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CARACTERIZAÇÃO GENÉTICA DA POPULAÇÃO DE BALEIAS JUBARTE (*MEGAPTERA NOVAEANGLIAE*) DA ÁREA DE REPRODUÇÃO DO OCEANO ATLÂNTICO SUL OCIDENTAL BASEADO EM MICROSSATÉLITES NUCLEARES

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DISSERTAÇÃO DE MESTRADO

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SUMÁRIO

In memorian ao meu irmão, Leonardo, que me incentivou a fazer biologia e sempre acreditou no meu potencial.

AGRADECIMENTOS

RESUMO

A principal área de reprodução das baleias jubarte no Oceano Atlântico Sul Ocidental está no Banco dos Abrolhos, na costa brasileira. A diversidade genética e a história demográfica da população de jubartes brasileiras de duas localidades geográficas (Banco dos Abrolhos, $n = 235$; Praia do forte, $n = 39$) foi investigada usando dez locos de microssatélites. Microssatélites foram também usados para avaliar a existência de diferenciação genética entre essa população e duas baleias da ilha Geórgia do Sul. A população brasileira apresentou um alto nível de diversidade alélica ($A = 12.1$) e uma elevada heterozigosidade média observada ($H_O = 0.735$), de acordo com outras áreas de reprodução estudadas no Hemisfério Sul. Apesar da população brasileira de baleias jubarte ter sido reduzida durante a caça comercial *bottleneck* genético não foi detectado com os diferentes procedimentos usados. Nossos dados não evidenciaram uma diferenciação temporal ao longo dos anos e diferenciação genética entre as baleias das duas localidades da área de reprodução brasileira e entre essas jubartes e aquelas duas da ilha Geórgia do Sul não foi encontrada. Em adição, uma fêmea amostrada nas proximidades da ilha Geórgia do Sul apresentou uma possível relação mãe-filha com uma fêmea do banco dos Abrolhos. Os dados obtidos através desse estudo não forneceram evidência para associação baseada em parentesco dentro dos grupos sociais. Nossos resultados suportam a homogeneidade genética da população brasileira de baleias jubarte e corrobora os dados de DNA mitocondrial, que sugerem a região das ilhas Geórgia do Sul/Sanduíche do Sul como principal área de alimentação para essa população.

ABSTRACT

Genetic characterization of the humpback whale (*Megaptera novaeangliae*) population of the Southwestern Atlantic Ocean breeding area based on nuclear microsatellites

The main breeding ground of the humpback whales in the Southwestern Atlantic Ocean is the Abrolhos Bank, off the Brazilian coast. The genetic diversity and demographic history of the Brazilian humpback whale population, from two geographic locations (Abrolhos Bank, $n = 235$; Praia do forte, $n = 39$) was assayed with ten microsatellite loci. Microsatellites were also used to evaluate the existence of genetic differentiation between this population and two whales off South Georgia Islands. This population showed a high level of allelic diversity ($A = 12.1$) and a high mean observed heterozygosity ($H_O = 0.735$), in agreement with other breeding areas studied in the Southern Hemisphere. Although the Brazilian population of humpback whales was reduced during the commercial whaling no genetic bottleneck was detected with the different procedures that we used. Our findings showed no evidence to the temporal differentiation along the years and no genetic differentiation was found between whales from the two geographic locations of the Brazilian breeding ground and between these humpback whales and those two from South Georgia Islands. In addition, a female sampled off South Georgia Islands showed a putative parent-offspring relationship with a female off Abrolhos Bank. These results support the hypothesis that South Georgia/ South Sandwich Islands area as the main feeding area for the Brazilian breeding stock and that it constitute a single population. The data obtained through this study provided no evidence of association within social groups based in kinship.

APRESENTAÇÃO

O propósito do presente estudo foi investigar a diversidade genética, a história demográfica, a existência de estruturação temporal, e a relação de parentesco entre os indivíduos dentro dos grupos sociais da população de baleias jubarte, *Megaptera novaeangliae*, da área de reprodução do Oceano Atlântico Sul Ocidental usando microssatélites nucleares. Adicionalmente, os microssatélites foram também usados para avaliar a existência de diferenciação genética entre essa população e duas baleias jubarte amostras nas proximidades da ilha Geórgia do Sul, provável área de alimentação das jubartes brasileiras. Outra proposta do nosso trabalho foi determinar o sexo de cada indivíduo e a proporção sexual dessa população.

As baleias jubarte são encontradas em todos os oceanos do mundo, sendo distribuídas dentro de três populações oceânicas principais: a do Pacífico Norte, a do Atlântico Norte e a do Hemisfério Sul, as quais estão separadas por barreiras físicas ou temporais (Baker *et al.*, 1994; Baker *et al.*, 1998). Elas realizam migrações entre as altas latitudes, onde se alimentam durante o verão, e as baixas latitudes, onde acasalam e têm seus filhotes durante os meses de inverno (Dawbin, 1966). O intercâmbio efetivo entre as populações do Hemisfério Norte e do Sul é limitado devido à oposição sazonal desse ciclo migratório (Baker *et al.*, 1994), pois, quando as jubartes do norte estão em águas tropicais, as suas coespecíficas do sul estão em águas polares, e seis meses mais tarde a situação se inverte.

No Hemisfério Sul as jubartes formam oito concentrações reprodutivas ou subpopulações conforme a sua distribuição em baixas latitudes durante o inverno, que foram nomeadas pela Comissão Internacional Baleeira (CIB) de áreas de reprodução de A a G no Hemisfério Sul e X no Mar da Arábia (IWC, 2005). A CIB também dividiu as águas Antárticas em seis extensas zonas de I à VI, que funcionavam como unidades controle na divisão das cotas de caça comercial baleeira na Antártida (Tonnessen & Johnsen, 1982). A principal área de reprodução das jubartes no Oceano Atlântico Sul Ocidental, considerada como o estoque reprodutivo A, está localizada no Banco dos Abrolhos (16°40' – 19°30'S e 37°25' - 39°45'W), no sul da Bahia e norte do Espírito Santo, Brasil (Martins *et al.*, 2001; Freitas *et al.,* 2004; Andriolo *et al.*, 2006). Dados históricos sugerem que a área de

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alimentação correspondente para essa população está próxima da Península Antártica (área I e II; 65°S, 60°W) e/ou da ilha Geórgia do Sul (área II; 54°20'S, 36°40'W) (Slijper, 1962; Mackintosh, 1965). Entretanto, estudos genéticos realizados através de DNA mitocondrial das jubartes brasileiras e das que freqüentam os lados oeste e leste da Península Antártica (respectivamente área I e II) demonstraram que essa região não constitui a área de alimentação das jubartes brasileiras. Além disso, duas jubartes que foram amostradas próximas à ilha Geórgia do Sul apresentaram haplótipos da população brasileira (Engel *et al.*, *in press*). Portanto a área de alimentação do estoque reprodutivo A deve ser localizada provavelmente nas águas adjacentes às ilhas Geórgia do Sul e Sanduíche do Sul, como indicado por recentes estudos de foto-identificação (Stevick *et al.*, 2006) e de rádio telemetria (Zerbini *et al.*, 2006).

O entendimento da origem e manutenção da diversidade genética original é o principal foco da genética da conservação, sendo esta diversidade requerida para a população se adaptar às mudanças ambientais, isto é, representa seu potencial evolutivo. (Frankham *et al*, 2002; Oliveira, *et al.* 2006). A aplicação de técnicas moleculares para investigar a diversidade genética de espécies em perigo de extinção tem sido largamente usada em pesquisas de conservação. Através de marcadores moleculares é possível analisar a estrutura e a dinâmica populacional, resolver incertezas taxonômicas, detectar hibridação entre espécies, definir unidades de manejo dentro das espécies, detectar caça ilegal (*forense*) e entender importantes aspectos da biologia da espécie (Frankham *et al*, 2002).

Amplamente usados como marcadores genéticos, os microssatélites apresentam uma taxa de mutação mais alta do que o resto do genoma. Estes marcadores são também conhecidos como *short tandem repeats* (STR) e são seqüências compostas de um a seis nucleotídeos do DNA nuclear repetidas em série (*in tandem*). Eles representam locos biparentais não ligados, codominantes e em geral seletivamente neutros. Logo se destacam nos estudos genéticos de populações, fornecendo informações relevantes para identificar unidades de conservação, para investigar processos genéticos como o fluxo gênico e a incidência da deriva genética, além de serem usados para as análises de parentesco (Schlötterer, 2004; Oliveira, *et al.* 2006).

Para definir estratégias adequadas de proteção e manejo das populações de cetáceos é muito importante o conhecimento da diversidade genética populacional e esta é uma das

ações prioritárias recomendadas pelo Plano de Ação de Mamíferos Aquáticos do Brasil (IBAMA, 2001). A diversidade genética das baleias jubarte que freqüentam o Brasil deverá constituir ferramenta importante de compreensão e conseqüente gestão de uma espécie presente na Lista Oficial de Espécies da Fauna Brasileira Ameaçadas de Extinção/IBAMA, e considerada "vulnerável" pela IUCN e pelo Plano de Ação de Mamíferos Aquáticos do Brasil.

O conhecimento do tamanho populacional histórico é importante para o manejo e a recuperação das espécies de cetáceos que foram muito caçadas e através de dados genéticos as flutuações históricas do tamanho da população podem ser investigadas (Rooney *et al.*, 2001; Roman & Palumbi, 2003; Alter *et al.*, 2007; Borge *et al.*, 2007). Os dados do tamanho histórico da população ajudarão a esclarecer as tendências atuais da população, determinando o provável impacto do ecossistema sobre uma espécie ecologicamente importante e ameaçada.

Diferente de outros mamíferos, as jubartes não vivem em grupos estáveis e o sistema social dessa espécie nas áreas de alimentação é caracterizado por pequenos grupos e associações efêmeras entre os indivíduos, relacionados com a distribuição das presas (Clapham, 1993; Weinrich *et al.*, 2006). Nas áreas de reprodução, um fluxo similar é observado na composição dos grupos, e machos competindo pelo acesso à fêmea entram e saem dos grupos competitivos, compostos por mais de dois machos e uma fêmea (Clapham, 1994). A análise do grau de parentesco entre indivíduos nos grupos deverá gerar informações importantes para os estudos de comportamento da espécie que já vêm sendo desenvolvidos.

A determinação do sexo nas baleias jubarte e em outros cetáceos na natureza é difícil ou até mesmo impossível dependendo das condições de avistagem, já que em muitas espécies o dimorfismo sexual está limitado ao tamanho e peso do corpo e a localização das regiões genital e anal. Nas jubartes as fêmeas também exibem outra característica sexual secundária, que consiste de um lobo hemisférico localizado na porção posterior da região genital (Glockner, 1983). No entanto, essa característica só pode ser observada em animais encalhados, ou através de imagens submarinas ou ainda quando o animal expõe a nadadeira caudal acima da superfície da água. Então, a análise de regiões sexo-específicas no DNA constitui uma alternativa eficiente para a determinação do sexo (Palsboll *et al.*, 1992;

Bérubé & Palsboll, 1996). A caracterização do sexo e a determinação da proporção sexual serão importantes para os estudos reprodutivos, fornecendo informações acerca de seu comportamento e estrutura social e, associada a fotoidentificação dos indivíduos biopsiados, permitirá compreender o padrão de distribuição das baleias jubarte na costa brasileira, nela incluída a região do Parque Nacional Marinho dos Abrolhos.

Por último, as comparações através de marcadores nucleares (microssatélites) das jubartes brasileiras e das que freqüentam a ilha Geórgia do Sul irão complementar estudos já realizados para determinação do sítio alimentar do estoque reprodutivo A. Isto permitirá uma melhor compreensão do padrão migratório desta espécie e os riscos e as ameaças a que ela pode estar sujeita quando fora da Zona Economicamente Exclusiva (ZEE) do Brasil.

A dissertação, escrita em formato de artigo cientifico, será submetida à "*Journal of Heredity*" e pretende contribuir para o conhecimento, manejo e conservação de uma espécie presente em todas as listas oficiais de fauna brasileira ameaçada de extinção, protegida da caça através da moratória internacional e que constitui um dos maiores ícones da conservação mundial. Os resultados obtidos neste trabalho também proporcionarão subsídios técnico-científicos para o órgão ambiental brasileiro (MMA/IBAMA) na tomada de decisões na política de conservação de cetáceos tanto a nível nacional como internacional.

Genetic characterization of the humpback whale (*Megaptera novaeangliae*) population of the Southwestern Atlantic Ocean breeding area based on nuclear microsatellites

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Running title: nuclear microsatellite diversity in Brazilian humpback whales

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Abstract

The main breeding ground of the humpback whales in the Southwestern Atlantic Ocean is the Abrolhos Bank, off the Brazilian coast. The genetic diversity and demographic history of the Brazilian humpback whale population, from two geographic locations (Abrolhos Bank, $n = 235$; Praia do forte, $n = 39$) was assayed with ten microsatellite loci. Microsatellites were also used to evaluate the existence of genetic differentiation between this population and two whales off South Georgia Islands. This population showed a high level of allelic diversity ($A = 12.1$) and a high mean observed heterozygosity ($H_O = 0.735$), in agreement with other breeding areas studied in the Southern Hemisphere. Although the Brazilian population of humpback whales was reduced during the commercial whaling no genetic bottleneck was detected with the different procedures that we used. Our findings showed no evidence to the temporal differentiation along the years and no genetic differentiation was found between whales from the two geographic locations of the Brazilian breeding ground and between these humpback whales and those from South Georgia Islands. In addition, a female sampled off South Georgia Islands showed a putative parent-offspring relationship with a female off Abrolhos Bank. These results support the hypothesis that South Georgia/South Sandwich Islands area as the main feeding area for the Brazilian breeding stock and that it constitute a single population. The data obtained through this study provided no evidence of association within social groups based in kinship.

Introduction

Humpback whales (*Megaptera novaeangliae*) are found throughout the world's ocean basins where they undertake annual migrations between the high latitude waters, where they feed during the summer, and the low latitude waters, where they breed and calve during the winter months (Dawbin 1966). Currently, the International Whaling Commission (IWC) divided the Southern Hemisphere waters into eight breeding grounds (A-G in the Southern Hemiphere, and X in the Arabian Sea) based on low latitude distributions (IWC 2005). Although humpback whales show high mobility and the apparent absence of geographic barries between ocean basins, gene flow between populations is minimal, and may even be restricted between different populations within the same ocean basin (Valsecchi et al. 1997). The Antarctic waters were also divided into six feeding grounds, known as Management Areas I to VI, which have functioned as political units for commercial whaling in the region (Tonnessen and Johnsen 1982).

In the Southwestern Atlantic Ocean the main mating and calving ground of this species is in the Abrolhos Bank ($16°40'$- $19°30'$S and $37°25'$- $39°45'$W) in Brazil (Martins et al. 2001; Freitas et al. 2004; Andriolo et al. 2006). This humpback whale population, considered as breeding stock "A", is distributed along the coast of the Brazil from approximately 5° to 21° S (Zerbini et al. 2004), with additional sightings to the north and east of 5° S, near the Archipelago of Fernando de Noronha (3°51' S) (Lodi 1994).

A comparative genetic study based in mitochondrial DNA (mtDNA) variability between the humpback population that breeds along the Brazilian coast and the populations that feed in the western and eastern part of the Antarctic Península (respectively Antarctica Area I and II; Donovan 1991), showed a lower differentiation between these two latter areas when both are compared with the Brazilian population (Engel et al. *in press*). This result corroborates the hypothesis that the Antarctic Peninsula does not constitute the main feeding ground of the Brazilian humpback whales. Furthermore two whales that were sampled very near South Georgia Islands matched with haplotypes from the Brazilian breeding ground (Engel et al. *in press*). Therefore the feeding ground for the Brazilian population should be located most likely in the South Georgia/South Sandwich Islands area, in the Scotia Sea, as indicated by recent photo-identification reports in these areas (Stevick et al. 2006) and a study using radio telemetry (Zerbini et al. 2006).

The commercial whaling during the 20th century reduced the humpback whale population of the world at less than 10% of the original size before of the worldwide protection in 1966 (Tonnessen and Johnsen 1982). The species is listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), it is considered "vulnerable" by the International Union for the Conservation of Nature and Natural Resources (IUCN) and by Brazilian Action Plan for Aquatic Mammals and it is in IBAMA Oficial List of Threatened to Extinction Species of the Brazilian Fauna (IBAMA 2001).

MtDNA variability of the Brazilian humpback whales showed high level of haplotypic and nucleotidic diversity (Engel et al. *in press*), in agreement with other breeding areas studied in the Southern Hemisphere (Baker et al. 1993; 1998; Rosenbaum et al. 1998; 2000; 2001). This result support the prediction that for most stocks of whales the population size was not sufficiently reduced or did not last for enough generations to significantly reduce their genetic diversity (Amos 1996). In addition, Engel et al. (*in press*) suggest that Brazilian population suffered the most intense bottleneck of three to six humpback whale generations, relative to the generation time estimated for this species of 12 to 24 years (Roman and Palumbi 2003). Moreover, gene flow between breeding grounds after whaling bottleneck could also contributed for the current high genetic variability in these populations, but this information is still unknown and more data from other Southern Hemisphere breeding grounds are needed (Engel et al. *in press*). The genetic structure of humpback whale populations is complex refleting long-distance migration from summer feeding grounds to winter breeding grounds (Baker et al. 1993), the genetic nuclear diversity of the Brazilian humpback whale population and its structure studied through molecular markes such as microsatellites is still unknown.

The present study aims to investigate the genetic diversity and the demographic history of this population based on the analysis of ten microsatellite loci. We also investigated the existence of temporal (annual) structure and whether genetic differentiation exists between three geographical locations, two off the Brazilian coast (Abrolhos Bank and Praia do Forte) and one off South Georgia Islands. Microsatellites were also used to evaluate if humpback whale associations are based on kinship and molecular sexing was performed to verify sexual proportion in the population.

Materials and methods

Sample collection, DNA extraction and sex determination

During seven breeding seasons (July to November), from 1999 to 2005, 278 skin samples of humpback whales (1999, $n = 89$; 2000, $n = 5$; 2001, $n = 37$; 2002, $n = 22$; 2003, $n = 61$; 2004, $n = 26$; 2005, $n = 38$), were collected by biopsy dart procedure (Lambertsen 1987) at two geographic locations off the Brazilian coast, the Abrolhos Bank ($n = 239$), in the southern Bahia and northern Espírito Santo states, and Praia do Forte ($n = 39$), northern coast Bahia (Fig 1a). Additionaly, some samples ($n = 32$) resulted from individuals stranded in Bahia and Espírito Santo states or from other locations on the Brazilian coast. During the feeding Summer season in 2006, two skin samples were collected four miles off South Georgia Islands (Fig 1b).

For the sampling of free-ranging whales, a Barnett Wildcat XL crossbow was used with stainless steel biopsy darts (8mm diameter, 15mm length sampling tip). Samples were kept in 70% ethanol or DMSO, according to the protocol established by Amos and Hoezel (1990) and later stored at -20°C until processed. Only adult animals were sampled within the social groups. It was not always possible to biopsy all the individuals in a group due to the behavior of the animals, size of the group, or weather conditions. It was also not possible to completely avoid resampling the same animal. For each whale sampled, date, GPS coordinates, and group composition were recorded. The social groups were classified in eight classes (singletons (S), mother-calf pairs (MC), mother-calf-escort (MCE), adult pairs (AP), groups of three or more individuals engaged (CG) or not (NCG) in competitive behaviours and mother-calf pairs with more than one scort (CGMC) engaged in competitive behaviours), based on the behaviours described for this species (Clapham et al. 1992).

Except for those specimens ($n = 149$) where DNA were already available (Engel et al. *in press*), the genomic DNA extraction ($n = 163$) was carried out following protocols modified from Palsboll et al. (1995), with lysis of cells in 1.0 % STE (10 mM Tris-HCl (pH 8.0), 150 mM sodium chloride, 1 mM EDTA (pH 8.0)) and the tissue digestion in 20 µg/ml of Proteinase K at 65^0 C for a minimum of four hours, followed by phenol/chloroform (3:1) extraction and ethanol precipitation.

The molecular sex determination was carried out by PCR amplification followed by *Taq*I digestion of the ZFX/ZFY region of the sex chromosomes following the protocol of Palsboll et al. (1992) modified by Bérubé and Palsboll (1996).

The total sexual rate was estimated (Clapham et al. 1995), and compared with those obtained in other breeding and calving areas and the Pearson chi-square test with Yate's correction was used to calculate the statistical significance of this rate with the expected ratio from 1:1.

Microsatellite analysis

All samples were screened for ten microsatellite loci: seven dinucleotides (EV1Pm, EV37Mm, EV94Mm, EV96Mm, Valsecchi and Amos 1996; 199/200, 417/418, 464/465, Schlöterrer et al. 1991) and three tetranucleotides (GATA028, GATA053, GATA417, Palsboll et al. 1997b). These ten loci were standardized for the use in other humpback whale population studies in the Southern Hemisphere (Pomilla and Rosenbaum, 2006). Foward primers were 5'-tailed with the M13 sequence (5'-CACGACGTTGTAAAACGAC-3') that is used in combination with an M13 primer marked with fluorescence (FAM, HEX, NED) (Boutin-Ganache et al. 2001). Amplifications were carried out in a 20 μL or 10 μL with the following conditions: 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.5 mM of reverse and M13-fluorescent primers, 0.0333 mM of the M13-tailed forward primer, 0.5 U of *Taq* DNA polymerase, 1X PCR buffer (Invitrogen) and 1 μl of DNA (approximately 50 ng). The ten loci were divided in two PCR profiles: BK50 profile (199/200, 464/465, EV1, EV37, GATA 417 and 417/418) and EV profile (EV94, EV96, GATA28 and GATA53). The BK50 profile consisted of initial denaturation at 94°C for 10 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s, with a final extension of 3 min at 72°C. The EV profile consisted of initial denaturation at 94°C for 10 min, followed by 10 cycles of denaturation at 92°C for 30 s, annealing at 60°C for 45 s, and extension at 72°C for 40 s, with an additional 15 cycles of denaturation at 91°C for 30 s, annealing at 56°C for 40 s, and extension at 72°C for 40 s, and 20 cycles of denaturation at 90°C for 30 s, annealing at 53°C for 40 s, and extension at 72°C for 40 s, with a final extension of 5 min at 72°C. All loci were amplified in separate reactions.

The PCR products were genotyped on a MegaBACE 1000 automated sequencer (Amersham Biosciences). The allele size in base pairs was identified with the software GENETIC PROFILER version 2.2 (Amersham Biosciences).

To minimize erros associated with PCR and scoring of loci some specific guidelines were followed during laboratory and scoring procedures. First, negative controls were run at the PCR step to control for exogenous contamination. Second, scoring was automated in GENETIC PROFILER, and alelle sizing was successively checked by hand. Finally, the program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) was used to identify possible nonamplified alleles (null alleles), large allele dropout and scoring errors due to stutter peaks.

Statistical analysis of microsatellite variation

Searches for genotype identity were performed using the program GENECAP (Wilberg and Dreher 2004), which uses an executable macro within Microsoft EXCEL that compares each multilocus genotype with all other genotypes within the dataset to identify matching samples. Pairs of matching were further compared for gender and with photographic matches. To quantify the power of our markers to distinguish between individuals, we calculated the probability of identity statistic $P_{(ID)}$, the probability that two individuals within the population shared the same multilocus genotype, using two different formulations (the HW $P_{(ID)}$ and a more conservative measure, the Sib $P_{(ID)}$) through the GENECAP program. Putative duplicated samples were excluded for the estimation of statistics of genetic variation and allele frequencies.

Genetic diversity was measured as the number of alleles per locus (K), the mean number of alleles per locus (allelic diversity, A), observed heterozygosity (H_O) and expected heterozygosity (H_E) under Hardy-Weinberg assumptions (Nei 1978). These analyses were performed using FSTAT version 2.9.3 (Goudet 2002) and GENEPOP version 3.4 (Raymond and Rousset 1995). The program FSTAT was also used to calculate Weir and Cockerham's (1984) measure of F_{IS}. The loci were tested for deviations from Hardy-Weinberg equilibrium (Guo and Thompson 1992) and linkage disequilibrium using the program ARLEQUIN 3.11 (Excoffier et al. 2005). Significance levels ($\alpha = 0.05$) for departure from HWE and for LD were corrected for simultaneous comparisons with the

sequential Bonferroni test (Rice 1989). Analyses were repeated excluding loci that were found to present possible null alleles.

Demographic history

Three methods based on changes in allele frequencies were used to detect the existence of historical demographic changes. Two methods were performed using the program BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996); one tests for excess of observed heterozygosity and the other tests the existence of a deficit of rare alleles in a sample of loci, therefore discriminating bottlenecked populations from stable populations (Luikart et al. 1998a, 1998b). The former estimates the expected heterozygosity for each locus through the observed number of alleles (k), given the sample size (n), assuming mutation-drift equilibrium, and compares it to the observed heterozygosity to establish whether there is a gene diversity excess or deficit at that locus. The probability of significant heterozygosity excess was calculated using a Wilcoxon signed rank test, and the computation was based on the two-phase mutation (TPM) model (Di Rienzo et al. 1994). The second is a graphical method, in which the allele frequency distribution is established in order to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (if recent bottlenecks caused a mode shift). A bottlenecked population has a deficit in rare alleles that causes a change in the distribution of allele frequencies seen as loss of low-frequency alleles and an increase in relative abundance of intermediate and high-frequency alleles. Described by Garza and Williamson (2001) the third method is based on the M-value which was calculated using the program AGARst (Harley 2001). The M-value is the mean ratio of number of alleles to total range of allele size ($M = k/r$), where k is the total number of alleles and r is the overall range of allele size. Bottlenecked populations have reduced values of M ($M < 0.68$), because k is expected to be reduced more quickly than r, since through the effect of genetic drift, the loss of any allele will contribute to a reduction in k but only a loss of the largest or smallest allele will contribute to a reduction in r.

Statistical analysis of population genetic structure

To evaluate the existence of any temporal (annual) structure in breeding ground A, three tests of a hierarchical analysis of molecular variance (AMOVA) were implemented using the program ARLEQUIN 3.11 (Excoffier et al. 2005), (1) grouping alternating years (1999-2001-2003 and 2000-2002-2004), (2) grouping two consecutive years (1999-2000; 2001-2002 and 2003-2004) and (3) grouping three consecutive years (1999-2000-2001 and 2002-2003-2004).

Genetic distance between the two geographic locations off the Brazilian coast (Abrolhos Bank and Praia do Forte) and off South Georgia Islands were also investigated by pairwise F_{ST} (Weir and Cockerham 1984) and AMOVA as implemented in ARLEQUIN 3.11.

We pooled all individual, including the two whales that were sampled very near South Georgia Islands, and tested for potencial population subdivision using a Bayesian model-based clustering method implemented in STRUCTURE 2.1 (Pritchard et al. 2000). The method does not require a priori population definitions and assumes the presence of K populations, where K may be unknown. The program uses a MCMC procedure to estimate $P\,(K/X)$, the posterior probability that the data fit the hypothesis of K clusters. The program also calculates the fractional membership of each individual in each cluster (Q). We conducted four independent runs for each K between 1 and 6 using no prior infomation, the admixture model, which allows individuals to have mixed ancestry, and correlated allele frequencies model, which assumes that frequencies in the different populations are likely to be similar due to migration or shared ancestry. Burn-in length and length of simulation were set at 500 000 and 1 000 000 steps, respectively. Additionally, we used the program STRUCTURAMA (Huelsenbeck and Andolfatto 2007) that infers population genetic structure from genetic data by allowing the number of populations to be a random variable with a Dirichlet process prior, a model first described by Pella and Masuda (2006). The program uses a particulary efficient variant of MCMC called Gibbs sampling, where each MCMC cycle involves a Gibbs scan of all of the individuals. Hence the total number of MCMC cycles for the analysis of this study is the product of the reported number of MCMC cycles and the number of individuals in the analysis. We run 1 000 000 cycles and we let α (the prior mean of the number of populations) be a random variable. The first 100 000 cycles were discarded as burn-in.

Relatedness analysis

The associations (affiliation between two individuals) for each social group containing a minimum of two sampled individuals without known calves (i.e. MCE, PR, CG, NCG, CGMC) were classified into three types according to the sex of the animals: female-female (F-F), male-female (M-F) and male-male (M-M). The coefficient of relatedness r_{LR} (Lynch and Ritland 1999) was calculated for each association within-population and within-group using the program IDENTIX (Belkhir et al. 2002). The program calculates relatedness between any two individuals by comparing the alleles shared by these individuals with the allele frequency of the population. The relatedness coefficients (r) range from -1 to 1, with 0.5 for parent-offspring or full-siblings, 0.25 for half-siblings and zero or negative coefficients for unrelated individuals. To test whether members of the same social group are more (or less) closely related to each other than the other members of the population we calculated the mean of the coefficients of relatedness (r) and their standard error. The mean difference between pairwise genetic relatedness within groups and within the population as a whole was evaluated using the two-sample randomization test within the program RT 2.1 (Manly 1997) with 10 000 randomizations.

We also tested whether individuals of the same sex (associations M-M and F-F) are more (or less) closely related to each other than the different sex (associations M-F) whithin-groups. Furthemore, we compared the degree of relatedness between the different classes of sex (M-M, M-F and F-F) within social groups. For this we calculated the coefficients of relatedness for each association, as well as the mean for each class of sex within social groups. The mean difference in pairwise genetic relatedness between the classes of sex was also evaluated using the two-sample randomization test within the program RT 2.1 with 10 000 randomizations.

We also searched for likely parent-offspring relationships within the whole population, where neither parent was known a *priori*. For this we used the AGARst program (Harley 2001), which looks for compatible parent-offspring genotypes. Only individuals for whom we genotyped all loci were included in the analysis, and were further inspected to verify if at least one allele was shared in all loci. The matches yielding a high relatedness coefficient ($r > 0.4$) for r_{LR} implemented in IDENTIX and r_{QG} (Queller and

Goodnight 1989), implemented in KINSHIP 1.3.1 were considered likely parent-offspring or full-siblings. To test these likely parent-offspring relationship we used a maximum-likelihood approach implemented in KINSHIP 1.3.1. Three hypotheses were tested, (I) parent-offspring relationship was the primary hypothesis against the null hypothesis of unrelated individuals; (II) full-sibling relationship against the null hypothesis of unrelated individuals; and (III) parent-offspring relationship against the null hypothesis of full-sibling relationship. The significance level of the obtained ratio was calculated by simulating 10 000 pairs of individuals using the primary hypothesis settings and the observed allele frequencies and determining the ratio needed to reject the null hypothesis with $P = 0.05$, 0.01, and 0.001.

We also compared mtDNA haplotypes already available (Engel et al. *in press*) for individuals from putative female-female and male-female matches. Shared haplotypes add weight to the parent-offspring association while different haplotypes either reject the relationship (e.g. two females) or suggest another relationship (e.g. a male and female with different haplotypes could be a father with his daughter but not a mother and her son).

Results

Microsatellite polymorphism and genetic variability

Only samples for which we obtained allele data information for six or more loci were included in the analysis, and therefore, 293 samples were used here. Individual multilocus genotypes were on average 97.9 % complete. Based on genotype identity and accessory information the samples were assigned to 277 individuals. Probability of identity calculations showed that the power of the employed loci to discriminate between individuals was high. HW $P_{(ID)}$ was 2.32 x 10^{-12} and the most conservative measure of the $P_{(ID)}$, the Sib $P_{(ID)}$ was 8.98 x 10^{-5}, indicating that the probability that even related individuals would have the same genotype is extremely low. Thus, different samples which produced duplicate multilocus genotypes (excluded from the analysis) can be assumed with high confidence to represent the same individual.

All 10 microsatellite loci were highly polymorphic and showed a fair amount of genetic variability in the Brazilian humpback whales, with the number of alleles per locus ranging from 4 (EV1) to 18 (GATA417 and EV37) with a mean of 12.1. The observed (H_O)

and expected (H_E) heterozygosity ranged from 0.527 (EV1) to 0.904 (EV37) with a mean of 0.735 and 0.519 (EV1) to 0.922 (EV37) with a mean of 0.747, respectively (Table 1). The MICRO-CHECKER analysis indicated that null alleles may be present at locus 417/418 as also suggested by the general excess of homozygotes for most allele size classes and by the significant ($P < 0.025$) combined probability of observed homozygote class frequencies using the binominal test. Moreover, the same locus showed a significant ($P = 0.000$) deviation from HWE expectations. Thus, this locus was discarded from all analyses. No signs of null alleles were seen at the other loci, and none of them deviated significantly from HWE expectations. Pairwise comparisons of allele frequencies revealed no significant linkage disequilibrium after Bonferroni correction. Further tests for errors in the data showed no evidence for stuttering or large allele dropout.

Demographic history

BOTTLENECK analysis did not provide evidence for a recent population decline. The test for excess heterozygosity based on the TPM model was not significant ($P = 0.455$) and the distribution of allele frequencies was clearly L-shaped (Figure 2). Furthermore, the M index for the ten polymorphic loci ($M = 0.85 \pm 0.20$) was higher than the critical value ($M = 0.68$) indicated by Garza and Williamson (2001).

Population genetic structure

The three AMOVA analyses supported the absence of the temporal differentiation among the years in this breeding ground, as almost all genetic variation was apportioned within the populations (Table 2). Absence of geographic structure was also found in the AMOVA considering the three locations (Abrolhos Bank, Praia do Forte, and South Georgia Islands), with genetic variation within populations around 100%, and non-significant pairwise F_{ST} (Table 3).

For the Bayesian STRUCTURE clustering analysis the highest posterior probability of the genetic data was obtained when the individuals were grouped in a single population ($K = 1$) (Table 4) instead of two or more ($K = 2$ to $K = 6$, Figure 3). The likelihood values were consistent among the 4 replicate runs, with similar values of cluster membership Q for all individuals (Figure 3). The alternative approach implemented in the program

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STRUCTURAMA, estimated that the number of populations with the highest posterior probability was $K = 1$ (P (K/X) = 0.9940). Furthermore, all the pairs of individuals shared high posterior probabilities (> 0.9) of being grouped in the same population and their Bayes factors were > 10 (167.976), supporting the hypothesis of that all individuals belong to the same population.

Molecular sexing

Sex was determined for 253 individuals resulting in 140 males and 113 females. The observed overall proportion of 55.3% males and 44.7% females (1.2:1 males to females) did not differ significantly ($x^2 = 1.12$, $\alpha = 0.05$, df = 1) from a 1:1 sex ratio.

Relatedness within social groups

A total of 37 social groups were surveyed, of which 22 (59.4%) had all group members sampled (two or three individuals). The groups contained 30 females and 46 males, totaling 76 whales. Table 5 shows the number of individuals, groups and associations analysed by group class and sex combination. Among the sampled pairs of adults, more than half were M-F associations (70.0%) followed by M-M (27.5%) and F-F (2.5%) associations. The only instance of F-F association was found in a mother-calf-escort (MCE) group (all others escorts were males).

The mean relatedness for within-group pairwise comparisons ($r = - 0.0588 \pm 0.1000$) was lower than the mean relatedness for within-population pairwise comparisons ($r = - 0.0038 \pm 0.1058$), but this difference was not significant ($P = 0.091$). The mean relatedness and standard error for the each class of sex comparison are reported in table 6. The class F-F was excluded from the analysis since it included a single association. The difference between the relatedness of the different sex classes was not significant ($P = 0.923$).AGAR$_{ST}$ identified 76 matches (at least one allele shared at each locus), of which 17 yielded a high relatedness coefficient ($r > 0.4$) in the analysis of the two programs. All these 17 matches presented high and significant levels ($P < 0.01$ or $P < 0.001$) of relatedness compatible with parent-offspring and full-sibling relationships (Hypothesis I and II, respectively; Table 7). However, three matches did not present significant parent-

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offspring relationship when full-sibling was the null hypothesis (Hypothesis III; Table 7). Therefore, 14 matches presented likely parent-offspring relationship.

However, we had mtDNA haplotype information for only five matches and among them four female-female matches had identical haplotypes, adding weight to a parent-offspring association, while the other female-female match had different haplotypes, refuting a parent-offspring relationship (Table 7). Significantly, one of the matches was between a female sampled off Abrolhos Bank in 2001 (ID=177) and a female sampled near South Georgia Islands in 2006 (ID=GS02), that present identical mtDNA haplotypes.

Discussion

Genetic diversity

Our data reveal high genetic nuclear diversity in the humpback whales population that overwinter off the Brazilian coast (Breeding Ground A), corroborating the mtDNA data, which showed high variability (Engel et al. *in press*). A large number of alleles per locus and a consequent high level of heterozygosity was observed in each of the 10 microsatellite loci. This genetic variation was similar to that of other large baleen whales, such as right whales, *Eubalaena glacialis* (Waldick et al. 2002), fin whales, *Balaenoptera physalus* (Bérubé et al. 1998), sei whales, *B. borealis* (Kanda et al. 2006) and Bryde's whales, *B. brydei* (Kanda et al. 2007).

Moreover, the population of this breeding area showed a high level of allelic diversity ($A = 12.1$) and a high mean observed heterozygosity ($H_O = 0.735$), in agreement with other humpback whale breeding areas studied in the Southern Hemisphere (Valsecchi et al. 2002; Garrigue et al. 2004; Pomilla and Rosenbaum 2006) and in the North Pacific Ocean (Cerchio et al. 2005). For the same loci analysed in this study, Pomilla and Rosenbaum (2006) found in the breeding areas of Regions B and C, a mean number of alleles per locus of 12.91 for both Gabon ($H_O = 0.745$) and Northeastern Madagascar ($H_O = 0.751$). Likewise, the two breeding areas in Region E, Eastern Australia ($A = 10.4$ and $H_O = 0.726$; Valsecchi et al. 2002) and New Caledonia ($A = 9.9$ and $H_O = 0.768$; Garrigue et al. 2004), had high level of allelic diversity and high mean observed heterozygosity, in both cases using 9 loci. Cerchio et al. (2005) found for 13 microsatellite loci an allelic richness

of 10.1 and a mean observed heterozygosity of 0.710 in the breeding area for humpback whales in the Mexican Pacific coast.

Although the Brazilian population of humpback whales had been reduced to less than 5% of its historical size by commercial whaling (Engel et al. unpuplished results) no signal of genetic bottleneck was detected with the different procedures that we used. These results were similar to other studies that tested for genetic bottlenecks on large baleen whale species that had been the target of commercial whaling worldwide (e.g. bowhead, Rooney et al. 1999; right, Waldick et al. 2002; and sei whales, Kanda et al. 2006), corroborating the prediction that commercial whaling did not last long enough to significantly reduce the genetic diverstity for most of these species (Amos 1996).

Population genetic structure

Our results showed no evidence of any genetic structure considering the years of sampling, with up to three years of interval. Some studies suggested that humpback whales may occasionally change destinations between different wintering seasons after migration to shared feeding grounds (Darling and Cerchio 1993; Garrigue et al. 2002). Temporal differences may also exist in occupancy of wintering areas between individuals arriving from different feeding grounds (Stevick et al. 2003). Furthemore, a complicated temporal genetic pattern during the migration season was observed in the bowhead whale, however an explanation for this pattern remains uncertain (Jorde et al. 2007). Clark and Clapham (2004) suggested that the Brazilian humpback whale population may share feeding grounds with Gabon humpbacks and some contact between Brazil and Gabon was supported by recent acoustical analyses (Darling and Sousa-Lima 2005), but this hypothesis remains to be more thoroughly tested.

The geographical population structure analyses (AMOVA and pairwise F_{ST}) showed non-significant differentiation among individual samples collected at the three geographical locations, including the South Georgia Islands, with virtually all variability allocated within populations. Moreover, the Bayesian clustering analysis yielded the highest posterior probability of the data when all individuals were grouped into a single population. However, we recommend caution in the evaluation of our results with STRUCTURE as this program is known to have poor resolution in partitioning the individuals when genetic

differentiation is weak (Waples and Gaggiotti 2006). The program could not detect more than one population at an $F_{ST} = 0.01$, and could not discern all populations at an $F_{ST} = 0.02$ (Latch et al. 2006). However, the alternative approach implemented in STRUCTURAMA agreed with the existence of a single population in our data.

Concerning the non-significant differentiation between the two geographic locations off the Brazilian coast (Abrolhos Bank and Praia do Forte), this result agreed with photographic data, in which whales that are found at the Abrolhos Bank matched whales off Praia do Forte (Freitas et al. 2004). Moreover, together with the increasing sightings further north on the Abrolhos Bank to the Fernando de Noronha Archipelago, these data indicate the likely return to areas occupied by the species before the depletion of this stock by whaling (Martins et al. 2001; Zerbini et al. 2004; Andriolo et al. 2006).

Our analyses did not find differentiation between both locations of the Brazilian breeding ground and the South Georgia Islands feeding area. In addition, a female sampled off South Georgia Islands showed a putative parent-offspring relationship with a female off Abrolhos Bank, with a high relatedness coefficient and identical mtDNA haplotype. Despite the small sample size for South Georgia Islands, these results agree with our previous suggestion that the likely feeding ground for the Brazilian population is near South Georgia Islands. On support of this, a study regarding modern sighting and stranding patterns off Brazil provided support for an offshore migration, suggesting a feeding area in south or southeast waters (Siciliano et al. 1999). Likewise, humpback whales tagged with satellite transmitters off the Brazilian coast migrated to feeding areas close to South Georgia and Sandwich Islands (Zerbini et al. 2006). Stevick et al. (2004) showed that individuals identified did not move off the Antarctic Peninsula to the Brazilian coast, but to northwest coast of South America. Furthemore, recent comparisons of whales photo-identified from Abrolhos Bank and Shag Rocks, off South Georgia Islands (Stevick et al. 2006) and between the Abrolhos Bank and the Sandwich Islands (Engel et al. unpuplished results) showed photographic matches. A larger sampling effort off South Georgia/ South Sandwich Islands will be necessary to genetically test this hypothesis.

Sexual proportion and relatedness of the groups

The observed proportion of males and females in the Brazilian humpback whales did not differ significantly from the 1:1 sex ratio generally accepted for humpback whales (Clapham and Mayo 1987; Clapham et al. 1995; Medrano et al. 1994). However, an overall higher number of males was found, in agreement with other breeding ground studies, due to the different pattern of migration in males versus females (Craig and Herman 1997). Female residency on the breeding ground is shorter than that of males and is temporally alternated among females, resulting in a excess of males in this region. This difference is due to the short female oestrus relative to residency time and is likely to be broadly asynchronous among females (Cerchio et al 2005). Another explanation is that this is a strategy aimed at increasing the female's probability of reproductive success by maximizing the time spent on the feeding grounds, and so acumulating energy that will be spent in migration and lactation for long periods without food sources (Craig and Herman 1997).

Most of the association types were of male-female pairs, in agreement with observations in other breeding grounds, where most of the groups found are M-F pairs in dyads or in large competitive groups (Pomilla and Rosenbaum 2006). All dyads were formed by unrelated males and females, generaly associated with courtship and mating behaviour. As male-female dyads also occur on the feeding grounds, Clapham (1993) suggested that bonds of males with females before the breeding season might increase a male's reproductive success, although this hypothesis has not been tested yet. With a single exception all escorts were males, suggesting that escorting behaviour is related to mating opportunities, that is, males in temporary groups competing for females access, suggesting that males secure bonds with females near oestrus in the migration to wintering areas or they guard the mate in the migration to feeding areas (Valsecchi et al. 2002).

In our study, a single social group had more than one female, found in a MCE group of unrelated individuals. Associations of females are known on the feeding ground (Clapham 1993; Weinrich et al. 2006), but they are rarely represented during the migrations and on the breeding grounds (Pomilla and Rosenbaum 2006). On feeding grounds, this behaviour seems to indicate cooperation during feeding (Clapham 1993). Despite a study on feeding grounds in the southern Gulf of Maine having indicated that females of the same

mtDNA haplotype are more often associated than predicted by chance, these results was not tested for relatedness and could be explained by spatial and temporal effects, which are due to the influence of maternal experience on feeding styles and prey preferences (Weinrich et al. 2006). Our results corroborate the low occurrence of associations between females on breeding grounds. For example, no group sampled on the West Indies breeding grounds included more than one adult female (Craig et al. 2002) and only one and three associations between females in a competitive group were found, respectively, in the Northeastern Madagascar and Gabon breeding grounds (Pomilla and Rosenbaum. 2006). None of these associations showed strong evidence for relatedness among females, with a single exception, where two females shared the same mtDNA haplotype and were related at the level of half-siblings. Pomilla and Rosenbaum (2006) suggested as an explanation for this association that females could travel together from the same feeding area to the breeding grounds, or they could associate upon arrival, diluting their chance of harassment by males.

Despite the interactions among males within competitive groups being typically agonistic, with more than two males competing for a single female, pairs of cooperating males have been observed within these groups (Clapham et al. 1992). Such observations suggest a social bond based on kin, where related males cooperating enhance the reproductive success of relatives. However, our results provided no evidence for this, as pairs of males within competitive groups were not more related on average than random individuals in the population. This is in agreement with the associations among males in the Gabon breeding ground, where the males showed low mean relatedness (Pomilla and Rosenbaum 2006). Alternatively, a reduced mean relatedness within these groups could be explained by kin avoidance to minimize competition among relatives (Pomilla and Rosenbaum 2006).

Finally, the mean relatedness within social groups was not significantly different than the mean relatedness within the whole population, indicating no support for the hypothesis that social groups are formed by related individuals (other than mother-calf), corroborating other studies (e.g. Clapham and Palsboll 1997, Valsecchi et al. 2002, Cerchio et al. 2005, Pomilla and Rosenbaum 2006).

The putative parent-offspring or full-sibling relationship found in this study suggest that calves return to their natal breeding ground, attesting to the maternal fidelity to

migratory destinations. An important finding to support this was the parent-offspring association among one female sampled off Abrolhos Bank in 2001 and one female sampled very near South Georgia Islands in 2006.

Conservation implications

Our analyses showed high genetic variability and no evidence to genetic bottleneck for the humpback whales that breed off the Brazilian coast. These findings indicate that this population has a good chance of long-term viability, something that is already occurring as could be seen through the signs of population recovery in this breeding stock (Engel et al. unpublished results). Conservation efforts must therefore focus on the maintenance of this genetic diversity through demographic stability and habitat protection. Furthemore, it is very important to monitor not only the level of genetic diversity but also changes of life history traits.

A management concern is the risk of depleting genetically distinct populations when exploiting the stock under the assumption of a single population. Migratory whale exploitation is mainly conducted on feeding grounds, where genetically diferent populations might co-occur (Hoezel 1998). The identification of management units needs to account for temporal and spacial factors. Moreover, the issue of potential structure also has important implications for understanding the biology and demography of this species. A better understanding of the pattern and amount of gene flow as well as an accurate identification of grounds of possible population admixture should be important for conservation and management of humpback whale populations. Our results support the hypothesis that the Southwestern Atlantic humpback whale is a single population, with no evidence of spatial or temporal differentiation across years.

Acknowledgements

References

Amos B and Hoezel AR (1990) DNA fingerprinting cetacean biopsy samples for individual identification. *Rep. Int. Whaling Comm.*, *Spec Iss*, **12**, 79-85.

Amos B (1996) Levels of genetic variability in cetacean populations have probably changed little as a result of human activities. *Rep. Int. Whaling Comm.*, **46**, 657-658.

Andriolo A, Martins CCA, Engel MH, Pizzorno JL, Más-Rosa S, Freitas A, Morete ME, Kinas PG (2006) The first aerial survey to estimate abundance of humpback whale (*Megaptera novaeangliae*) in the breeding ground off Brazil. *J. Cetacean Res. Manage*, **8** (3), 307-311.

Baker CS, Perry A, Bannister JL, Weinrich MT, Abernethy RB, Calambokidis J, Lien J, Lambertsen RH, Urban-Ramirez J, Vasquez O, Clapham PJ, Alling A, O´Brien SJ, Palumbi SR (1993) Abundant mitochondrial DNA variation and worlwide population structure in humpback whales. *Proc. Nat. Acad. Sci. USA* **90**, 8239-8243.

Baker CS, Flóres-Gonzáles L, Abernethy B, Rosenbaum HC, Slade RW, Capella J, Bannister JL (1998) Mitochondrial DNA variation and maternal gene flow among humpback whales of the Southern Hemisphere. *Mar. Mamm. Sci.*, **14**, 721-737.

Belkhir K, Castric V, Bonhomme F (2002) IDENTIX, a software to test for relatedness in a population using permutation methods. *Mol. Ecol.*, **2**, 611-614.

Bérubé M and Palsboll P (1996) Identification of sex in cetaceans by multiplexing using ZFX and ZFY specific primers. *Mol. Ecol.*, **5**, 283-287.

Bérube M, Aguilar A, Dendantos D, Larsen F, Di Sciara GN, Sears R, Sigurjónsson J, Urban-R J, Palsboll PJ (1998) Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus, 1758): analysis of mitochondrial and nuclear loci. *Mol. Ecol.*, **7**, 585-599.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-Tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *BioTechniques* **31**, 26-28.

Cerchio S, Jacobsen JK, Cholewiak DM, Falcone EA, Merriwether DA (2005) Paternity in humpback whales, *Megaptera novaeangliae*: assessing polygyny and skew in male reprodutive success. *Ani. Behav.*, **70**, 267-277.

Clapham PJ and Mayo CA (1987) Reproduction and recruitment of individually identified humpback whales, *Megaptera novaeangliae*, observed in Massachussets Bay, 1979-1985. *Can. J. Zool.*, **65**, 2853-2863.

Clapham PJ, Palsboll PJ, Mattila DK, Vasquez O (1992) Composition and dynamics of humpback whale competitive groups in the West Indies. *Behavior*, **122**, 182-194.

Clapham PJ (1993) Social organization of humpback whales on a North Atlantic feeding ground. *Sym. Zoo. Soc. Lon.*, **66**, 131-145.

Clapham PJ, Bérubé M, Mattila DK (1995) Sex ratio of the Gulf of Maine humpback whale population. *Mar. Mamm. Sci.*, **11** (2), 231-240

Clapham PJ and Palsboll PJ (1997) Molecular analysis of paternity shows promiscuous mating in humpback whales (*Megaptera novaeangliae*, Borowski). *Proc. Roy. Soc. Lon.*, **264**, 95-98.

Clark CW and Clapham PJ (2004) Acoustic monitoring on a humpabck whale (*Megaptera novaeangliae*) feeding ground shows continual singing into late spring. *Proc. Roy. Soc. Lon.*, **271**, 1051-1057.

Cornuet JM and Luikart G (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001-2014.

Craig AS and Herman LM (1997) Sex differences in site fidelity and migration of humpback whales (*Megaptera novaeangliae*) to the Hawaiian Islands. *Can. J. Zoo.*, **75**, 1923-1933.

Craig AS, Herman LM, Pack AA (2002) Male mate choice and male-male competition coexist in the humpback whale (*Megaptera novaeangliae*). *Can. J. Zoo.*, **80**, 745-755.

Darling JD and Cerchio S (1993) Movement of a humpback whale, *Megaptera novaeangliae*, between Japan and Hawaii. *Mar. Mamm. Sci.*, **9**, 84-89.

Darling JD and Souza-Lima RS (2005) Songs indicate interaction between humpback whale (*Megaptera novaeangliae*) populations in the western and eastern South Atlantic Ocean. *Mar. Mamm. Sci.*, **21**, 557-566.

Dawbin W (1966) The seasonal migratory cycle of the humpback whale. In: *Whales, Dolphins and Porpoises.* (ed. Norris KS), pp 145-170. University of California Press, Berkeley.

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple sequence repeat loci in human populations. *Proc. Nat. Acad. Sci. USA*, **91**, 3166-3170.

Donovan GP (1991) A Review of IWC Stock Boundaries. *Rep. Int. Whaling Comm., Spec. Iss.*, **13**, 39-68.

Engel MH, Fagundes NJR, Rosenbaum HC, Leslie MS, Ott PH, Schmitt R, Secchi E, Dalla Rosa L, Bonatto SL (*in press*) Mitochondrial DNA diversity of the Southwestern Atlantic humpback whale (*Megaptera novaeangliae*) breeding area off Brazil, and the potential connections to Antartic feeding areas. *Cons. Gen.*.

Excoffier LGL and Schneider S. (2005) ARLEQUIN ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47-50.

Freitas AC, Kinas PG, Martins CCA , Engel MH (2004) Abundance of humpback whales on the Abrolhos Bank wintering ground, Brazil. *J. Cetacean Res. Manage*., **3**, 225-230.

Garrigue C, Aguayo-Lobo A, Amante-Helwing VLU (2002) Movements of humpback whales in Oceania, South Pacific. *J. Cetacean Res. Manage*, **4**, 255-260.

Garrigue C, Dodemont R, Steel D, Baker CS (2004) Organismal and 'gametic' capture-recapture using microsatellite genotyping confirm low abundance and reproductive autonomy of numpback whales on the wintering grounds of New Caledonia. *Mar. Ecol. Progr. Ser.*, **274**, 251-262.

Garza JC and Williamson EG (2001) Detecting of reduction in population size using data from microsatellite loci. *Mol. Ecol.*, **10**, 305-318.

Goudet J (2002) FSTAT: a program to estimate and test gene diversities and fixation indices. Lausanne: Institute of Ecology.

Guo S, and Thompson E (1992) Performing the exact test of Hardy-Weinberg proportion for multiples alleles. *Biometrics* **48**, 361-372.

Harley EH (2001) AGARst. A program for calculating allele frequencies, G_{ST} and R_{ST} from microsatellite data. Version 2.0. University of Cape Town, Cape Town.

Hoezel AR (1998) Genetic structure of cetacean populations in sympatry, parapatry and mixed assemblages: implications for conservation policy. *J. Hered.*, **89**, 451-458.

Huelsenbeck JP and Andolfatto P (2007) Inference of population structure under a Dirichlet process model. *Genetics*, **175**, 1787-1802.

IBAMA (2001) *Plano de Ação de Mamíferos Aquáticos do Brasil*. Versão II. Ministério do Meio Ambiente e dos Recursos Naturais Renováveis, Brasília.

International Whaling Commission (2005) Report of the Scientific Committee. Annex H. Report of the sub-committee on other Southern Hemisphere whale stocks. *J. Cetacean. Res .Manage.* **7** (Suppl.), 235-46.

Jorde PE, Schweder T, Bickham JW, Givens GH, Suydam R, Hunters D, Stenseth NC (2007) Detecting genetic structure in migrating bowhead whales off the coast of Barrow, Alaska. *Mol. Ecol.*, **16**, 1993-2004.

Kanda N, Goto M, Pastene LA (2006) Genetic characterstics of Western North Pacific Sei whales, *Balaenoptera borealis*, as revealed by microsatellites. *Mar. Bio.*, **8**, 86-93.

Kanda N, Goto M, Kato H, McPhee MV, Pastene LA (2007) Population genetic structure of Bryde's whales (*Balaenoptera brydei*) at the inter-oceanic and trans-equatorial levels. *Cons. Gen.*, **8**, 853-864.

Lambertsen RH (1987) A biopsy sistem for large whales and its use for citogenetics. *J. Mamm.*, **68**, 443-445.

Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE Jr (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Cons Gen.*, **7**, 295-302.

Lodi L (1994) Ocorrências de baleias-jubarte, *Megaptera novaeangliae*, no Arquipélago de Fernando de Noronha, incluindo um resumo de registros de capturas no Nordeste do Brasil. *Biotemas*, **7**, 116-123.

Lynch M and Ritland K (1999) Estimation of pairwise relatedness with molecular markers. *Genetics*, **152**, 1753-1766.

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998a) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Hered.*, **89**, 238-247.

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998b) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Mol. Ecol.*, **7**, 963-974.

Manly BFJ (1997) RT, a program for randomization testing, version 2.1. Centre for Applications of Statistics and Mathematics. University of Otago, Otago.

Martins CCA, Morete ME, Engel MH, Freitas A, Secchi ER, Kinas PG (2001) Aspects of habitat use patterns of humpback whales in the Abrolhos bank, Brazil, breeding ground. *Mem. Queensl. Mus.*, **47**, 563-570.

Medrano L, Salinas M, Salas I, Ladrón de Guevara P, Aguayo A (1994) Sex identification of humpback whales, *Megaptera novaeangliae*, on the wintering grounds of the Mexican Pacific Ocean. *Can. J. Zool.*, **72**, 1771-1774.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583-590.

Palsboll PJ, Vader A, Bakke I, El-Gewely MR (1992) Determination of gender in cetaceans by polymerase chain reaction. *Can. J. Zool.*, **70**, 2166-2170.

Palsboll PJ, Clapham PJ, Mattila DK, Larsen F, Sears R, Siegismund HR, Sigurjónsson J, Vásquez O, Artander P (1995) Distribution of mtDNA haplotypes in North Atlantic humpback whales: the influence of behaviour on population structure. *Mar. Ecol. Prog. Ser.*, **116**, 1-10.

Palsboll PJ, Bérubé M, Jorgensen H (1997) Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. *Mol. Ecol.*, **6**, 893-895.

Pella J and Masuda M (2006) The Gibbs and split-merge sampler for population mixture analysis from genetic data with incomplete baselines. *Can. J. Fish. Aquat. Sci.*, **63**, 576-596.

Pomilla C and Rosenbaum HC (2006) Estimates of relatedness in groups of humpback whales (*Megaptera novaeangliae*) on two wintering grounds of Southern Hemisphere. *Mol. Ecol.*, **15**, 2541-2555.

Pritchard JK, Stephens M, Donnely P (2000) Inference of a population structure using multilocus genotype data. *Genetics*, **155**, 945-959.

Queller DC and Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258-275.

Raymond M and Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.*, 86, 248-249.

Rice WR (1989) Analyzing table of statistical tests. *Evolution*, **43**, 223-225.

Roman J, Palumbi S (2003) Whales before whaling in North Atlantic. *Science* **301**, 508-510.

Rooney AP, Honeycutt RL, Davis SK, Derr JN (1999) Evaluating a putative bottleneck in a population of bowhead whales from patterns of microsatellite diversity and genetic disequilibria. *J. Mol. Evol.*, **49**, 682-690.

Rosenbaum HC, Razafindrakoto Y, Flórez-González L, Capella J, Garrigue C, Greaves J, Jenner C, Jenner M-N, Robles-Saavedra MR, De Salle R, Baker CS (1998) Variation and geographic structure of humpback whale mitochondrial DNA from the wintering grounds of Areas III, IV, V and VI in the Southern Hemisphere. *Rep. Int. Whaling Comm.*, SC/50/CAWS35.

Rosenbaum HC, Best PB, Findlay KP, Engel MH, Pomilla C, Razafindrakoto Y, Morete ME, Vely M, Freitas AC, Baker CS, Jenner C, Jenner M-N, Bannister J (2000) Mitochondrial DNA variation among humpback whales from the wintering grounds in the South Atlantic and Southwestern Indian Oceans. *Rep. Int. Whaling Comm.*, SC/52/IA11.

Rosenbaum HC, Best PB, Pomilla C (2001) A preliminary analysis of mtDNA variation among humpback whales of the southeastern Atlantic Ocean from the wintering grounds along the coast of west Africa. *Rep. Int. Whaling Comm.*, SC/53/IA32.

Schlötterer C, Amos B, Tautz D (1991) Conservation of polymorphic simple sequence loci in cetacean species. *Nature*, **354**, 63-65.

Siciliano S, Pizzorno JLA, Barata PCR (1999) Distribution and possible migratory routes of humpback whales *Megaptera novaeangliae* in the western South Atlantic. *Rep. Int. Whaling Comm.*, SC/51/CAWS4.

Stevick PT, Allen J, Bérubé MC (2003) Segregation of migration by feeding ground origin in North Atlantic humpback whales (*Megaptera novaeangliae*). *J. Zoo.*, **259**, 231-237.

Stevick PT, Aguayo A, Allen J, Avila IC, Capella J, Castro C, Chater K, Dalla Rosa L, Engel MH, Félix F, Flórez-González L, Freitas A, Haase B, Llano M, Lodi L, Munoz E, Olavarria C, Secchi E, Scheidat M, Siciliano S (2004) Migrations of individually identified humpback whales between the Antartic Peninsula and South America. *J. Cetacean Res. Manage.*, **6** (2), 109-113.

Stevick PT, Pacheco de Godoy L, McOsker M, Engel MH, Allen A (2006) A note on the movement of a humpback whale from Abrolhos Bank, Brazil to South Georgia . *J. Cetacean Res Manage*, **8** (3), 297-300.

Tonnessen JN and Johnsen AO (1982) *The History of Modern Whaling.* University of California Press, Berkeley.

Valsecchi E and Amos W (1996) Microsatellite markers for the study of cetacean populations. *Mol. Ecol.*, **5**, 151-156.

Valsecchi E, Pasboll P, Hale P, Glockner-Ferrari D, Ferrari M, Clapham P, Larsen F, Mattila D, Sears R, Sigurjonsson J, Brown M, Corkeron P, Amos B (1997) Microsatellite genetic distances between oceanic populations of the humpback whale (*Megaptera novaeangliae*). *Mol. Bio. Evol.,* **14**, 355-362.

Valsecchi E, Hale P, Corkeron P, Amos W (2002) Social structure in migrating humpback whales (*Megaptera novaeangliae*). *Mol. Ecol.*, **11**, 507-518.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping erros in microsatelite data. *Mol. Ecol.Notes*, **4**, 535-538.

Waldick RC, Kraus S, Brown M, White BN (2002) Evaluating the effects of historic bottleneck events: an assessment of microsatellite variability in the endagered, North Atlantic right whale. *Mol. Ecol*., **11**, 2241-2249.

Waples RS and Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol. Ecol.*, **15**, 1419-1439.

Weinrich MT, Rosenbaum HC, Baker CS, Blackmer AL, Whitehead H (2006) The influence of maternal lineages on social affiliations among humpback whales (*Megaptera novaeangliae*) on their feeding grounds in the southern Gulf of Maine. *J. Hered.*, **97**, 226-234.

Weir BS and Cockerham CC (1984) Estimating F-statistics for the Analysis of Population Structure. *Evolution*, **38**, 1358-1370.

Wilberg MJ and Dreher BP (2204) GENECAP: a program for analysis of multilocus genotype data for non-invasive sampling and capture-recapture population estimation. *Mol. Ecol.Notes*, **4**, 783-785.

Zerbini AN, Andriolo A, da Rocha JM, Simões-Lopes PC, Siciliano S, Pizzorno JL, Waite JM, DeMaster DP, VanBlaricon GR (2004) Winter distribution and abundance of humpback whales (*Megaptera novaeangliae*) off Northeastern Brazil. *J. Cetacean Res. Manage*, **6**, 101-107.

Zerbini AN, Andriolo A, Heide-Jorgensen MP, Pizzorno JL, Maia YG, VanBlaricom GR, DeMaster DP, Simões-Lopes PC, Moreira S, Bethlem C (2006) Satellite-monitored movements of humpback whales *Megaptera novaeangliae* in the Southwest Atlantic Ocean. *Mar. Ecol. Prog. Ser.,* **313**, 295-304.

Figure Legends

Figure 1. Map of the surveyed areas. Sampling sites comprise the (a) breeding ground off the Brazilian coast and the (b) Antarctic feeding area off South Georgia, in the Scotia Sea.

Figure 2. Allele frequency distribution in the Brazilian population of humpback whales. The bars represent the proportion of all alleles detected (only for the loci in HW equilibrium), in each allele frequency class calculated for the population sampled.

Figure 3. Summary plot of estimates of Q for five scenarios ($K = 2$ to $K = 6$). Each individual is represented by a single vertical line broken into K colored segments, with lenghts proportional to each of the K inferred clusters.
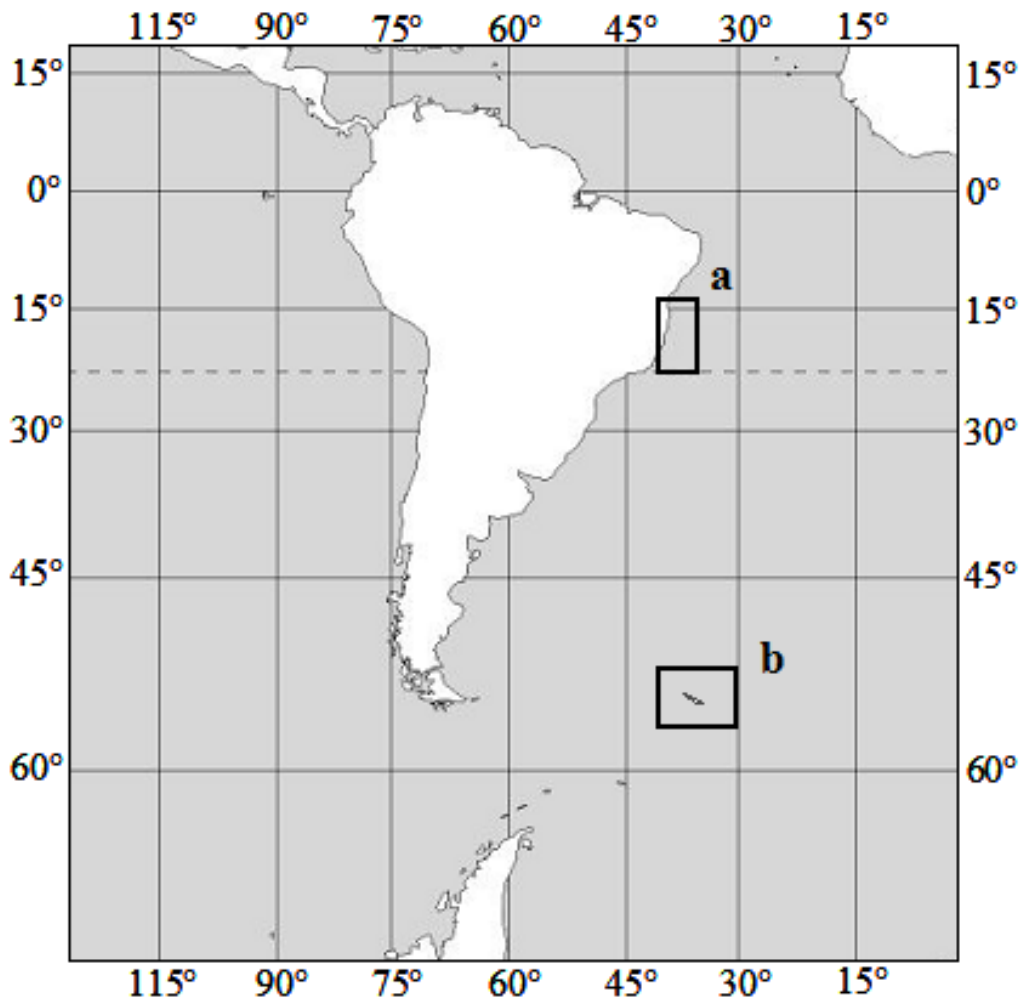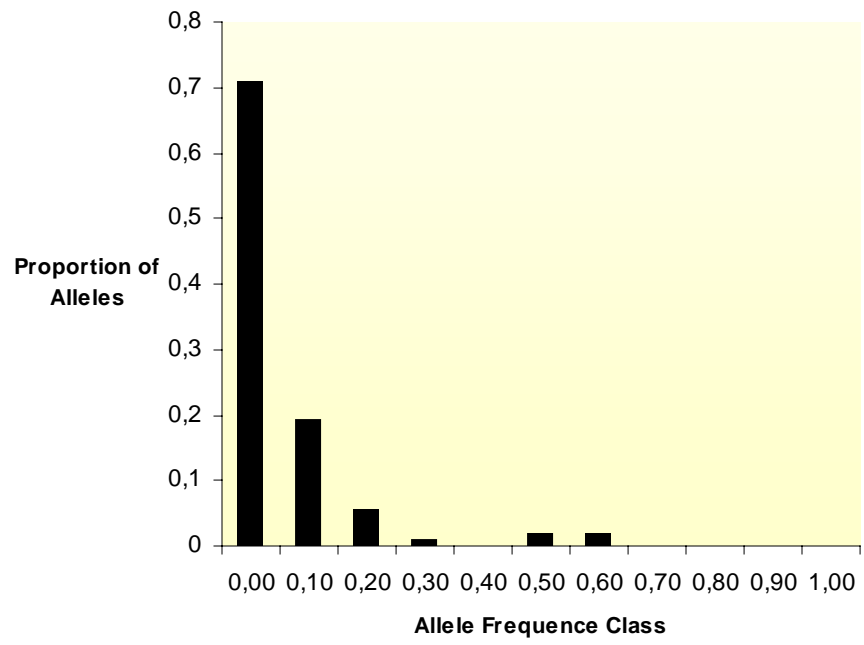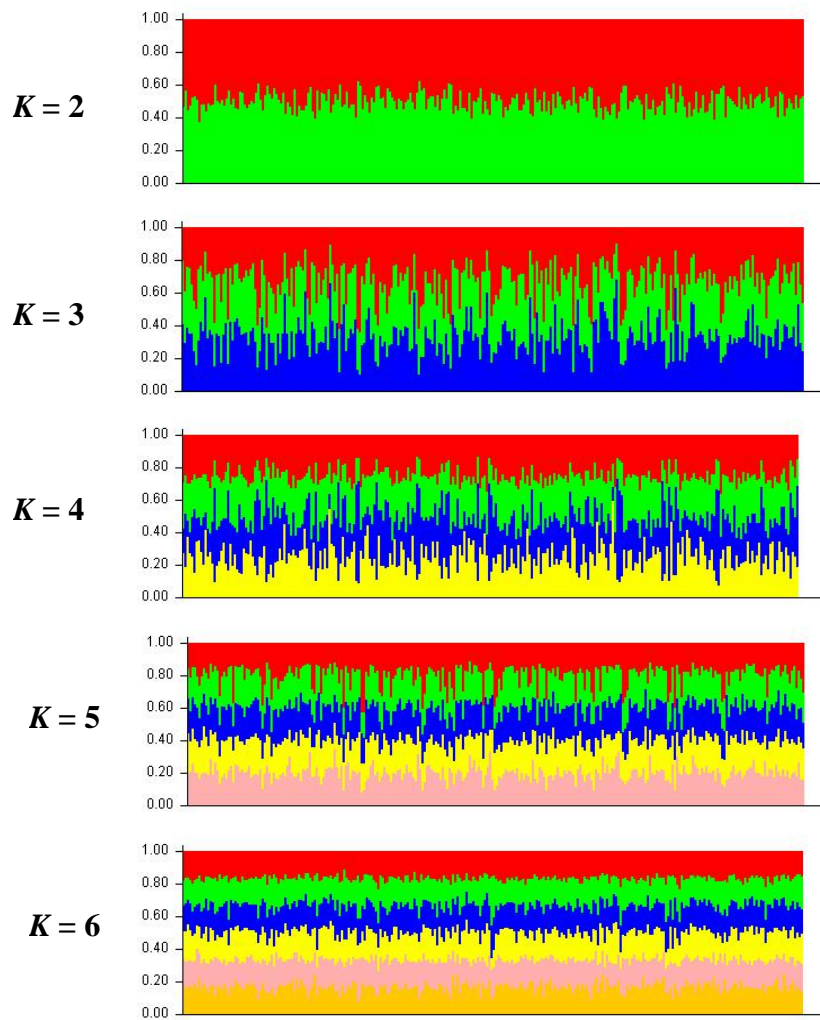
Figure 1

Figure 2

K = 2

K = 3

K = 4

K = 5

K = 6

Figure 3

Table 1. Summary statistics for ten microsatellite loci genotyped for humpback whale population of Brazil.

Locus	Rep	Allele range	K	H_O	H_E	F_{IS}
GATA 28	4	143-203	15	0.637	0.624	- 0.021
GATA 53	4	231-287	12	0.777	0.820	0.053
GATA 417	4	186-280	18	0.905	0.906	0.001
199/200	2	102-118	8	0.590	0.574	- 0.028
417/418	2	178-204	11	0.755	0.818	0.077
464/465	2	130-152	9	0.573	0.606	0.055
EV1Pm	2	123-129	4	0.527	0.519	- 0.017
EV37Mn	2	190-224	18	0.904	0.922	0.020
EV94Mn	2	201-221	11	0.813	0.815	0.002
EV96Mn	2	185-215	15	0.871	0.871	- 0.001

Rep, repeat motif length in base pairs; k, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient.

Table 2. AMOVA results for three alternative schemes of grouping different years.

	Among groups	Among populations within groups	Within populations
	F_{CT} (P) (% variation)	F_{SC} (P) (% variation)	F_{ST} (P) (% variation)
Alternating years			
1999, 2001 and 2003	0.0007 (0.68)	-0.0023 (0.95)	-0.0016 (0.92)
2000, 2002 and 2004	(0.07)	(-0.24)	(100.16)
Two following years			
1999 and 2000			
2001 and 2002	0.0009 (0.58)	-0.0028 (0.82)	-0.0019 (0.91)
2003 and 2004	(0.10)	(-0.29)	(100.19)
Three following years			
1999, 2000 and 2001	0.0023 (0.09)	-0.0035 (0.95)	-0.0011 (0.91)
2002, 2003 and 2004	(0.023)	(-0.35)	(100.11)

Table 3. Pairwise F_{ST} values between geographic locations.

	South Georgia	Praia do Forte
Praia do Forte	0.02137*	
Abrolhos Bank	0.02189*	-0.00241*

*P>0.05 based on 10,000 replications

Table 4. Results of the Bayesian analysis in program STRUCTURE. The posterior probability for each scenario and subsequent standard error are noted.

Scenarios	Estimated Ln Prob of Data	Standard error
$K = 1$	- 9791.4	± 7.4
$K = 2$	-9905.0	± 18.2
$K = 3$	-10167.8	± 29.7
$K = 4$	-10331.3	± 34.5
$K = 5$	-10649.8	± 42.8
$K = 6$	- 9954.7	± 20.0

Table 5. Number of individuals (*n*), groups (N), and associations (A) inclued in the genetic relatedness analysis for all group classes and sex combinations.

Association type	*n* (%)	N (%)	A (%)
F-F			
MCE	2 (2.6)	1 (2.7)	1 (2.5)
F-M			
MCE	20 (26.4)	10 (27.0)	10 (25.0)
CG	10 (13.2)	5 (13.5)	5 (12.5)
CGMC	8 (10.5)	4 (10.8)	4 (10.0)
AP	18 (23.7)	9 (24.4)	9 (22.5)
M-M			
CG	13 (17.1)	6 (16.2)	7 (17.5)
NCG	3 (3.9)	1 (2.7)	3 (7.5)
CGMC	2 (2.6)	1 (2.7)	1 (2.5)
TOTAL	76 (100)	37 (100)	40 (100)

F, female; M, male; MCE, mother-calf-escort; CG, competitive group; CGMC, competitive group with mother and calf; NCG, noncompetitive group; AP, adults pair.

Table 6. Observed mean relatedness (*r*) and standard
error for each class of sex within-group.

	Mean *r*	Standard error
F-M	-0.0721	±0.1310
M-M	-0.0682	±0.1030

Table 7. Putative parent-offspring or full-sibling relationships and their respective relatedness coefficients r_{QG} and r_{LR} calculated in KINSHIP 1.3.1 and IDENTIX respectively. Hypothesis I tested parent-offspring relationship against unrelated individuals; hypothesis II tested full-sibling relationship against unrelated individuals and hypothesis III tested parent-offspring against full-sibling relationship.

ID	Sex	r_{QG}	r_{LR}	Shared mtDNA haplotype	Hypothesis I	Hypothesis II	Hypothesis III
58/300	M-M	0.4321	0.45	Without	***	**	**
64/69	F-F	0.5582	0.53	Yes	***	***	*
64/188	F-M	0.4458	0.44	Without	***	***	**
99/128	F-F	0.5359	0.48	Yes	***	***	*
100/153	F-F	0.5518	0.55	Yes	***	***	**
119/327	M-M	0.8678	0.66	Without	***	***	N.S.
127/310	F-F	0.4023	0.41	Without	***	**	***
153/366	F-M	0.4813	0.42	Without	***	***	**
177/GS02	F-F	0.4067	0.64	Yes	***	***	**
195/197	F-F	0.4221	0.53	No	***	***	**
205-02/363	M-F	0.4210	0.5	Without	***	***	*
282/335	F-M	0.4045	0.4	Without	***	**	**
282/346	F-M	0.4254	0.41	Without	***	**	**
284/21	?-F	0.5899	0.64	Without	***	***	*
289/361	?-M	0.5835	0.48	Without	***	***	N.S.
302/327	F-M	0.4866	0.48	Without	***	**	*
321/10	F-M	0.4672	0.42	Without	**	**	N.S.

Yes: identical mtDNA haplotype; No: different mtDNA haplotype; Without: at least one mtDNA haploype absent.

* $P<0.05$, ** $P<0.01$ and *** $P<0.001$ based on 10 000 simulated pairs of individuals.

CONCLUSÃO

Os resultados obtidos no presente trabalho revelam uma elevada variabilidade genética nuclear na população de baleias jubarte do estoque reprodutivo A, que é semelhante às observadas em outras espécies de grandes cetáceos (Waldick *et al*., 2002; Bérubé *et al*., 1998; Kanda *et al*., 2006; Kanda *et al*., 2007) e às observadas em outros estoques reprodutivos de jubartes no Hemisfério Sul (Valsecchi *et al*., 2002; Garrigue *et al*., 2004; Pomilla & Rosenbaum, 2006). Essas observações corroboram as predições de que a caça comercial não reduziu suficientemente o tamanho das populações ou não durou gerações suficientes para significantemente reduzir a variabilidade genética dessas populações de baleias (Amos, 1996).

O nossos resultados não apresentaram evidência de estruturação temporal (ao longo dos anos) bem como geográfica, corroborando a hipótese de ser uma população homogênea. O presente trabalho concorda com estudos anteriores, notadamente os dados de DNA mitocondrial (Engel *et al*., *in press*), que sugerem a região das ilhas Geórgia do Sul/Sanduíche do Sul, como principal área de alimentação para essa população. Uma fêmea amostrada nas proximidades da ilha Geórgia do Sul e uma fêmea amostrada no banco dos Abrolhos apresentaram uma possível relação de mãe-filha, com um elevado coeficiente de parentesco e haplótipos compartilhados. No entanto, comparações genéticas entre as jubartes brasileiras e outras populações do Oceano Atlântico Sul serão de grande importância para testar esta hipótese.

Os resultados obtidos com relação às proporções sexuais e às análises de parentesco concordam com aqueles revelados em outras áreas de reprodução (Valsecchi *et al*., 2002, Cerchio *et al*., 2005; Pomilla & Rosenbaum, 2006), onde a proporção de machos e fêmeas na população foi de aproximadamente 1:1, com um pequeno excesso de machos, e a maioria das associações dentro dos grupos sociais foi de pares de machos e fêmeas em duplas ou em grandes grupos competitivos. Além disso, nenhuma relação de parentesco entre os indivíduos do mesmo grupo existe exceto aquela entre as fêmeas com filhotes.

As possíveis relações de pais e filhos encontradas neste estudo sugerem que os filhotes retornam ao seu estoque reprodutivo natal, corroborando a fidelidade maternal aos destinos migratórios. Isto fica mais evidente na provável relação de mãe e filha entre uma

fêmea amostrada no banco dos Abrolhos em 2001e uma fêmea amostrada nas proximidades da ilha Geórgia do Sul em 2006.

Os esforços voltados para a conservação da espécie devem focar na manutenção da alta diversidade genética dessa população de baleias jubarte através da continuação da proibição da caça, oferecendo assim estabilidade demográfica, além da proteção de seu habitat, no caso o banco dos Abrolhos. Além disso, é extremamente importante monitorar não somente o nível de diversidade genética, mas também, conhecer a história de vida da espécie. Então, os resultados obtidos deverão ser relacionados com informações disponíveis sobre a biologia e a ecologia desta espécie, contribuindo assim para a elaboração de estratégias adequadas para sua conservação.

REFERÊNCIAS BIBLIOGRÁFICAS

Alter, S.E.; Rynes, E.; Palumbi, S.R. 2007. DNA evidence for historic population size and past ecosystem impacts of gray whales. *Proceedings of National Academy of Sciences*, *USA*, **104** (38): 15162-15167.

Amos, B. 1996 Levels of genetic variability in cetacean populations have probably changed little as a result of human activities. *Report International Whaling Committee*, **46**: 657-658.

Andriolo, A.; Martins, C.C.A.; Engel, M.H.; Pizzorno, J.L.; Más-Rosa, S.; Freitas, A.; Morete, M.E.; Petta, C.B. & Kinas, P.G. 2006. The first aerial survey to estimate abundance of humpback whale (*Megaptera novaeangliae*) in the breeding ground off Brazil. *Journal of Cetacean Researche and Managenment*, **8** (3): 307-311.

Baker, C.S.; Slade, R.W.; Bannister, J.L.; Abernethy, R.B.; Weinrich, M.T.; Lien, J.; Urbán-Ramirez, J.; Corkeron, P.; Calmabokidis, J.; Vasquez, O. & Palumbi, S.R. 1994. Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. *Molecular Ecology*, **3**: 313-327.

Baker, C.S.; Flóres-Gonzáles, L.; Abernethy, B.; Rosenbaum, H.C.; Slade, R.W., Capella, J. & Bannister, J.L. 1998. Mitochondrial DNA Variation and Maternal Gene Flow among humpback whales of the Southern Hemisphere. *Marine Mammal Science,* **14** (4): 721-737.

Bérubé, M. & Palsboll, P.J. 1996. Identification of sex in cetaceans by multiplexing using ZFX and ZFY specific primers. *Molecular Ecology*, **5**: 283-287.

Bérube, M.; Aguilar, A.; Dendantos, D.; Larsen, F.; Di Sciara, G.N.; Sears, R.; Sigurjónsson, J.; Urban-R, J.; Palsboll, P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus, 1758): analysis of mitochondrial and nuclear loci. *Molecular Ecology*, **7**: 585-599.

Borge, T.; Bachmann, L; Bjornstard, G & Wiig, O. 2007. Genetic variation in Holocene bowhead whales from Svalbard. *Molecular Ecology*, **16**: 2223-2235.

Cerchio, S.; Jacbsen, J.K.; Cholewiak, D.M.; Falcone, E.A. & Merriwether, D.A. 2005. Paternity in humpback whales, *Megaptera novaeangliae*: assessing polygyny and skew in male reprodutive success. *Animal Behaviour*, **70**: 267-277

Clapham, P.J. 1993. Social organization of humpback whales on a North Atlantic feeding ground. *Sym. Zoo. Soc. Lon.*, **66**: 131-145.

Clapham, P.J. 1994. Maturational changes in patterns of association in male and female humpback whales, *Megaptera novaeangliae*. *Journal of Zoology of London*, **234**: 265-274.

Dawbin, W. 1966. The seasonal migratory cycle of the humpback whale. In: *Whales, Dolphins and Porpoises.* (ed. Norris KS), pp 145-170. University of California Press, Berkeley.

Engel, M.H.; Fagundes, N.J.R.; Rosenbaum, H.C.; Leslie, M.S.; Ott, P.H.; Schmitt, R.; Secchi, E.; Dalla Rosa, L. & Bonatto, S.L. (*in press*) Mitochondrial DNA diversity of the Southwestern Atlantic humpback whale (*Megaptera novaeangliae*) breeding area off Brazil, and the potential connections to Antartic feeding areas. *Conservation Genetics*.

Frankham, R.; Ballou, J.D. e Briscoe, D.A. 2002. *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, 617 pp.

Freitas, A.C.; Kinas, P.G.; Martins, C.C.A.; Engel, M.H. 2004. Abundance of humpback whales on the Abrolhos Bank wintering ground, Brazil. *Journal of Cetacean Research and Management*, **3**: 225-230.

Garrigue, C.; Dodemont, R.; Steel, D. & Baker, C.S. 2004. Organismal and 'gametic' capture-recapture using microsatellite genotyping confirm low abundance and reproductive autonomy of humpback whales on the wintering grounds of New Caledonia. *Marine Ecology Progress Series*, **274**: 251-262.

Glockner, D.A. 1983. Determining the sex of humpback whales (*Megaptera novaeangliae*) in their natural environment. *In* Behavior and communication of whales. *Edited by* R. Payne. *AAAS Sel. Symp.* **76**: 447-464.

IBAMA, 2001. *Mamíferos Aquáticos do Brasil: Plano de Ação*, versão 2, 2ª ed., Brasília.

International Whaling Commission 2005. Report of the Scientific Committee. Annex H. Report of the Sub-Committee on Other Southern Hemisphere Whale Stocks. *Journal Cetacean of Research and Management* (Suppl.) **7**: 235-46.

Kanda, N.; Goto, M.; Pastene, L.A. 2006. Genetic characterstics of Western North Pacific Sei whales, *Balaenoptera borealis*, as revealed by microsatellites. *Marine Biology*, **8**: 86-93.

Kanda, N.; Goto, M.; Kato, H.; McPhee, M.V.; Pastene, L.A. 2007. Population genetic structure of Bryde's whales (*Balaenoptera brydei*) at the inter-oceanic and trans-equatorial levels. *Conservation Genetics*, **8**: 853-864.

Mackintosh, N.A. 1965. *The stocks of whales*. Fishing News Books. London.

Martins, C.C.A.; Morete, M.E.; Engel, M.H.; Freitas, A.; Secchi, E.R.; Kinas, P.G. 2001. Aspects of habitat use patterns of humpback whales in the Abrolhos bank, Brazil, breeding ground. *Memoirs of the Queensland Museum*, **47**: 563-570.

Oliveira, E.J.; Pádua, J.G.; Zucchi, M.I.; Vencovsky, R. & Vieira, M.L.C. 2006. Origin, evolution and genome distribuition of microsatellites. *Genetics and Molecular Biology*, **29** (2): 294-307.

Palsboll, P.J.; Vader, A.; Bakke, I. & El-Gewely, M.R. 1992. Determination of gender in cetaceans by polymerase chain reaction. *Canadian Journal of Zoology*, **70**: 2166-2170.

Pomilla, C. & Rosenbaum, H.C. 2006. Estimates of relatedness in groups of humpback whales (*Megaptera novaeangliae*) on two wintering grounds of Southern Hemisphere. *Molecular Ecology*, **15**: 2541-2555.

Roman, J. & Palumbi, S. 2003. Whales before whaling in North Atlantic. *Science* **301**: 508-510.

Rooney, A.P.; Honeycutt, R.L.; Derr, J.N. 2001. Historical population size change of bowhead whales inferred from DNA sequence polymorphism data. *Evolution*, **55** (8): 1678-1685.

Schlötterer, C. 2004. The evolution of molecular markers just a matter of fashion? *Nature Reviews,* **5**: 63-69.

Slijper, E.J. 1962. *Whales*. Hutchinson, London.

Stevick, P.T.; Pacheco de Godoy, L.; McOsker, M.; Engel, M.H.; Allen, A. 2006. A note on the movement of a humpback whale from Abrolhos Bank, Brazil to South Georgia . *Journal of Cetacean Research and Management*, **8** (3): 297-300.

Tonnessen, J.N. & Johnsen, A.O. 1982. *The History of Modern Whaling.* University of California Press, Berkeley.

Waldick, R.C.; Kraus, S.; Brown, M.; White, B.N. 2002. Evaluating the effects of historic bottleneck events: an assessment of microsatellite variability in the endagered, North Atlantic right whale. *Molecular Ecology*, **11**: 2241-2249.

Valsecchi, E.; Hale, P.; Corkeron, P.; Amos, W. 2002. Social structure in migrating humpback whales (*Megaptera novaeangliae*). *Molecular Ecology*, **11**: 507-518.

Weinrich, M.T; Rosenbaum, H.C.; Baker, C.S.; Blackmer, A.L. & Whitehead, H. 2006. The influence of maternal lineages on social affiliations among humpback whales (*Megaptera novaeangliae*) on their feeding grounds in the southern Gulf of Maine. *Journal of Heredity*, **97**, 226-234.

Zerbini, A.N.; Andriolo, A.; Heide-Jorgensen, M.P.; Pizzorno, J.L.; Maia, Y.G.; Van Blaricon, G.R.; DeMaster, D.P.; Simões-Lopes, P.C.; Moreira, S. & Bethlem, C. 2006. Satellite-monitored movements of humpback whales, *Megaptera novaeangliae* in Southwest Atlantic Ocean. *Marine Ecology Progress Series*, **313**: 295-304.