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First molecular estimate of sex-ratio of southern right whale calves, *Eubalaena australis*, for Brazilian waters

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*The southern right whale (*Eubalaena australis*) was one of the most intensively hunted whales between the 17th and 20th centuries in the southern hemisphere. Recent estimates indicate that today there are around 7000 whales, representing 5 to 10% of its original population. On the other hand, recent studies estimated that the population that migrates to the Brazilian coast grew by 14% from 1987 to 2003. However, there is no information about sex-ratio for adults or for calves in this region, which is an important parameter for understanding the biology of the species. We present here the first estimate of calves' sex-ratio of southern right whales found along the southern Brazilian coast, one of the most important wintering grounds for the species. Sex was molecularly indentified for 21 biopsies collected from calves between 1998 and 2002, along the coast of Rio Grande do Sul and Santa Catarina States, in southern Brazil. The sex-ratio was two females for one male, however, it was not statistically different (χ^2 test, $\alpha = 0.05$; $df = 1$) from the expected ratio of 1:1. This result is in accordance with the sex-ratio estimated for the species of all ages using external morphology (and behaviour information), as well as for most species of baleen whales.*

Keywords: cetaceans, calf, *Eubalaena australis*, genetic sex identification, sex-ratio, southern right whale

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INTRODUCTION

Estimation of the sex-ratio in natural populations of mammals is essential for understanding population dynamics, behaviour, breeding systems and demography, therefore, it is a population parameter of great importance to management and conservation (Brown *et al.*, 1991a; Gompper *et al.*, 1998; Hughes, 1998). However, identification of the sex of each individual in a population is difficult in some species, such as some free-ranging cetaceans, since usually there is no remarkable external sexual dimorphism, and if there is, like in beaked whales (family Ziphiidae), it remains typically invisible to the observer above-water. Although females attain lengths generally 5% larger than males in baleen whales (Chivers, 2002) and may be recognized when they are accompanied by calves, size difference usefulness is often limited by age related variation in size, and the use of associations between adults and calves is only valid to reproductively active females (Knowlton *et al.*, 1994). In addition, the direct observation of the genital region is extremely opportunistic. For this reason gender identification through the analyses of molecular markers is becoming a powerful tool in the study of wild populations of cetaceans (Shaw *et al.*, 2003).

In many wild populations the sex-ratio deviates from 1:1 (i.e. the numbers of females and males are not equal). Deviations in the sex-ratio accelerate the effects of inbreeding on extinction rate because of the effect of sex-ratio on effective population size (Mills & Smouse, 1994). For example, many mammals have harems where one male mates with many females (polygyny), while many other males make no genetic contribution to the next generation (Frankham *et al.*, 2002). In this sense, sex-ratio is a very important parameter, mainly to the species which had drastic population declines and nowadays are in expansion, like several whales such as the southern right whale, *Eubalaena australis* (Desmoulins, 1822). However, gender identification and sex-ratio in baleen whales were studied for just a few species,

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the most studied one being the humpback whale (*Megaptera novaeangliae*) (e.g. Clapham *et al.*, 1995; Valsecchi *et al.*, 2002; Roman & Palumbi, 2003; Pomilla & Rosenbaum, 2006). Molecular sex-ratio estimates were also obtained from grey whale (*Eschrichtius robustus*) (Steeves *et al.*, 2001; Weller *et al.*, 2008), fin whale (*Balaenoptera physalus*) (Bérubé *et al.*, 1998) and from North Atlantic right whale populations (*Eubalaena glacialis*) (Brown *et al.*, 1994). For the southern right whale, the first estimate of sex-ratio was obtained by morphology of foetuses and non-foetuses sampled during whaling activities (Tormosov *et al.*, 1998) and the genetic sex-ratio was estimated exclusively for Australia and New Zealand wintering grounds (Baker *et al.*, 1999). Differences in the observed sex-ratio for the same species in different grounds were reported for fin whales hunted in the Gulf of St Lawrence and off Iceland, with the overrepresentation of females in the first area (Bérubé *et al.*, 1998). The authors explain this result as a possible whalers' preference of hunting females, because they are larger than males, and not by a real biological trend. In this sense, the comparison of sex-ratio estimates and other population parameters among different grounds could confirm (or not) demographic patterns and trends, which are essential information for management and conservation actions for currently or historically hunted species.

The southern right whale was one of the most intensively hunted whales between the 17th and 20th centuries in the southern hemisphere. Recent estimates indicate that there are now about 7000 specimens, which may represent 5 to 10% of its original population size (IWC, 2001). Although some populations have shown strong signs of recent recovery (e.g. IWC, 2001), the species is listed as 'Lower Risk/Conservation Dependent' (LR/cd) in the *IUCN Red List of Threatened Species* (IUCN, 2007), and it is included in the regional *Brazilian Red List of Threatened Species* (Danilewicz & Oliveira, 2003; IBAMA, 2003).

Southern right whales were once widely distributed across the three ocean basins in the southern hemisphere: the South Atlantic, the Indian Ocean, and the South Pacific (Townsend, 1935). The species dwells in coastal waters for calving and breeding at medium and low latitudes in austral winter/spring months (from May to December) and tends to migrate offshore to feeding grounds in the Subtropical and Antarctic Convergence zones during austral summer months (from January to April) (Tormosov *et al.*, 1998; IWC, 2001). Migration routes between winter calving and summer feeding grounds may include north–south (e.g. Australian calving ground to Southern Ocean feeding grounds; Bannister *et al.*, 1997, 1999) as well as east–west movements (e.g. South Africa calving ground to Gough Island feeding ground; Best *et al.*, 1993).

The southern Brazilian coast is one of the most important wintering grounds of the species in the western Atlantic due to its shallow and protected waters (Greig *et al.*, 2001). For this reason, it was subject to intensive commercial whaling in this region until 1973, when the whale population appeared to be extirpated (Palazzo & Carter, 1983). In the early 1980s, whales were 'rediscovered' in this region and have been studied since 1982 (Castello & Pinedo, 1979; Câmara & Palazzo, 1986). Recent evidence suggests a growth of 14% from 1987 to 2003 (Groch *et al.*, 2005) of the population that migrates to the southern Brazilian coast. However, there is no information about sex-ratio for adults or calves in this region. In this study we present the first estimate of calf sex-ratio of southern right whales for the Brazilian coast using molecular methods.

MATERIALS AND METHODS

Twenty-one biopsies were collected from live calves of southern right whales, between 1998 and 2002, along the coast of Santa Catarina and Rio Grande do Sul States (from 27°55′S to 29°22′S), the most important wintering ground for the species in Brazilian waters (Groch *et al.*, 2005). Skin biopsies were collected using a crossbow and lightweight pneumatic darts (Brown *et al.*, 1991b). Individuals were considered calves if they were between 1/3 to just over 1/2 of their (presumed) mother's length. Samples were stored in NaCl-saturated solution of 20% dimethyl sulphoxide (Amos & Hoelzel, 1991) and frozen at −20°C until DNA was extracted. Biopsies were taken from the research vessel only, and all biopsied whales were photographically identified.

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 by Pasbøll *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) to fragment size estimation. 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 X chromosomes, whereas males have two bands, one corresponding to the X intron and the other to the ZFY intron on the Y chromosome (Figure 1). DNA from individuals of known gender (e.g. stranded animals) 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 expected ratio of 1:1.

RESULTS

The results of the restriction-enzyme analysis of the ZFX/ZFY amplification product from calves of southern right whale are illustrated in Figure 1. The ZFX intron has approximately 490 pair bases (pb), whereas the ZFY has roughly 640 pb. From the 21 calves of southern right whales biopsied, 14 were

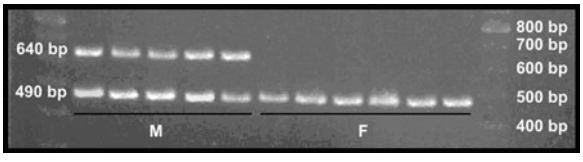

Fig. 1. Molecular gender identification of 11 southern right whale calves, from biopsies collected along the Brazilian coast between 1998 and 2002: females (F) have one band whereas males (M) have two bands (see text).

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genetically identified as females and seven as males. The observed sex-ratio was two females to one male, which was not statistically different from the expected ratio of 1:1 (χ^2 test, $\alpha = 0.05$; df = 1).

DISCUSSION

According to Groch *et al.* (2005), the southern right whales in the Brazilian wintering ground are not just transients, since 10% of the individuals have been re-sighted. Even those females with two, four and five years' calving interval (Knowlton *et al.*, 1994) returned to Brazil for their subsequent calving. Most of the re-sighted females with calves had three-years' calving intervals, which is consistent with successful reproduction, suggesting that the Brazilian coast represents an important breeding ground for the species in the southern hemisphere (Groch *et al.*, 2005).

We found twice as many females than males among newborn calves in Brazilian waters. Nevertheless, this sex-ratio is not statistically different from the expected ratio of 1:1. Our results are not likely influenced by any hidden bias due to the molecular technique used, since all five positive controls were correctly identified and this sexing method was successfully applied in several similar studies in cetaceans (e.g. Pasbøll *et al.*, 1992; Cunha & Solé-Cava, 2007; Patenaude *et al.*, 2007). Similarly, we could not identify any possible gender bias in the sampling strategy, since as many mother–calf pairs as possible were sampled.

The sex-ratio for calves found in this study cannot be extrapolated to adult whales, since the differential mortality during the growth of immature whales could change the sex-ratio for the adults (Ralls *et al.*, 1980). The earliest information about sex-ratio for *E. australis* was provided by direct analyses of whales captured by Soviet whaling activity in the southern hemisphere (Tormosov *et al.*, 1998). The authors found sex-ratios of 108 males to 105 females (1.03:1) in foetuses and 585 males to 403 females (1.45:1) in non-foetuses. In contrast with our results, there was a predominance of males, with statistically significant difference for non-foetuses. However, the sex proportion between foetuses found by Tormosov *et al.* (1998) and calves (from our results) was not statistically different (χ^2 test, $\alpha = 0.05$; df = 1). Another sex-ratio estimate for *E. australis* was conducted by Baker *et al.* (1999) for two wintering grounds of Australia, as well as around the Auckland Islands, New Zealand. Based on DNA markers, these authors found a sex-ratio of 21 males to 20 females (1:1.05), which was not statistically significant.

Similarly, Patenaude *et al.* (2007) studying population structure and gene diversity of *E. australis* from individuals of four winter calving grounds (Argentina, South Africa, Western Australia and the New Zealand sub-Antarctic) and two summer feeding grounds (South Georgia and south of Western Australia) genetically identified the sex of the sampled individuals. Their data indicate that the sex-ratio was 1:1 (56 males to 56 females), but differently from our sample, they pooled individuals from all ages (excluding foetuses). For the North Atlantic right whale, *E. glacialis*, Brown *et al.* (1994) identified by the observation of the genital morphology and DNA techniques 36 male and 34 female calves, a ratio which does not differ significantly from 1:1.

Besides the right whales, gender identification and sex-ratio have been studied in other cetaceans. Weller *et al.* (2008) found among calves (N = 59) of western grey whales in Russian waters that 66% of the individuals were males and 34% females, a statistically significant result. For adults (N = 142), the sex-ratio was also biased towards males (58%), but not significantly so. The sex-ratios found for adult individuals of other species, such as humpback (Clapham *et al.*, 1995; Valsecchi *et al.*, 2002; Roman & Palumbi, 2003), grey (Steeves *et al.*, 2001; Weller *et al.*, 2008) and fin whales (Bérubé *et al.*, 1998), at feeding and breeding grounds, as well as during migration, are similar to those found for southern right whale calves, with results not statistically different from the expected 1:1 ratio. Nevertheless, for the common minke whales (*Balaenoptera acutorostrasta*) in West Greenland, although the foetal sex-ratio is not significantly different from even, the proportion of females in the catch has varied around 3/4 since the beginning of the hunt in 1948, and is likely to be a result of a geographical sub-structuring of the two sexes during the summer (Witting, 2006).

The sex-ratio found in the Brazilian population could likely be extended to other western South Atlantic breeding sites because, according to Ott (2002), the Argentinean and Brazilian individuals are part of a single genetic population due to the high gene flow among these breeding sites.

Finally, it is important to mention that the sex-ratio biased towards females in the calves of Brazilian right whales could be real and the lack of statistical significance may be explained by the relatively small size of our sample. Therefore the continuity of this study, in terms of analysing more calves and also the adult sex-ratio for this population, is essential to better understand the spatial distribution of males and females in the region, as well as to establish biological parameters such as effective population size (the number of breeding males and females of a population). This information, in conjunction with accurate abundance estimates, will be essential to any future plan for the conservation and management of southern right whales in Brazilian waters.

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