Effective population size of an offshore population of bottlenose dolphins, *Tursiops truncatus*, from the São Pedro and São Paulo Archipelago, Brazil

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Abstract. The São Pedro and São Paulo Archipelago (SPSPA) (00°56′N, 29°22′W) lies approximately 1010km northeast off the coast of Rio Grande do Norte State in Brazilian waters. Recently, through photo-identification and group size analysis, around 20-30 individual bottlenose dolphins, *Tursiops truncatus*, from SPSPA were recognized as a resident, and potentially genetically isolated, population. The effective population size (\(N_e\)), not the census number (\(N\)), as well the sex ratio, are of primary concern from an evolutionary and conservation management perspectives. The estimate of \(N_e\) reflects the number of individuals responsible for the maintenance of genetic diversity of a species or population as well its evolutionary potential. For this reason, we present here the first \(N_e\) and sex ratio estimates for the bottlenose dolphin population from SPSPA. Sex was molecularly determined for 19 biopsy samples collected from bottlenose dolphins from SPSPA between January and February 2005. The \(N_e\) was estimated by direct counting of reproductive adults sexed by DNA analysis. The resulting \(N_e\) was 12 individuals and the sex ratio was 1.11 male to 1 female, however, it was not significantly different from the expected 1:1 ratio (\(\chi^2\) test, \(\alpha= 0.05; df = 1\)). The effective population size based on the genetic diversity of 19 sequences of the mtDNA control region resulted in a female effective population size of 223 individuals, and the total long-term effective size of ~470 individuals. We believe that the estimated \(N_e\) for the SPSPA population is a critical value, because it is significantly lower than the mean minimum viable population (MVP) suggested for vertebrates (around 5000 breeding age adults). This small \(N_e\) is of great concern and should be taken into account in future management plans to ensure the conservation and protection of this small population at SPSPA.

Resumo. O Arquipélago de São Pedro e São Paulo (SPSPA) (00°56′N, 29°22′W) encontra-se distante cerca de 1010km da costa norte brasileira. Recentemente, a partir das análises de foto-identificação e dos tamanhos de grupo avistados, verificou-se a existência na região de uma população residente de cerca de 20 a 30 espécimes do golfinho-nariz-de-garrafa (*Tursiops truncatus*), a qual possivelmente está isolada geneticamente. Para o cenário evolutivo, o tamanho efetivo (\(N_e\)) de uma população, e não o número total de indivíduos (\(N\)), é o fator fundamental. A estimativa do número de indivíduos responsáveis pela produção da próxima geração é determinante para a manutenção da variabilidade genética. Neste sentido, apresentamos a primeira estimativa de \(N_e\) e da proporção sexual da população do golfinho-nariz-de-garrafa do SPSPA, através da determinação genética.
do sexo de seus espécimes. Biópsias de 19 espécimes do golfinho-nariz-de-garrafa foram coletadas entre janeiro e fevereiro de 2005 no SPSPA. A proporção sexual observada foi de 1,11 macho para 1 fêmea, porém como não foi estatisticamente diferente da proporção esperada de 1:1 (teste $\chi^2$, $\alpha=0.05$, gl = 1), não se pode refutar a hipótese de que esta proporção deva-se ao acaso. O tamanho efetivo estimado pela contagem direta foi de 12 espécimes reprodutivamente adultos. Contudo, esta mesma estimativa feita através da análise da diversidade genética de 19 sequências da região controladora do mtDNA de espécimes coletados em SPSPA resultou num tamanho efetivo de fêmeas de 223 indivíduos, o que totalizou ~470 golfinhos (considerando machos e fêmeas). Nós acreditamos que os valores estimados de tamanho efetivo para os golfinhos de SPSPA são críticos para conservação desta população, porque são significativamente menores que o mínimo populacional viável (MPV) estimado para vertebrados (cerca de 5000 adultos em idade reprodutiva). Neste sentido, este resultado pode ser considerado preocupante e deve ser levado em consideração em qualquer futuro plano de manejo da região para que sejam asseguradas a conservação e viabilidade da pequena população do golfinho-nariz-de-garrafa do SPSPA.

Introduction

The São Pedro and São Paulo Archipelago - SPSPA (also known as St. Paul’s Rocks) is a small and isolated group of rocky islets just north of the Equator (00°56’N, 29°22’W) that has as total land area, and shallow waters surrounding them, less than 0.5km$^2$ (Edwards and Lubbock, 1983). The archipelago lays approximately 1010km northeast of the coast of Rio Grande do Norte State, Brazil, and 1824km southwest of Guinea-Bissau, Africa, in the equatorial Atlantic Ocean. The nearest landmass is the Fernando de Noronha Archipelago, 630km to the southwest, while Cape Verde Islands, off northwestern Africa, are about 1850km to the north-northeast (Campos et al., 2009). In order to provide a comprehensive review about the cetacean species around the SPSPA, dedicated surveys were undertaken from 1999 to 2005$^1$, with special attention to the bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) (Moreno et al., 2009; Ott et al., 2009). This offshore bottlenose dolphin population was studied mainly by photo-identification and genetic analysis$^2, 3$ (Moreno et al., 2009; Ott et al., 2009).

The bottlenose dolphin is a highly social species with a worldwide distribution in cold temperate to tropical waters, as well as inshore and offshore areas (Wells and Scott, 2009). Differences between nearshore and offshore populations have been found for this species in many geographic locations$^4$ (Ross, 1977; 1984; Duffield et al., 1983; Ross and Cockroft, 1990; Van Waerebeek et al., 1990; Mead and Porter, 1995; Hoelzel et al., 1998). This great geographical variation has led some authors in the past to divide the genus *Tursiops* into as many as 20 different species (Hershkovitz, 1966; Rice, 1998). Nevertheless, only two species are currently recognized, *T. truncatus*, the ‘common bottlenose dolphin’, and *T. aduncus* (Ehrenberg, 1882), the ‘Indian Ocean bottlenose dolphin’ (Wang et al., 1999; 2000a, b; Natoli et al., 2004; Wells and Scott, 2009). Recently a third potential species of *Tursiops* was formally described for southern Australian coastal waters, *T. australis*, the ‘Burrunan dolphin’ (Charlton-Robb et al., 2011). However, it was not considered as a valid species of *Tursiops* by the Committee on Taxonomy of The Society for Marine Mammalogy (2016). Despite the broad geographic coverage of all these studies, bottlenose dolphins have mostly been studied in peri-continental and shallow waters, and very little is known about offshore populations (Klatsky et al., 2007; Quérouil et al., 2007; Baird et al., 2009).

The bottlenose dolphins from the SPSPA have been studied since 1999 and the first genetic data from mitochondrial DNA (mtDNA) analysis of this population indicated an extremely low gene diversity in the control region of the mtDNA ($h = 0.1053$ e $\pi = 0.0007$) (Oliveira et al., 2008; Moreno et al., 2009; Ott et al., 2009). The 19 sampled animals from the SPSPA presented only two haplotypes, which were not shared with other populations along the Brazilian coast (Oliveira et al., 2008; Moreno et al., 2009; Ott et al., 2009). These results suggest that the bottlenose dolphins from SPSPA are likely to be genetically isolated from Brazilian populations. In addition, the sighting

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$^1$Project Occurrence and saisonality of cetaceans in the vicinity of St Paul’s Rocks conducted by GEMARS with support of SECIRM and CNPq (Proc. # 680037/2004-3).

$^2$Oliveira, L.R., Ott, P.H., Moreno, I.B., Tavares, M., Danilewicz, D., Almeida, R., Siciliano, S. and Bonatto, S. (2008) Variabilidade genética e estrutura populacional do golfinho-nariz-de-garrafa, *Tursiops truncatus*, em águas brasileiras. Page 80 in Resumos, XII Reunião de Trabajo de Especia- listas en Mamíferos Acuáticos de América del Sur 13-17 October 2008, Montevideo, Uruguay.

$^3$Ott, P.H., Oliveira, L.R., Almeida, R.S., Tavares, M., Moreno, I.B., Danilewicz, D., Siciliano, S., Apolinário, M. and Bonatto, S.L. (2006) Os golfinhos-nariz-de-garrafa do Arquipélago de São Pedro e São Paulo são uma população isolada? Page 196 in Resumos, 5º Congresso Brasileiro de Genética, 3-6 September 2006, Foz do Iguacu, PR, Brazil.

$^4$Walker, W.A. (1981) Geographic variation in morphology and biology of bottlenose dolphins (*Tursiops*) in the eastern North Pacific. NOAA/NMFS Southwest Fisheries Science Center Administrative Report no. LJ 81-5c. 21 pp.
Sample collection and DNA extraction

Nineteen biopsy samples were collected from live bottlenose dolphins, between January and February 2005 in offshore waters of the SPSPA. Skin biopsies were collected using a crossbow and lightweight darts (CETA-DART®) (Brown et al., 1991b; Ott et al., 2009). Samples were stored in NaCl-saturated solution of 20% dimethyl sulphoxide (Amos and Hoelzel, 1991) and frozen at -20°C until DNA was extracted. Biopsies were taken from a research vessel, and most biopsied dolphins were photographically identified at the same time. All 19 samples used in these analyses represent 19 different individuals, according to the photo-identification data as well by the screening of eight microsatellite loci (unpublished results).

DNA extraction followed the phenol/chloroform method described by Sambrook et al. (1989) and modified by Shaw et al. (2003). The sex of each individual was identified by the amplification of introns from ZFX and ZFY genes, which are located on the X and Y mammalian chromosomes, respectively (Page et al., 1987), using primers ZFY1204 and ZFY0097 under PCR conditions described in Pasboll et al. (1992). Amplification products were digested by Taq I restriction endonuclease and visualized in 2% agarose gel with 100 base-pair ladder (GE Healthcare) for fragment size estimation.

Gender determination

Gender identification was based on the number of bands for a given sample. Females have a single band that corresponds to the ZFX intron on the X chromosome, whereas males have two bands, one corresponding to the X intron and the other to the ZFY intron on the Y chromosome (Page et al., 1987). DNA from bottlenose dolphins of known gender (e.g. stranded animals in southern Brazil) were amplified, digested and visualized in gel as positive controls to validate the technique.

Sex ratio was calculated by the division between the total number of males and females identified. The Chi-square test was used to verify if the resulting sex ratio deviates significantly from an expected 1:1 ratio.

\[ N_r \]

Calculations

We calculated the contemporary \( N_r \) after the estimate of sex ratio, because in organisms with separate sexes one sex may be more common than other and need an equation that accounts for the effects of an unequal sex ratio (Wright, 1931).
However, there was no statistically significant deviation from 1:1 (see Results), and therefore the estimate of \( N_e \) was calculated by counting all the adult males and females genetically sexed (Wright, 1931). The age classes of the dolphins were visually estimated based on body size (Siciliano et al., 2007) relative to the boat and the amount of tooth rake scarring (e.g. Scott et al., 2005). Individuals estimated to be longer than 2.5m and ‘heavily’ scarred were classified as adults, as well as animals accompanied by a calf.

We also estimated the long-term effective population size based on genetic diversity of the 19 sequences from the mtDNA control region (316bp) (Oliveira et al., 2008; Moreno et al., 2009), collected from specimens of SPSPA. Haplotype \((h)\) and nucleotide \((\pi)\) diversities (Nei, 1987) were estimated using Arlequin 3.5 (Excoffier and Lischer, 2010).

Based on mtDNA diversities we estimated the female effective population size \((N_e)\) for this population using the formula: \(N_e = \theta/2\mu g\), where \(\mu\) = mutational substitution rate per generation and \(\theta = \text{genetic diversity} \) (estimated here by \(\pi\)) (Avise et al., 1988). Generation time \((g)\) estimated for bottlenose dolphin and used for calculation was 10 (Cassens et al., 2005) with a mutation rate of 1.5E-7 (Hoelzel et al., 1991).

**Results**

From the 19 bottlenose dolphins biopsied, nine were genetically sexed as females and ten as males. The observed sex ratio was 1.11 male to one female, which was not significantly different from the expected ratio of 1:1 \((\chi^2\text{ test, } \alpha=0.05; \text{ df}=1)\).

The contemporary \(N_e\) from bottlenose dolphins of the SPSPA was 12 individuals, accounting for the effects of an equal sex ratio since 12 from the 19 individuals were potential breeding adults from both sexes (seven males and five females). Sequence analysis of the mitochondrial DNA control region (316 base pairs) of the 19 individuals revealed a total of two polymorphic sites defining two different haplotypes, leading to extremely low genetic diversities \((h = 0.1053 \text{ and } \pi = 0.00067)\). This resulted in a female effective population size of 223 individuals, and considering the 1.11:1 sex ratio estimated above, to a total long-term effective size of ~470 individuals. None of the haplotypes were shared between SPSPA and the other known Brazilian populations (Oliveira et al., 2008; Ott et al., 2009).

**Discussion**

We present the first estimates of sex ratio and effective population size for the SPSPA population of bottlenose dolphins and suggest that the slight predominance of male individuals (1.11:1) is likely random.

Despite the significant amount of studies on bottlenose dolphins, most of them are on coastal populations, and very little is known about sex ratio of bottlenose dolphin populations in distant offshore regions. The only similar study conducted at an offshore area was made by Quérouil et al. (2007), who analyzed the population structure of bottlenose dolphins in two of the most isolated archipelagos of the North Atlantic: the Azores and Madeira. Sex ratio estimated by molecular sexing for bottlenose dolphins in the Azores clearly indicated a sampling bias in favor of males. Excluding the samples from stranded animals (two males from Madeira), there were 61 males and 22 females in the Azores (sex ratio = 2.77:1) and 13 males and 12 females in Madeira (sex ratio = 1.08:1). The authors attributed this result probably to a sampling artifact, as it seemed that adult females tended to avoid the boat, especially when accompanied by young calves. However, in the case of the dolphins from SPSPA, the presence of females and calves was relatively common in the groups sighted around the archipelago, including during sampling.

Our estimate of a contemporary \(N_e\) of 12 dolphins for the SPSPA population does not account for two other possible factors: i) the variation in reproductive success among individuals, and ii) gene flow, i.e. the possibility of peripheral males or females (from other geographically close populations) being reproductively active. In both cases, it is very difficult to obtain empirical information. If individual variance in reproductive success exists, its effect will reduce the estimate of \(N_e\) to a more critical value. Regarding gene flow, the relationship between the dolphins from SPSPA and other offshore populations is still unclear. We did not find sharing of mtDNA haplotypes between SPSPA and some Brazilian inshore populations from southern region (Oliveira et al., 2008; Moreno et al., 2009; Ott et al., 2009). However, bottlenose dolphins have been occasionally sighted in other oceanic regions, such as the biological reserve of Rocos Atoll (03°50’S; 33°49’W) (Baracho et al., 2008), located about 720km from the SPSPA. Furthermore, our long-term \(N_e\) result based on genetic diversity from specimens of SPSPA was much larger (~470 individuals), reinforcing the possibility of gene flow, at least in the past. Therefore, we can not presently reject the hypothesis that some gene flow exists or existed between SPSPA and other populations.

The data from group size and photo-identification indicated that the bottlenose dolphin population in the SPSPA contains about 20-30 that are potentially residents to the area (Caon et al., 2009; Moreno et al., 2009; Ott et al., 2009). Therefore, it is unlikely that the current \(N_e\) for this population would be much higher than estimated here. Nevertheless, fewer than ten dolphins were resighted between 1999 and 2005 in the area (Caon et al., 2009; Moreno et al., 2009; Ott et al., 2009).

Interestingly, contrary to the usual situation (Hare et al., 2011), the SPSPA contemporary \(N_e\) estimated by direct count is much lower than the long-term \(N_e\) estimated by mtDNA nucleotide diversity. However, long-term \(N_e\) is usually not a reliable indicator of short-term or contemporary \(N_e\) for a population (Hare et al., 2011), since the latter is more similar to a \(N_e\) averaged over tens to hundreds of generations.
Unfortunately, there is very few information on \( N \) for vertebrates in general and even less so for delphinids, with available information generally focused on the diversity of genetic markers as an alternative method to the demographic estimation by counting (e.g. Galvão et al., 2011; Caballero et al., 2012; Martien et al., 2012). These previous studies concentrated on species or populations of the continental shelf and found a large range of values for \( N \). For example, for north Pacific killer whales (Orcinus Orca) \( N \) could vary from high (>1000) to very low (<50) (Hoelzel et al., 2007). For bottlenose dolphins, there are very few studies that present molecular estimates of \( N \), and one presents this estimation for the Black Sea population, with the value oscillating between 162 and 2273, according to the mutation rate used (Viaud-Martinez et al., 2008).

Considering data from other studies, as well as the small size and geographic isolation of the SPSPA, the very small \( N \) estimated for bottlenose dolphins in this area is not unexpected and does not seem to result from anthropogenic factors. Nevertheless, the extremely low \( N \) for the SPSPA population represents a critical value, since it is significantly lower than the MVP estimated for vertebrates (e.g. Reed et al., 2003).

Originally, Franklin (1980) proposed the so-called ‘50/50/50’ rule, whereby an \( N \) of 50 adult individuals is required to prevent damaging effects of inbreeding, while a long-term \( N \) of 500 individuals is required to ensure overall genetic variability. However, more recent assessments suggest that this number should be higher, usually approaching 5000 adult individuals (Reed et al., 2003; Traill et al., 2007; 2010).

The small \( N \) for the bottlenose dolphin population from SPSPA, as well as its very low genetic diversity, are reasons of great concern and should be taken into account in future management plans to ensure the conservation and protection of this population at the SPSPA. In addition, the available genetic information (Oliveira et al., 2008; Moreno et al., 2009; Ott et al., 2009) strongly indicates that this population is an ‘evolutionary significant unit’ (ESU, sensu Moritz, 1994) and as such should be considered ‘distinct’ for conservation management purposes.

Acknowledgments

We greatly acknowledge the logistic and financial support from the Secretaria da Comissão Intermisterial para os Recursos do Mar – SECIRM, the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (National Council for Scientific and Technological Development) (CNPq Proc. 480037/2004-3 and CNPq Proc. 557176-2009-3) and ‘Projeto Mamíferos e Quêniais Marinhos’, coordinated and supported by ‘Gerência de Avaliação e Monitoramento Ambiental do CENPES/PETROBRAS’. We are also grateful to the crew of the fishing vessels Transmar I, II and III for the logistic support during the sampling expeditions. We also thank Matheus Williams de Oliveira, Marcus Rodrigo Guidoti and Cladinara Sarturi Roberts for their laboratory help. L.R. Oliveira was supported by CNPq (Proc. 151307/2005-9). This paper is dedicated to the memory of Raquel Santos de Almeida, who was involved in the laboratory work of the present study. With Raquel’s passing, we have lost an excellent student and very good friend.

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