Journal of Experimental Marine Biology and Ecology 455 (2014) 29–37

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Molecular techniques reveal cryptic life history and demographic processes of a critically endangered marine turtle

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ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form 17 February 2014
Accepted 20 February 2014
Available online 19 March 2014

Keywords:
Effective population size
Marine turtles
Mating systems
Paternity reconstruction
Population genetics

ABSTRACT

The concept of 'effective population size' (N_e), which quantifies how quickly a population will lose genetic variability, is one of the most important contributions of theoretical evolutionary biology to practical conservation management. N_e is often much lower than actual population size: how much so depends on key life history and demographic parameters, such as mating systems and population connectivity, that often remain unknown for species of conservation concern. Molecular techniques allow the indirect study of these parameters, as well as the estimation of current and historical N_e. Here, we use genotyping to assess the genetic health of an important population of the critically endangered hawksbill turtle (*Eretmochelys imbricata*), a slow-to-mature, difficult-to-observe species with a long history of severe overhunting. Our results were surprisingly positive: we found that the study population, located in the Republic of Seychelles, Indian Ocean, has a relatively large N_e, estimated to exceed 1000, and showed no evidence of a recent reduction in N_e (i.e. no genetic bottleneck). Furthermore, molecular inferences suggest the species' mating system is conducive to maintaining a large N_e, with a relatively large and widely distributed male population promoting considerable gene flow amongst nesting sites across the Seychelles area. This may also be reinforced by the movement of females between nesting sites. Our study underlines how molecular techniques can help to inform conservation biology. In this case our results suggest that this important hawksbill population is starting from a relatively strong position as it faces new challenges, such as global climate change.

1. Introduction

Small populations lose genetic variation much more rapidly than large populations, as they are more susceptible to inbreeding and more strongly affected by genetic drift (Wright, 1931). Importantly, almost all populations will lose genetic variation more quickly than expected from their census population size N, due to factors that include variation between individuals in reproductive success, fluctuations in population size, unequal sex ratios, and population structure. This greater rate of loss is quantified as the population's effective size N_e (Wright, 1931), which is often substantially lower than N (Frankham, 1995; Hartl, 1988). Given that low genetic diversity increases the risk of population extinction and may reduce adaptability to future environmental change (Frankham et al., 1999; Franklin and Frankham, 1998), N_e and its implications for genetic diversity are important considerations in the management of species of conservation concern (Frankham et al., 2002).

Amongst conservation-priority species, demography and life history are often not well known enough for their impacts on N_e to be assessed, which restricts the potential for adjusting management plans to help solve specific conservation problems (Hare et al., 2011; Palstra and Ruzzante, 2008). In such situations, molecular techniques are essential tools, allowing mating systems to be assessed, migration and dispersal patterns to be explored, and inbreeding and genetic diversity to be quantified. Of particular value to conservation managers is the utility of molecular methods for inferring connectivity/structure amongst populations, to identify and measure the breeding contributions of unseen individuals, to derive estimates of N_e directly from molecular data, and to infer past changes in N_e such as population bottlenecks (e.g. Frankham et al., 2002; Piry et al., 1999; Waples, 1989).

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http://dx.doi.org/10.1016/j.jembe.2014.02.012
0022-0981/

Population declines driven by overhunting, habitat loss, and other anthropogenic factors have made marine turtles a global conservation priority (Wallace et al., 2011). However, little is known about N_e in most populations of these taxa, or about how N_e might relate to census counts. This makes it difficult to quantify loss of genetic variation, or assess how low levels of variation may slow population recovery and reduce adaptability to future perturbations such as global climate change (see Hawkes et al., 2009; Wright et al., 2012b). To estimate N_e and adjust conservation management accordingly, we require more information about specific key life history and demographic parameters than is currently available for many marine turtle populations. For example, male reproductive skew is a key parameter influencing effective population size, with N_e being larger the more evenly reproduction is distributed amongst males within the population (Hartl, 1988). In marine turtles, the vast majority of paternity studies have focused on data from a single nesting season (but see Lasala et al., 2013; Wright et al., 2012a), but accurate assessment of skew in such long-lived species requires assessing paternity across years. If the same set of males sires the offspring of a given nesting site across years, skew will be higher and N_e lower than if the number/local turnover of males is greater. Furthermore, the ability to estimate N_e directly from molecular data (e.g. Wang, 2009; Waples, 1989), and to use these data to infer past changes to N_e (e.g. Garza and Williamson, 2001; Piry et al., 1999), have rarely been applied to marine turtles (but see Rivalan et al., 2006; Theissinger et al., 2009).

The hawksbill turtle (*Eretmochelys imbricata*) occurs throughout the world's tropical oceans, and is IUCN-listed as critically endangered following substantial population declines driven by anthropogenic factors (Mortimer and Donnelly, 2008). Many aspects of the hawksbill's life history are poorly known, and most published genetic work involves hawksbill populations in the Caribbean (Blumenthal et al., 2009; Bowen et al., 2007). In the Indo-Pacific, little is known about the distribution of genetic variation beyond the existence of broad-scale structure between several major rookeries (Vargas et al., 2013), and gene flow between both juveniles and nesting females of two of the region's most important populations, those of Seychelles and Chagos (Mortimer and Broderick, 1999; Sheppard et al., 2012). However, a study of mating systems based on one year's data from hawksbills in the Republic of Seychelles indicated that the number of males in this population was large, based on evidence that the majority of females were fertilised by a single male each but that no male fertilised more than one female (Phillips et al., 2013). Here, we use a four-year dataset from the same population to quantify N_e and compare it to census data, to test for changes in N_e in the recent past that might indicate genetic effects of population declines, and to assess key processes affecting N_e, such as dispersal and between-year patterns of parentage. Using samples collected from nesting beaches spanning several hundred kilometres across Seychelles, we also assess population genetic structure and consider the implications of our results for N_e and for ongoing hawksbill conservation management in the region. Our results help us move towards a fuller understanding of demographic and life history parameters in a species that is inherently difficult to study, and reiterate the value of molecular techniques to conservation biologists.

2. Methods

2.1. Field sampling

Tissue samples were collected from female hawksbills and ca 20 hatchlings per nest on Cousine Island (04°21′S, 55°38′E), Republic of Seychelles, over four nesting seasons (Sep–Apr) spanning Sep 2007–Apr 2011. For a full field protocol as used on Cousine, see Phillips et al. (2013). Over the first three years, sampling of females and nests was near exhaustive. In 2010/11, samples were only collected from previously unsampled adult females, and from hatchlings from the nests of females observed on Cousine in any of the three previous seasons of the study.

For analysis of population structure, tissue samples were collected in the 2010/11 and 2011/12 seasons from females nesting on additional islands across the Seychelles (Fig. 1): in the Granitic Seychelles (the region that includes Cousine; Fig. 1C), from Frégate (04°35′S, 55°57′E) and North Islands (04°24′S, 55°15′E); and in the Amirantes (the outer coralline islands; Fig. 1B), from D'Arros/St. Joseph (05°25′S, 53°19′E), Desroches (05°42′S, 53°40′E), and Alphonse/St. François (07°04′S, 52°44′E). Additionally, a small number of juvenile hawksbills were hand-captured and sampled in the waters around Aldabra Atoll (09°26′S, 46°23′E). These samples were collected by removing a small section of tissue from the trailing edge of a flipper with a sterile scalpel, ideally during nesting for adult females. On captive turtles, no long-term harm has been detected from comparable tissue sampling (Bjorndal et al., 2010).

2.2. Molecular analysis

Following DNA extraction (ammonium acetate method; Nicholls et al., 2000), individuals were genotyped at 32 microsatellite loci in three polymerase chain reaction multiplexes, as described in Phillips et al. (2013). An individual's genotype for a given multiplex was not used in downstream analyses if more than four loci (out of the 10–11 loci) from that multiplex failed to amplify, and individuals were removed entirely if two multiplexes were discounted, or if more than ten loci failed in total. Where possible, we genotyped at least 20 offspring per female from the 2007/08 and 2008/09 seasons. Time and cost constraints meant that we were unable to do this for 2009/10: instead, we genotyped 3 offspring from every female, and an additional 10–12 offspring from a subsample of 20 families.

2.3. Parentage assessment and reconstruction of male genotypes

After checking estimated null allele frequencies (CERVUS 3.0; Marshall et al., 1998) and assumptions of Hardy–Weinberg and linkage equilibria (GENEPOP 4.2; Raymond and Rousset, 1995; Rousset, 2008), parentage analysis was conducted on the entire Cousine dataset using COLONY 2.0 (Wang and Santure, 2009). This programme uses a maximum likelihood method to group offspring into full- and half-sib clusters, assign parentage, and reconstruct the genotypes of unsampled parents. COLONY was provided with per-locus estimates of genotyping error (Phillips et al., 2013) and allowed to update allele frequencies during the analysis. We ran the programme with three different random number seeds, with each run of 'medium' length and 'medium' precision. COLONY reconstructs the genotypes of unsampled parents on a locus-by-locus basis and provides a confidence value for each reconstruction. When assembling these into multilocus male genotypes, we only incorporated single-locus genotypes with confidence of ≥ 0.90, and only used multilocus genotypes in downstream analyses if they contained $\geq 29/32$ loci and were reconstructed from ≥ 10 offspring (see Phillips et al., 2013).

The programme COANCESTRY 1.0 (Wang, 2011) was used to screen the data for related adults prior to running any subsequent analyses, as some population genetics and N_e estimation methods can be adversely affected by the presence of close kin. Allele frequencies for use in COANCESTRY were obtained from three runs of COLONY on the entire dataset, with all adult females as candidate mothers and the Aldabra juveniles as offspring.

2.4. Population structure

Pairwise F_{ST} values and absolute number of migrants exchanged (M; Slatkin, 1991) were computed between all population pairs in ARLEQUIN 3.5 (Excoffier et al., 2005). The inbreeding coefficient F_{IS} (Wright, 1965) was also computed for each population. Male genotypes