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Population at risk: low genetic diversity in bottlenose dolphins of Bocas del Toro, Panama

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Abstract

Bottlenose dolphins are among the most studied cetaceans around the globe. Across its distribution there has been an increasing understanding of many aspects of their biology, however the lack of molecular studies is hindering adequate management planning regionally and locally. A recent study in the Caribbean has shown the existence of two forms an "inshore ecotype" and a "worldwide distributed form". Here we use molecular tools to determine the stock structure and the conservation status of a small population of bottlenose dolphins in Bocas del Toro (Panama), that is daily exposed to intense and aggressive dolphin-watching activities. Previous studies have found that dolphins from this

population react negatively to these activities suggesting long-term negative impacts that may result in population decline. Here we report results from initial genetic analyses of the Control Region of the maternally inherited mtDNA (mitochondrial DNA). Skin samples from individuals were obtained using a remote biopsy system (PAXARMS). Our results from the mtDNA control region analyses show this population has only one haplotype, not previously described anywhere in the Caribbean. This haplotype is shared between males and females suggesting high philopatry. The Neighbor-Joining analyses grouped this haplotype in a unique clade with previously described "inshore ecotype" haplotypes from Cuba, Bahamas and Mexico. Our results suggest that the bottlenose dolphin population of Bocas del Toro could may be the result of a "founder effect" from individuals from Caribbean northern populations. Microsatellite analyses are currently underway. Our results show the vulnerability of this bottlenose dolphin population and the importance to implement strategies to reduce negative effects of dolphin-watching in this population.

Introduction

The bottlenose dolphin (*Tursiops truncatus*) is probably the most extensively studied cetacean species in the world. Consequently, *T. truncatus* has been listed as a species of "Least Concern" (LC) by the IUCN Red List (IUCN 2013). However, it is also listed under Appendix II of CITES (CITES 2000). While the global population of bottlenose dolphin appears to be under no immediate threat, there are still worrying risks that may affect populations especially at the local level. This may be concerning given that some coastal populations worldwide show a high degree of genetic differentiation (e.g., Natoli et al. 2004, Segura et al. 2006, Parsons et al. 2006, Tezanos-Pinto et al. 2009, Caballero et al. 2011). This fine scale genetic structure has raised concerns about their vulnerability to human activities, such as unregulated dolphin-watching (Culik 2004). This is a particularly important industry in Latin American countries like Costa Rica and Panama, where tourism is an important source of income for local communities (Lemay 1998). However, the unregulated increase of this activity has potentially negative consequences for cetaceans, due principally to an increase in boat traffic (Hoyt & Hvenegaard 2002).

In the Archipelago of Bocas del Toro lives a small population of Bottlenose dolphins (~150 individuals), which is regularly subject to dolphin watching activities (May-Collado et al. SC64WW2). Previously obtained data shows that the mode of approach by the boats may have negative effects on dolphin communication, and more recently, shifts in their distribution have been recorded (e.g. May-Collado & Wartzok 2008, Quiñones-Lebrón & May-Collado 2011, May-Collado et al. SC64WW2). In addition, a reduction on mother-calf communication in the presence of boats can make calves more vulnerable to boat strikes (Slensland & Berggren 2007, Tseng et al. 2011), which have been documented in this population, and could consequently have a negative impact on the population's vital rates. The potential risk of decline of this population due to uncontrolled dolphin-watching activities raised concerns at the IWC Scientific Committee-Small Cetacean sub-committee (Panama, 2012) resulting in a recommendation to the government of Panama in order to further evaluate this particular situation in Bocas del Toro.

Hence, establishing dolphin population studies based on genetic information is fundamental for improving local and regional conservation and management strategies. Several studies have shown how coastal and pelagic bottlnose dolphin populations differ in their genetic structure and diversity, with coastal population showing significantly low variability (e.g., Natoli et al. 2004, 2005, Caballero et al. 2011, Segura et al. 2006, Tezanos-Pinto et al. 2009). Moreover, local studies indicate even finer genetic structuring within coastal waters, where bottlenose dolphins seem to form small and genetically distinct populations (e.g., estuaries, islands) (Gubbins 2002, Zolman 2002). A previous study on the plylogeography of *T. truncatus* in the Caribbean revealed the presence of “inshore” and “worldwide distributed form” haplotypes in Wider Caribbean waters (Caballero et al 2011). Based on mtDNA analyses Caballero et al (2011) proposed three management units for bottlenose dolphins: Puerto Rico, Cuba/Colombia/Bahamas/Mexico, and Honduras. Nevertheless, in local areas like Bocas del Toro it is difficult to define appropriate managements units due principally to the scarce information about population genetic structure and lack of abundance estimates of bottlenose dolphins (Oviedo & Silva 2005, Caballero et al. 2011).

Understanding how bottlenose dolphin populations are structured in the Caribbean is a top priority for IWC Scientific Committee meeting (St Kitts, 2006) where the sub-committee on small cetaceans highlighted the need for biopsy sampling of bottlenose dolphins from the area. The committee also highlighted the importance of documenting the status of small vulnerable dolphin populations in the Caribbean. Given that bottlenose dolphin in Bocas del Toro are exposed to an intense dolphin-watching, we conducted molecular analyses in order to provide key information about the status and population structure of these groups which are vulnerable, to provide important information to define adequate management units for their conservation.

Methods

Workshops with the community

In order to be able to conduct the research with the support of local communities, we offered informational workshops to the community of Bocas del Toro about the project. This allowed the community to become part of the project and understand the sampling techniques.

Biopsy sampling

Skin samples were obtained by firing remote biopsy darts from a modified rifle (Krützen et al. 2002). The biopsy system uses a modified 0.22 veterinary rifle with adjustable pressure. The biopsy darts have a hollow polycarbonate body and a small stainless steel biopsy tip (5 mm diameter, 9 mm length) (Krützen et al. 2002, Tezanos-Pinto & Baker 2011). This system allows penetrate the dolphin epidermis leaving behind a small wound (Tezanos-Pinto & Baker 2011). However, the effect on dolphins is low, because the polycarbonate body of dart to spread the impact over a wider area and therefore, reduce the risk of injury by penetration (Krützen et al. 2002, Parsons et al. 2003, Tezanos-Pinto & Baker 2011). Dolphin reactions have been scored on a 5 point scale from 0 (no reaction) to 5 reaction (strenuous reaction) (Krützen et al. 2002, Tezanos-Pinto & Baker 2011), being mild

reaction (level 1) more frequent (99% on 225 observation realized on bottlenose dolphins in New Zealand, Tezanos-Pinto & Baker 2011). The biopsies were obtained only if the individual was photo-identified to avoid sampling the same dolphin repeatedly (Krützen et al. 2002). The samples were preserved in alcohol 70% and stored at -20 ºC (Amos & Hoelzel 1991) for subsequent laboratory analysis.

DNA Extraction and Amplification

DNA was extracted from skin samples using the DNeasy kit (QIAGEN, Valencia, CA, USA). A 650pb *D-loop* hypervariable portion of the *mtDNA CR* was amplified by the polymerase chain reaction (PCR), using two pairs of primers, t-Pro-whale M13Dlp1.5 (5′-TGTAAAACGACAGCCAGTTCACCCAAAGCTGRARTTCTA-3′) and Dlp8G (5′-GGAGTACTATG TCCTGTAACCA-3′), following amplification conditions proposed by Baker et al. (1998). All samples were sexed following the protocol by Gilson et al (1998). Previously published sequences from the Caribbean were obtained from NCBI (accession numbers JN596281 to JN596321).

Data analyses

We used sequences of Control Region (mtDNA) from bottlenose dolphins in whole Caribbean obtained by Caballero et al. (2011), which were edited and aligned with the sequence obtained from Bocas del Toro (Drummond et al. 2009), to identify haplotypes using Geneious version 4.8.5 (Maddison & Maddison 2008). In order to understand the relationships among Bocas del Toro haplotypes and previously described haplotypes from the Caribbean, a Neighbor-Joining tree was constructed using PAUP, version 4.0 (Bandelt et al. 1999).

Results

We obtained 11 biopsy samples from bottlenose dolphins distributed in several locations in Bocas del Toro Archipelago, including Dolphin bay, Almirante bay, Pastores Island and Tierra Oscura. Additionally, one sample was obtained from a stranded dolphin found in the

area. All samples were sexed and five males and six females were identified among the 11 samples obtained. Haplotype analyses revealed only one haplotype detected in all samples obtained in in Bocas del Toro (fig. 1). This unique haplotype has not been previously described anywhere in the Caribbean, and is shared between males and females. Neighbor-Joining analyses grouped Bocas del Toro haplotype in a unique clade with previously described "inshore ecotype" haplotypes from Cuba, Bahamas and Mexico.

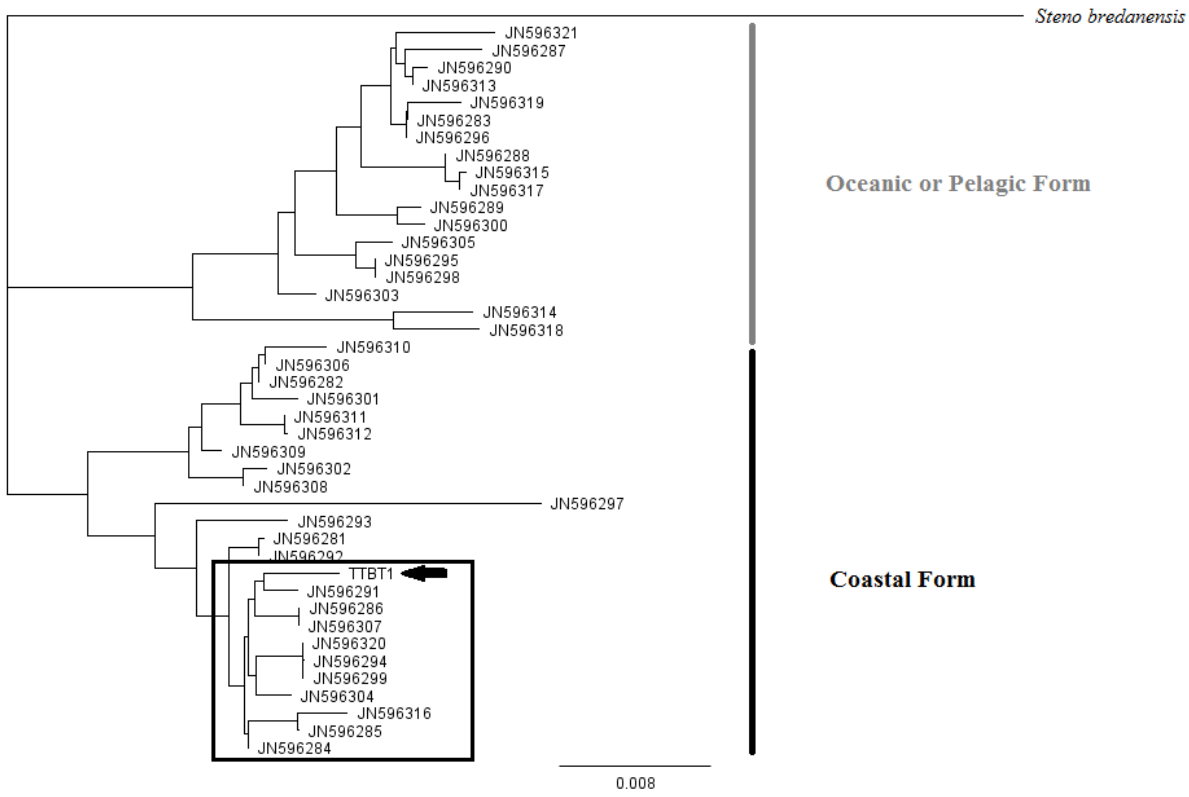

Figure 1. Neighbor-Joining reconstruction showing grouping of Wider Caribbean Control Region haplotypes. Black arrow indicates Bocas del Toro haplotype, which is grouped with haplotypes defined as belonging to the "inshore" ecotype. These other haplotypes have been reported in Cuba (JN596284, JN596285, JN596291, JN596294, JN596299), Bahamas (JN596285) and Mexico (JN596284, JN596286, JN596291, JN596304, JN596307, JN596320).

Discussion

Caballero et al. (2011) described two forms of bottlenose dolphins found in the Caribbean: the "inshore" and "worldwide distributed form". Specifically, the "inshore" ecotype

commonly found in the Bahamas, Mexico and the Western North Atlantic is also present in many of the Caribbean regions. The haplotype found in Bocas del Toro grouped with these "inshore" ecotype haplotypes. Based on mtDNA analyses, Caballero et al. (2011) proposed three management units for bottlenose dolphins: Puerto Rico, Cuba/Colombia/Bahamas/Mexico, and Honduras. Particularly, the Cuba/Colombia/Bahamas/Mexico mtDNA population unit presented a considerable number of individuals that were assigned to the 'inshore' ecotype. It is and it is possible that the bottlenose dolphin population of Bocas del Toro could be the result of a "founder effect" from individuals from Caribbean northern populations. In fact, migration analyses conducted by Caballero et al. (2011) suggested that the direction of female migration is from Puerto Rico to the Cuba/Colombia/Bahamas/Mexico mtDNA population unit and from Honduras to the Cuba/Colombia/Bahamas/Mexico mtDNA population unit.

Our work provides initial evidence that bottlenose dolphin in Bocas del Toro is highly phylopatric and is isolated from neighboring populations. These results agree with seven years of Photo-ID data that indicate this is a small and relatively isolated population (May-Collado et al. in prep.). These results have great implications for these dolphins, since boat traffic seems to be negatively affecting this population, and boat traffic could be an important factor in bottlenose dolphin population decline around the world (Lusseau 2003). Despite the IUCN Red List of Threatened Species considers *T. truncatus* like a Least Concern species, it is necessary to establish adequate conservation plans, since human activities have a negative effect on bottlenose dolphin populations around the world, as population of bottlenose dolphin in Fiordland (New Zealand), which was categorized recently as "Critically Endangered" because population decline by human activities (boat traffic and pollution), and genetic studies showed strong evidence of isolation (Lusseau 2003, 2003*b*, 2004, 2005).

In Panama, the Authority of Aquatic Resources created a legislation "Gaceta 25731" (not the appropriate word to protect cetaceans during interactions with boats), but this law is not being followed by the people, and this could have negative consequences on dolphin

population from Bocas del Toro. The increasing of boat traffic not controlled has affected the population of bottlenose dolphins in Bocas del Toro. Indeed, during 2012, there were three reports of death of dolphin calves by boat traffic. Nevertheless dolphin-watching is an important economic activity for local communities. For this reason, it is urgently establish regulations for reduce boat traffic impacts on dolphins need to be established urgently, principally because our results show bottlenose dolphin from Bocas del Toro would have to be managed like a population in risk and as an independent stock for conservation. However, to be conclusive we need to get a higher number of biopsies from dolphins of Bocas del Toro population. We are currently undertaking analyses of nuclear DNA markers (11 microsatellite loci) in order to further understand and corroborate these initial findings.

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