Biological Conservation 225 (2018) 66–79

Contents lists available at ScienceDirect

Biological Conservation

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

Population declines, genetic bottlenecks and potential hybridization in sea snakes on Australia's Timor Sea reefs

Vimoksalehi Lukoschek

ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland 4811, Australia

ARTICLE INFO

Keywords:
Aipysurus
Emydocephalus
Population genetics
Ashmore Reef
Bottleneck
Hybridization

ABSTRACT

Population bottlenecks can result in loss of genetic variation, increased extinction risk, and hybridization with related sympatric species. Many challenges are associated with empirical detection of population declines, thus conservation biologists often use molecular approaches as surrogates. This study explored whether declines in abundances of viviparous sea snakes on Australia's Timor Sea reefs could have been foreshadowed using genetic surveys. Ashmore Reef (the largest Timor Sea reef) once hosted large breeding populations of sea snakes. Abundances have declined precipitously since 1994 and Ashmore Reef has been devoid of snakes since 2012. Moreover, high rates of hybridization between two sympatric species have been documented on Timor Sea reefs, possibly associated with sea snake declines. I analysed mitochondrial DNA and 11 nuclear microsatellites for > 250 sea snakes from three species, *Aipysurus laevis*, *Aipysurus fuscus* and *Emdocephalus annulatus*, sampled on four Timor Sea reefs in 2002 and 2010. While there was strong spatial genetic structure among reefs, there was little temporal genetic divergence for *A. laevis* at Ashmore Reef, despite the massive declines in abundance during that temporal window. Positive Tajima's D and Fu's F_S values at Ashmore Reef indicated demographic contraction for: *A. laevis* in 2002 and 2010; *E. annulatus* (2002); but not *A. fuscus* (2002). Microsatellites showed inbreeding depression (positive *Fis* values) and non-random mating (heterozygote deficit) for all three species at Ashmore Reef, consistent with population declines. Bottleneck tests were equivocal, with significant heterozygous excesses at Ashmore Reef, but non-significant M-ratios or mode-shifts in allele frequencies, with the significance of tests differing markedly with microsatellite mutation models. Thus genetic analyses alone would not have been sufficient to provide managers with unequivocal evidence of population declines. There was little evidence for hybridization between *A. laevis* and *A. fuscus*, despite previous research suggesting that the Endangered *A. fuscus* was at risk of reverse speciation secondary to the highly porous reproductive barriers between these species.

1. Introduction

Populations that experience sudden large reductions in effective size, often referred to as population bottlenecks (Frankham et al., 2002), can be subject to a multitude of changes. These changes include the loss of molecular genetic variation (Bouzat, 2010); increased identity by descent (Lande, 1988); compromised ability to adapt environmental change (Frankham et al., 1999; Reusch and Wood, 2007); and increased likelihood of extinction via a host of genetic and demographic processes (Keller and Waller, 2002). Reductions in population size may also reduce the availability of conspecific mates thereby increasing the probability of hybridization with related sympatric species, often referred to as Hubbs' principle or “desperation hypothesis” (Hubbs, 1955). Hybridization may further reduce the fitness of populations and impede their ability to adapt to environmental change, particularly where parental species are highly divergent and/or adapted to contrasting environments (Allendorf et al., 2010), resulting in greater risk of extinction. Alternatively hybridization may give rise to new genetic combinations, resulting in novel traits that impart new ecological potential absent from parental species, thereby increasing fitness (Nolte and Tautz, 2010). Understanding genetic and demographic changes in endangered species, and detecting possible associated hybridization, is therefore important for biodiversity conservation (Allendorf et al., 2010).

Viviparous sea snakes (Elapidae; Hydrophiinae) are an important group of mesopredators facing significant conservation challenges throughout their ranges in the shallow-water marine habitats of the Indo-West Pacific (Heatwole, 1999). Hydrophiine sea snakes are the most biodiverse extant group of marine reptiles, comprising two evolutionary lineages. The larger *Hydrophis* group has 46 nominal species in two genera (Lukoschek and Keogh, 2006; Sanders et al., 2013), while the much smaller *Aipysurus* group has 11 nominal species in two genera

E-mail address: Vimoksalehi.lukoschek@jcu.edu.au.

https://doi.org/10.1016/j.biocon.2018.06.018
Received 13 December 2017; Received in revised form 26 May 2018; Accepted 16 June 2018

(*Aipysurus* – nine species; *Emydocephalus* – two species), of which nine are endemic to Australasia (including the Coral Sea, New Guinea, and New Caledonia) (Cogger, 2014). Highest species diversity and endemism occurs on a handful of Timor Sea reefs off coastal Western Australia (Cogger, 2014). Historical records indicate that almost one quarter of the world's sea snake species occurred on Timor Sea reefs (Minton and Heatwole, 1975; Smith, 1926), and all have been recorded at Ashmore Reef (the largest Timor Sea reef) (Cogger, 2014), which has long been regarded as the sea snake capital of the world. While some species were likely vagrants, until recently Ashmore Reef hosted large resident breeding populations of at least nine sea snake species (Guinea, 2007; Lukoschek et al., 2013), with an estimated standing stock of almost 40,000 sea snakes on its 174 km^2 reef flat in 1994 (Guinea and Whiting, 2005). Between 1994 and 2010 sea snakes at Ashmore Reef declined precipitously (Guinea, 2007; Lukoschek et al., 2013), despite the reef's habitats and biota being highly protected since 1983, when Ashmore Reef was declared a National Nature Reserve (IUCN Category 1a) (Anon, 2002). Sighting rates of sea snakes at Ashmore Reef in 1973 (Minton and Heatwole, 1975) and 1994 (Guinea and Whiting, 2005) were between 42 and 46 snakes day^{-1}, whereas by 2002 sighting rates had halved to 21 snakes day^{-1}, and subsequently declined to < 5 snakes day^{-1} by 2005 (Lukoschek et al., 2013). Species richness followed a similar trajectory of decline, with nine species recorded in 1973 (Minton and Heatwole, 1975) and 1994 (Guinea and Whiting, 2005), whereas in 2002 only three species (*A. laevis*, *A. fuscus*, *E. annulatus*) remained in significant numbers (Lukoschek et al., 2013). By 2010 only *A. laevis* remained in highly reduced numbers in a small area of the Ashmore Reef complex (Lukoschek et al., 2013), with no snakes recorded in subsequent surveys (Guinea, 2013). Other Timor Sea reefs have been less well surveyed but available data indicate that, while Scott Reef continues to host large numbers of sea snakes, Cartier and Hibernia reefs may also be experiencing declines (Guinea, 2013). Previous genetic studies of *A. laevis* on the Great Barrier Reef, Gulf of Carpentaria, and Timor Sea reefs found different demographic histories during the Pleistocene, with sea snakes on the Great Barrier Reef and Gulf of Carpentaria having undergone range expansions associated with sea level rise since the last glacial maximum, whereas Timor Sea reefs appear to have had older more stable populations (Lukoschek et al., 2007b, 2008). Yet, *A. laevis* and *E. annulatus* also appear to have undergone local extinctions from some southern Great Barrier Reef reefs between 1970 and the early 2000s (Lukoschek et al., 2007a).

The disappearance from Ashmore Reef of three small-range endemic species [once thought to occur only on Timor Sea reefs but see D'anastasi et al., 2016 and Sanders et al., 2015] resulted in their listing as Critically Endangered (*A. apraefrontalis*, *A. foliosquama*) or Endangered (*A. fuscus*) (IUCN, 2011). In a recent study Sanders et al. (2014) used genetic data from 11 microsatellite loci for 80 snakes from two closely related species, *A. laevis* and *A. fuscus*, on Timor Sea reefs to infer high rates of hybridization at Hibernia (95%), Scott (55%) and Seringapatam (42%) reefs in 2012 and 2013, and at Ashmore Reef in 1998 and 2001. The majority of putative hybrids resembled the *A. laevis* phenotype, and Sanders et al. (2014) concluded that the two species had highly fragile reproductive boundaries with evidence of 'reverse speciation' and potentially dire conservation implications for the Endangered *A. fuscus*.

The challenges of obtaining accurate and/or precise estimates of abundance for large mobile marine vertebrates, combined with low power to detect changes in abundance (Lukoschek and Chilvers, 2008), means that even in intensively surveyed populations, declines may remain undetected until abundances reach catastrophically low levels (Taylor et al., 2007). This situation is compounded in the absence of regular standardized surveys, as has historically been and continues to be the case for sea snakes in most locations throughout their extensive ranges. Indeed, the absence of published survey data for sea snakes at Ashmore Reef meant that sighting rates obtained in 2002 [21 snakes day^{-1} Lukoschek et al., 2013] did not trigger concerns about population declines. It was not until sighting rates dropped below 5 snakes day^{-1} in 2005 that concerns about the disappearance of sea snakes were triggered (Guinea, 2006, 2007) and the much higher sighting rates from the 1990s (~45 snakes day^{-1}) were published (Guinea and Whiting, 2005). Moreover, the logistical challenges and expense of conducting field surveys to monitor sea snake abundances means that genetic surveys may play an important role in detecting population declines.

This study capitalizes on sea snake samples collected on Timor Sea reefs, during the period that snakes on Ashmore Reef underwent precipitous declines in census size, to retrospectively evaluate whether signals from genetic data could have foreshadowed these population bottlenecks. I used genetic analyses of mitochondrial DNA and nuclear microsatellites for > 250 sea snakes from *A. laevis*, the olive sea snake, *A. fuscus*, the dusky sea snake, and *E. annulatus*, the turtleheaded sea snake, sampled on Ashmore, Cartier, Hibernia and Scott reefs in the Timor Sea in 2002 and Ashmore Reef in 2010, to evaluate whether the massive declines in abundance at Ashmore Reef could be detected in spatial and temporal patterns of genetic diversity and divergence. Theory predicts that measures of genetic diversity (nucleotide/haplotype diversity, heterozygosity, allelic richness) would be lower in the bottlenecked populations than in comparatively stable populations (Bouzat, 2010) while there would be increased identity by descent (Lande, 1988). In addition, formal demographic and bottleneck analyses were used to evaluate the presence of bottlenecks on Timor Sea reefs. Patterns of genetic diversity and formal demographic and bottleneck analyses conducted for *A. laevis* on Timor Sea reefs were further compared with new analyses of genetic data from reefs in the Great Barrier Reef and Gulf of Carpentaria that are known to have experienced different demographic histories (Lukoschek, 2018; Lukoschek et al., 2007b). In addition, I investigated whether the findings of high levels of hybridization between *A. laevis* and *A. fuscus* in 1998–2001 and 2012–2013 (Sanders et al., 2014) could be replicated using larger sample sizes for both species from Timor Sea reefs in 2002 and 2010.

2. Methods

2.1. Sampling

Sampling was conducted on four Timor Sea reefs: Ashmore, Cartier, Hibernia and Scott (Fig. 1A_C). A total of 254 sea snakes from three species were sampled in 2002 and 2010 (Tables 1, S1). In 2002, samples were obtained from 98 *A. laevis* (Ashmore $n = 57$, Cartier $n = 9$, Hibernia n = 9, Scott $n = 23$); 29 *A. fuscus* (Ashmore $n = 26$, Scott $n = 3$); and 81 *E. annulatus* (Ashmore $n = 34$, Cartier $n = 6$, Hibernia $n = 33$, Scott $n = 8$). In 2010, samples were obtained from 43 *A. laevis* (Ashmore $n = 42$, Cartier $n = 1$) and 3 *E. annulatus* (Cartier n = 3). Tissue samples were collected from live snakes on SCUBA or snorkel (Lukoschek et al., 2007b) and stored at room temperature in 70% ethanol.

2.2. DNA extraction, mitochondrial DNA sequencing, and microsatellite genotyping

Samples were sequenced for mitochondrial ND4 (~700 bp) and adjacent 3' tRNA-His and tRNA-Ser (~120 bp) (Lukoschek et al., 2007b) and genotyped for 11 polymorphic nuclear microsatellites (Lukoschek and Avise, 2012). DNA extractions, PCR amplifications and mtDNA sequencing were conducted using the methods described in Lukoschek et al. (2007b). Sequences were edited and aligned in Geneious ver. R9.0.5 (Kearse et al., 2012) and visually refined. Nuclear microsatellites, developed for *E. annulatus* and previously shown to amplify in all *Aipysurus* species, were genotyped using the methods described in Lukoschek and Avise (2012). Alleles were sized using a ROX labeled GS500 internal standard and scored using GeneMapper 4.0 (Applied Biosystems).