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Estimating the abundance of humpback whales in New Caledonia using DNA genotyping and photo-identification

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ABSTRACT

The abundance of humpback whales in New Caledonia was estimated using a catalogue of 214 individuals identified by microsatellite genotyping, 217 individuals identified by fluke photographs and combined records of both, collected from 1995 and 2001. A small number of errors were detected (and corrected) in the photo-identification records and in the field and laboratory records of the genotypes. Estimates from the weighted mean of Petersen capture-recapture model ranged from 319 to 520 individuals. Two estimates from the combined genotypes and photo-identification records provided the greatest precision but were likely biased downward as only resights were included in dataset extensions. The estimate obtained from the genotype estimate (n = 520, CI: 366 – 674) was larger and less precise that the photo-identification estimated (n = 355, CI: 279-432). The genotype estimates of males and females (n = 278 and n = 248, respectively) were similar although the female estimate was less precise. We suggest that the effect of any heterogeneity in female migration is reduced by the long-term sampling records and the capture-recapture model used in the present study.

INTRODUCTION

Photo-identification of natural markings has been used extensively to estimate population abundance, reproductive rates and describe migratory movement of cetaceans (e.g. Hammond et al., 1990). In recent years, the development of genetic tools for sex and individual identification have provided information that can confirm and extend photo-identification records (e.g. Amos et al., 1993; Baker et al., 1991; Palsboll et al., 1997; Richards et al., 1994; Richards et al., 1996).

The waters of Oceania (south of the equator) include a number of known and suspected wintering grounds for humpback whales that feed in Areas V, VI and I of the Antarctic (i.e., Groups V, VI and I stocks). Whaling during the 20th century, including the illegal Soviet whaling, reduced these stocks to very low numbers. In the last several years, a number of projects have been initiated to assess the status of humpback whales in various parts of Oceania, including New Caledonia, Tonga, New Zealand, the Cook Islands and French Polynesia (Garrigue et al. in press). These projects have employed photo-identification and the collection of sloughed skin and biopsy samples to study the occurrence, distribution, behaviour, abundance, genetics and habitat use of humpbacks at each study site. Preliminary estimates of abundance are available for two regions based on capture-recapture analyses of photo-identification records: New Caledonia (approximately 314 in the year 2000, Garrigue et al., 2001) and Tonga (approximately 770 in the year 2000, Baker Donoghue and Constantine, 2001). In other regions (i.e. Cook Islands, Fiji, New Zealand, Samoa, Niue, French Polynesia) observers note from sighting density data that the abundance of humpback whales seems to be low (Garrigue et al., in press). This is in contrast with the increase in numbers exhibited in the western portion of Area V and in Areas III and IV (Bannister 1994; Best 1993; Bryden et al. 1990; Paterson et al. 1994; IWC 2000).

Here we present a comparison of capture-recapture estimates of abundance for New Caledonia using photo-identification, genotypes and combining the two. Our objective was to improve our understanding of the dynamics of this population and to compare the usefulness of combining microsatellite genotyping (e.g., DNA profiling) and photo-identification. For this purposes, sex identification and genotyping for 11 microsatellites was completed for 321 humpback whales skin samples collected in New Caledonia between 1995 and 2001.

MATERIAL AND METHODS

Data collection.

Small–boat surveys of humpback whales were conducted in New Caledonia (Fig.1) for two to three months each winter from 1995 to 2001 (see Garrigue et al, 2001). Individual humpback whales were identified by taking photographs of the unique markings on the ventral surface of their tail flukes (Katona et al., 1979). Tissue samples were collected using a crossbow and specially adapted bolt and dart (Lambertsen et al., 1994.) or by skimming water following an active surface behaviour (Clapham et al., 1993). Biopsy collection was combined with fluke photographs (photo-identification) when available (Garrigue and Greaves, 1999) but some biopsy samples were collected from whales that were not photo-identified, because these did not lift their flukes.

Laboratory analysis

A total of 321 genetic samples of humpback whales were collected in New Caledonia between 1995 and 2001 using a biopsy darting (n = 285) or sloughed skin (n = 36). The samples were preserved in ethanol 70% and frozen until extraction. DNA extraction with ProK digestion, phenol/chloroform extraction and ethanol precipitation followed the protocol described by Sambrook et al. (1989) as modified for small samples by Baker et al. (1991). A set of 11 fluorescent labelled microsatellites loci was standardised with collaborators (Anderson et al. 2001; Medrano et al. 2001) and amplified from most samples: 3 tetranucleotides (GATA28, GATA53, GATA417, Palsboll et al., 1997), 1 trinucleotide (TAA 31, Palsboll et al., 1997) and 6 dinucleotides (464/465, Schloterrer et al. 1991; and EV1, EV14,EV21, EV37, EV94, EV104, Valsecchi and Amos, 1996). Molecular identification of the sex was carried out using the SRY system and ZFX positive control described in Gilson and Syvanen, (1998).

The polymerase chain reaction (PCR) was carried out in 20µl volumes under the following conditions: PCRII 2µl, MgCl2 2µl, MgCl2 (1mM), 0.4µl of each oligonucleotide primer (4mM), 0.2µl of dNTP (0.2mM), 0.1µl of AmpliTaq or GoldTaq DNA polymerase (0.5u) and 1µl of working DNA extract. Temperature profiles were one initial cycle of 3 or 10' (for Taq Gold) at 94°C followed by 35 cycles each of 30'' at 94°C, 30'' at 50°C, 30'' at 72°C), then one final cycle of 10' at 72°C. All samples were amplified as individual reactions (i.e. PCR reactions were not multiplexed).

All PCR products were run on 1.6% agarose gels and visualised with EtBr. Intensity of the products was assessed by eye in preparation for ABI genotyping in two multiplexed sets of loci. The two multiplexed sets were as follows; Set 1 - GATA28 (FAM), GATA53 (TET), EV14 (FAM), EV104 (TET), and EV94 (FAM); Set2 - EV1 (HEX), EV21 (FAM) , EV37 (HEX), 464/465 (FAM), GATA417 (FAM), and TAA31 (TET). These sets were run for 3.5hrs on 6% acrylamide gels on an ABI373 automated sequencer with a TAMRA350 size standard (Applied Biosystems). Data collected by Genescan (Applied Biosystems) was analysed with Genotyper 2.5 (Applied Biosystems) and peaks were automatically assigned sizes by comparison with the size standard. Initially peaks were binned to the nearest whole bp. A standard set of samples was used to establish an "allelic ladder" to further assist in binning of alleles.

Individual identity

The level of variation was estimated as the number of alleles per locus and the expected heterozygosity. Two loci (GATA53 and EV14) gave uncertain resolution of allele sizes and were omitted from this analysis. The probability of identity for the other 9 loci was calculated following Paetkau and Strobeck (1994). This represents the probability that two unrelated animals have the same genotype at one single locus more by chance alone. The expected number of random matches calculated was adjusted for multiple comparisons following Palsboll (1999). Identity of the animals was inferred using Cervus 2.0 software (Marshall, 1998-2001).

Population estimate

Identification by genotyping and photographs was conducted independently and then compared. Then population abundance was estimated using the weighted mean of the Petersen estimate (Seber, 1982). For this, four data matrices were used. The first contains only information coming from photo-identification mark-recapture study. Only the good and medium quality photographs were used to assure individual distinctiveness (Friday et al., 2000; Perry et al., 1990). In this matrix the first identification of an individual by photograph constitutes the capture, and the followings photographic identification of this animal corresponds to the recapture. The second matrix contains only information coming from genotypes. In this matrix each genotype is treated as a mark and a recapture is recorded whenever an identical genotype is found in subsequent DNA samples. A third "genotype extended by photo-ID" matrix merges part of the information coming from the photo-identification matrix to the genotype matrix. The data coming from the photo-identification were add to the genetic matrix for all individuals for which photo-identification were combined with a genotype. For example a whale photo-identified and biopsied in year 1 and subsequently identified in year 3 only by photographs was enter in the genotype complemented by photo-identification matrix. Finally, a last matrix "photo-identification extended by genotype" was created. This merged the photo-identification matrix complemented by the recapture done only by genotype.

Population estimates were also calculated separately for each sex using the results of the molecular identification. In order to search for any preference of females for residence time in breeding grounds we sorted all the information where females were resighted by genotype or photo-ID. We then track the date from one sighting to another to estimate the calendar differences of sighting in between years.

RESULTS

Probability of identity

A total of 321 skin samples were analyses for the nine microsatellites loci. The number of alleles per locus varied from 4 (EV1) to 20 (EV37) (Table 1) and the observed level of heterozygosity ranged from 0.371 to 0.900 (Table 1). Average heterozygosity was 77% over the 9 loci. The loci did not show any deviations from the Hardy-Weinberg equilibrium. The probability of identity for each locus is given in Table 1. The combined probability of identity for the 9 microsatellite loci combined reached $2.5x10^{-10}$. For multiple comparisons of genotypes, the expected number of random matches is $1.3X10^{-5}$. Based on this low probability the samples with identical genotype were considered to be replicates of the same individual.

Comparisons of genotypes showed that the 321 samples were derived from 229 unique individuals (including calves), of which 175 were also photo-identified. Most of the replicates genotypes come from animals that were resighted in the same year or in different years (n = 58) and confirmed by photo-identification. By careful comparison of field records and laboratory replication seven field errors and six laboratory errors were discovered. Field errors included double sampling of individuals or inversion of samples by attribution of samples to the wrong animal. Laboratory errors included mislabelling or mixing of tubes during the extraction or pipe ting. 17 genetic samples collected without accompanying fluke photographs were linked by subsequent or previous sightings where both biopsies and photographs were collected. Five "missed" identification errors were discovered in the photo-identification catalogue (i.e., photographs previously considered to represent different individuals were found to be from the same individual). These missed identification were all white flukes of the Type I category and some involved showed substantial changes in marks (Figure 2). No "mis-identifications" were found in the photo-identification records (i.e., no photographs previously considered to represent the same individual were found to be from different individuals).

After correction for errors, the full photo-identification catalogue contained 250 individual whales, 175 of which were also genotyped. The full genetic catalogue of humpback whales contained 229 individual genotypes. For the purposes of the capture-recapture estimation, calves were then removed from both catalogues as were photographs judged to be of marginal quality. Finally, the photos taken before 1995 were removed from the catalogue as the biopsy collection was not initiated until that year. In total, the available records of non-calf individuals for population estimate included 217 for the photo-identification catalogue and 214 for the genetic catalogue (Table 3). The number of individual whales sighted and resighted by photo-identification and genotyping for each year of the survey is presented in Table 2.

Population estimate

The 259 genotype sightings were 64% males and 36% females. The 302 photo-identification sightings included 239 sightings of known sex with 59% males and 38% females (Table 3). After accounting for resights, a similar number of individuals of both sexes were identified by photo-identification (n = 217, 61% males and 39% females) and genotyping (n = 214, 62% males 38% females) during the seven-year study. Although the sex ratios were similar in the two sets of records, comparisons showed that not all individuals were equally available to the two methods. For example, 55 individuals were photo-identified but not biopsied and 54 individuals were biopsied but not photo-identified. In this last set, more than 25% of the animals were mother and calf pod.

The population abundance was estimated using the weighted mean of the Petersen model for the four matrixes. Results are presented in Table 3. The estimates of the New Caledonian population ranged from 319 to 520. The genotype estimation was larger and less precise (i.e., a larger CV) than the photo-identification estimate. The estimates based on genotyping and calculated by sex were comparable (males, n = 278; females n =248) despite the biased sex ratio of the sample.

To investigate potential capture heterogeneity due to differences in migratory arrival of individual females (e.g., Mattilla et al. 2001) we sorted all the information on females resighted by genetic or photo-identification. We end up with 22 individuals sighted between 2 and 5 times in different years. Fourteen sightings of nine females were mother and calf. With the exception of these, 34 sightings representing a total of 14 females were available to estimate the interval between the dates of first sightings in different years. The results indicate that sightings occurred few days apart between one year and the other (in 5 cases), in an interval of 1 to 2 weeks (in 3 cases), less than a month (4 cases) and between 1 and 2 months (5 cases).

DISCUSSION

The five missed identifications found in our catalogue, are considered as false negative errors (Stevick et al., 2001; Perry et al., 1990). They resulted from lack of distinctiveness as it was the case for some type I flukes (Friday et al., 2000), photographic quality or angle, and changes in markings over time (Carlson et Mayo, 1990). Changes in natural marks overtime can lead to unequal capture probabilities when natural marks are used in capture-recapture studies (Gowans et al., 2001). This change of appearance have also been documented in calves

that presents large colour changes in the ventral fluke pattern during the firsts few months of life (Carlson and Mayo, 1990). These errors had not been detected prior to the genotyping, despite careful review of the catalogue. However, the number of errors was small and would have introduced only a slight positive bias in the capture-recapture estimates (e.g., see Garrigue et al. 2001, for an uncorrected estimate). Similarly, a small number of errors in laboratory and field records of genotypes were detected by comparison with photographic records. Overall, our results support the general need for scrutiny in the application of both photo-identification and genotyping but do not support the suggestion that the predominately white flukes of humpback whales from Group V are an obstacle to robust photo-identification.

Our results suggest that somewhat different groups of whales are available for genotyping and photo-identification in New Caledonia. Cow/cal pairs are conspicuous members of a group with a reduced probability of identification by photo-identification. In our case most of the cow and calves were not available for photo-identification (except using underwater photo-ID) as they never fluke. However it was often possible to get skin samples from one or both members of a cow/calf pair. Genotyping also provided information on less conspicuous groups with differing probabilities of capture. This includes individuals that appear to be "en route". In the field, these animals follow a constant bearing, at a constant speed, and rarely lift their flukes. Further investigation is required to determine if these whales are members of the New Caledonia "breeding" stock or if they are in transit through to other regions.

Considering a two-year sampling from the Caribbean breeding grounds Palsboll et al. (1997) found a nearly two-fold difference between males and females estimates (4894 males; 2804 females). Mattila et al (2001) hypothesised that this difference could be due, in part, to a higher degree of preferences with respect to region and/or residence time on the breeding ground by females. Unlike the Caribbean estimate, our genetic population estimate for New Caledonia, based on a seven-year study, gave an adjusted sex ratio of 1.1 male to 1 female. This is comparable to observations by Chittleborough (1958, 1965) who found 52.8% males among 18,136 humpbacks taken from the Antarctic Area IV population. Further, our results do not indicate a strong preference in arrival time for the females in New Caledonia, although the set of data used in our analysis is small. We suggest that a long-term sampling design and the use of the weighted Petersen model (e.g., Baker et al. 1992) will reduce heterogeneity due to individual and sex-specific migration patterns. The relatively small population and the length of the field effort (covering most of the seasonal presence of humpbacks) in New Caledonia are also likely to have reduced capture heterogeneity.

A higher population estimate emerges from genetic approach with wider confidence limits compare to the use of classic photo-identification mark-recapture. Similar results have been reported previously (Pearse et al., 2001; Palsboll et al 1997). The two calculation combining datasets gave estimates comparable to the photo-identification estimate but with a narrower confidence interval. However, we are aware that the inclusion of only resighted individuals in the extended datasets will result in a negative bias in the estimate despite the apparent increase in precision. We suggest that further consideration should be given to analytical methods that combine datasets, taking advantage of the long-term photo-identification records available in some populations while using genotyping to correct for heterogeneity or sex biases in capture probabilities.

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Table 1. Sample size (N = individual whales) and diversity of microsatellites loci.

Loci	N	Observed heterozygosity	Expected heterozygozyty	Total number of alleles	PI
GATA28	229	0.533	0.549	9	0.221
GATA417	229	0.895	0.902	15	0.018
TAA31	229	0.860	0.870	14	0.031
464/465	229	0.629	0.639	6	0.186
EV1	229	0.520	0.530	4	0.284
EV21	229	0.703	0.673	6	0.146
EV37	229	0.900	0.929	20	0.010
EV94	227	0.855	0.784	10	0.061
EV104	229	0.371	0.356	5	0.407
Mean		0.696	0.768	9.9	

Table 2. Number of individual whales sighted and resighted for each year.

Years	Photo-identification sightings	Photo-identification resights	Genotype sighting	Genotype resights
1995	29	0	5	0
1996	50	9	37	0
1997	52	17	29	4
1998	49	17	43	9
1999	19	7	23	3
2000	38	15	42	10
2001	68	19	80	19

Table 3. Population estimate of New Caledonia humpback whales using weighted mean of the Petersen model based on photo-identification and genotyping.

Matrix Used	Number of individual whales	Total number of sightings	Total number of resights	Estimated abundance (95% CI)
Photographic	217	302	84	355 (279-432)
Only female	63	91	28	92 (57-127)
Only male)	101	151	50	139 (100-178)
Genotype	214	259	45	520 (366-674)
Only female	80	92	12	248 (101-394)
Only male	133	166	33	278 (182-374)
Genotype extended by photo-ID	214	305	88	319 (252-386)
Photographic extended by genotype	217	318	93	344 (274-415)

Figure 1. Location of New Caledonia.

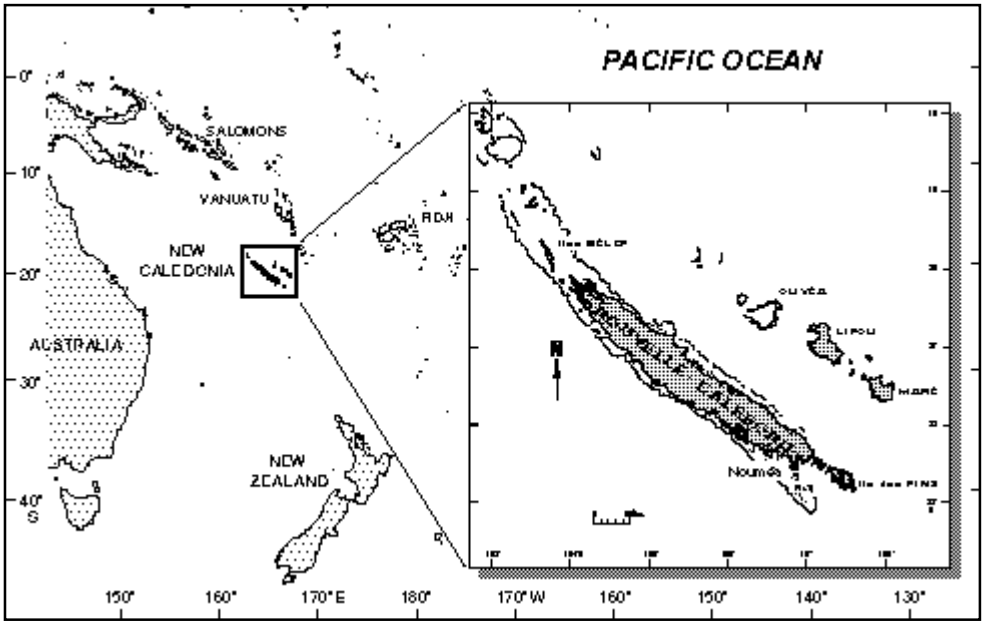

Figure 2. A missed identifification involving two photographs of the same individual whale, confirmed by genotyping.