males), but not in the other nesting sites. Comparison of the UAE population with published data from other populations suggests that population structure exists both within the Gulf and between the Gulf and Indian Ocean populations, and that the UAE population has lower genetic variability than the Seychelles population. Finally, the data suggest that the UAE population, and the Gulf population overall, experienced a bottleneck/founder event. The observed overall low genetic variability, evidence of population structure in the Gulf, and strong differentiation between the Gulf and the Indian Ocean populations, raises concerns about the sustainability of this species in this near-enclosed basin. These results highlight the need for regional collaboration in the development of management measures for the long-term conservation of this Critically Endangered species.

1. Introduction

The hawksbill turtle (*Eretmochelys imbricata*) occurs throughout the world's tropical oceans (Witzell, 1983), and is considered Critically Endangered across its range by the International Union for the Conservation of Nature Red List (IUCN, 2016). Worldwide, hawksbill populations have been drastically reduced by the harvesting of eggs for food and the hunting of adult turtles for the use of their carapace as curios (McClenachan et al., 2006). As hawksbill populations continue to decline in many parts of the world, there is a need to better understand this species' biology, life history, nesting ecology, population trends, and movements/migrations, as well as population structure and connectivity, in order to develop appropriate management measures and assist the recovery of populations.

Inferences from molecular genetics have transformed the study of sea turtles (Bowen and Karl, 2007; Lee, 2008), and advanced the knowledge on topics such as natal philopatry (Meylan et al., 1990), migration patterns (Bowen et al., 2005), sex-biased gene flow (FitzSimmons et al., 1997a), mating systems (Phillips et al., 2013; Tedeschi et al., 2015), effective population size (Phillips et al., 2014), and even hybridization (Lara-Ruiz et al., 2006). Despite this body of research, the sea turtle molecular ecology literature remains biased towards green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles (Bowen and Karl, 2007; Jensen et al., 2016; Lee, 2008; Matsuzawa et al., 2016; Shamblin et al., 2015; Tedeschi et al., 2015). By contrast, hawksbill turtles have been less well studied, with a bias towards populations of the western Atlantic (Bowen and Karl, 2007; Velez-Zuazo et al., 2008; Vilaça et al.,

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2013). Until recently, hawksbill molecular research in the Indian Ocean consisted of a set of location-specific studies with a non-standardised set of markers (e.g. mtDNA, various suites of microsatellites; Mortimer and Broderick, 1999; Phillips et al., 2014; Tabib et al., 2011; Zolgharnein et al., 2011). A recent Indo-Pacific-wide mtDNA study set out a much broader picture of regional population structure, but also highlighted the lack of country-specific information on hawksbill populations at both nesting and foraging grounds (Vargas et al., 2015).

The hawksbills of the Arabian/Persian Gulf (henceforth 'the Gulf') were one of the eight Indo-Pacific genetic stocks identified by Vargas et al. (2015). Despite potentially harsh conditions (e.g. a 22 °C range in annual water surface temperatures (Carpenter et al., 1997; Sheppard et al., 2010)), the area supports considerable numbers of hawksbills, with 100–1000 individuals nesting each year in each of Saudi Arabia, Iran, the United Arab Emirates (UAE), and Qatar (e.g. Al-Ghais, 2009; Al-Merghani et al., 2000; EAD, 2007, 2015; Miller, 1989; Mobaraki, 2004; Pilcher, 1999, 2000; Pilcher et al., 2015; SCENR, 2006), with smaller numbers (<10 annual nesters) on the offshore islands of Kuwait (Meakins and Al Mohanna, 2004). In recent years, anthropogenic threats, including the harvesting of eggs on remote islands (EAD, 2007; Pilcher et al., 2014), the stranding of juvenile turtles due to cold stunning (hypothermic reaction in cold water temperature; Caliendo et al., 2010), drowning in fishing gear (EAD, 2007), and accelerating coastal development (Sheppard et al., 2010) have negatively affected populations and their habitats, threatening the future of the species in the area. However, many aspects of the ecology of the hawksbills in the Gulf, including movements, migrations, and population connectivity, are poorly known. Addressing some of these outstanding questions will help the design and implementation of effective management plans for the area's hawksbills.

In the UAE, monitoring has shown that hawksbills nest on the mainland in Dubai, on Abu Dhabi's inshore and offshore islands, and on the offshore island of Sir Bu Nair (EAD, 2007, 2015; Pilcher et al., 2014). Still, the numbers of females and males that may be contributing to these nesting beaches is not known, and nor is the degree to which these nesting beaches are interconnected. Work on Iranian hawksbills has indicated genetic differentiation between nesting beaches only 350 km apart (Zolgharnein et al., 2011), but studies in other regions have detected no significant differentiation at 500 km (Phillips et al., 2014). It is also unknown how juvenile turtles feeding in Gulf waters relate to the region's nesting beaches (e.g. see Bowen and Karl, 2007) and how many breeding sites the area sustains. Establishing such boundaries and connections is important in defining management units and in assessing the benefits/risks associated with particular environmental interventions/impacts (e.g. Bowen and Karl, 2007; Godfrey et al., 2007; Mortimer et al., 2007a, 2007b). This is particularly true in the UAE, where large-scale coastal developments, increasing effluents from desalination and electricity generation, and other human stressors are substantially changing the environment (Sheppard et al., 2010).

Here, molecular markers were used to investigate the hawksbill population of the UAE. The parentage patterns, population connectivity among nesting beaches, and the relationship of juveniles to those nesting beaches were assessed. Then, the UAE population was compared with other hawksbill populations from elsewhere in the Gulf and Indian Ocean using published molecular datasets. The aim of this study is to provide information on the genetic and demographic health of the UAE hawksbill population, and contribute to a better understanding of this species within and beyond the Gulf.

2. Materials and methods

2.1. Study sites and samples

Tissue samples were collected from hatchling and stranded juvenile hawksbill turtles in the UAE. Samples were preserved in DMSO 20% $NaCl_2$ 5 M.

2.1.1. Hatchling sampling

Samples were collected during nest monitoring by the Emirates Marine Environmental Group (EMEG), which undertook daily beach patrols from 6 pm to 6 am at these sites and a number of others in the UAE during the nesting season from early March to April (Fig. 1). Nests were excavated and checked for dead hatchlings one week after the first observed hatchling emergence. One to five freshly dead hatchlings per nest for each of 68 nests across three nesting areas were sampled: (1) Dubai = 23 nests, 2008–2010; (2) Abu Dhabi (Sir Bani Yas, Bu Tinah, Saadiyat Island) = 5 nests, 2009–2010; (3) Sir Bu Nair Island = 40 nests, 2010 (total hatchling samples = 295).

2.1.2. Juvenile sampling

Samples were collected from 123 stranded juvenile hawksbills reported from Abu Dhabi (n = 16), Dubai (n = 100), Sharjah (n = 5), Ras Al Khaimah (n = 1) and Sir Bu Nair (n = 1) in the winter seasons between 2007 and 2010. After rehabilitation and prior to release, tissue was taken from the trailing edge of the forelimb using a sterile 6 mm biopsy punch. Based on carapace dimensions and body weight, all sampled juveniles were considered to be less than one year old at the time of stranding (Caliendo et al., 2010) and therefore to have been born during the previous nesting season.

2.2. Molecular methods

DNA was extracted using an ammonium acetate method (Nicholls et al., 2000) and diluted to a working concentration of 10 ng/μl. Samples were genotyped at 33 variable microsatellite loci in three multiplex PCRs, following the methodology of Phillips et al. (2013). Amplification was conducted using Qiagen Multiplex PCR kit in 2 μl PCRs (Kenta et al., 2008; Phillips et al., 2013). PCR products were separated and sized on an ABI 3730 automated sequencer with ROX 500 size standard, and the resulting genotype traces scored in GeneMapper 3.7 (all Applied Biosystems). Individuals were removed entirely from subsequent analysis if data were missing for more than ten loci in total. Loci were checked for the presence of null alleles in CERVUS 3.0 (Marshall et al., 1998) using a subsample of 32 juveniles.

All juvenile samples and one hatchling sample per nest were amplified for the mitochondrial control region (D-loop) using the primer pair LCM 15382/H950 (Abreu-Grobois et al., 2006). Amplification was conducted following the methodology described in Abreu-Grobois et al. (2006). PCR products were purified with QIAgen PCR purification columns and sequenced using the ABI dye-terminator method as implemented by MACROGEN. Mitochondrial DNA sequences were aligned using ClustalX (Thompson et al., 1997) and edited with BioEdit Alignment Editor v.7.0.9 (Hall, 1999). New haplotypes have been submitted to GenBank (accession numbers: KY363929, KY363930, KY363931, KY363932, KY363933, Supplementary Table 1).

2.3. Data analysis

2.3.1. Parentage analysis

Parentage analysis was conducted in COLONY 2.0 (Wang and Santure, 2009), which uses a maximum-likelihood method to assign parentage and sibship groups. Hatchling microsatellite genotypes were entered into COLONY, along with: A) maternal sibships known from field data, B) excluded maternal sibships known from mtDNA data, and C) per-locus estimates of genotyping error (0.011–0.023) derived from repeat PCR of 96 samples. The program was allowed to infer both polyandry and polygyny, and to estimate and update allele frequencies during analysis. Five runs of COLONY were performed, with all runs having 'medium' length and 'medium' likelihood precision, and each run having a different random number seed. A second batch of five runs was performed that included stranded juvenile genotypes.

Estimates of the number of females contributing to the hatchlings samples were obtained directly from the COLONY outputs, after