FOR CONSIDERATION BY THE SCIENTIFIC COMMITTEE OF
THE INTERNATIONAL WHALING COMMISSION
BERLIN, GERMANY

Organismal and 'gametic' capture-recapture using microsatellite genotyping confirm demographic closure and reproductive autonomy of a humpback whales wintering ground

CLAIRE GARRIGUE1, 2, RÉMI DODEMONT1, DEBBIE STEEL2 AND C. SCOTT BAKER2
1 Opération Cétacés BP 12827 98802 Nouméa, New Caledonia
2 School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

ABSTRACT

The abundance and reproductive autonomy of humpback whales from the New Caledonia wintering grounds were investigated using capture-recapture models and paternity inference based on nuclear microsatellite genotyping, mitochondrial DNA sequencing and photographs of natural markings (photo-identification). The analyses included records of 213 individuals (excluding 16 calves used in paternity inference) identified by genotypes (9 loci), and 210 identified by fluke photographs collected from 1995 to 2001. Using the Chapman's correction of the Petersen two-sample model the estimate of abundance based on genotyping (N = 643, CV = 0.18) was slightly larger than the estimate based on photo-identification (N = 574, CV = 0.18). A sex-specific estimate of abundance based on genotypes gave comparable numbers of males and females although the variance of the female estimate was greater (N_m = 382, CV = 0.22; N_f = 239, CV = 0.29). The likely paternity of 5 calves from 16 cow/calf pairs was assigned to five individual males (one offspring each) from the total sample of 133 non-calf males. The number of assigned paternities provided an alternate 'gametic recapture' estimate of male abundance. This gametic recapture estimate (N_m = 379, CV = 0.30) was almost identical to the sex-specific estimate based on the organismal recapture using a two-sample model (N_m = 382, CV = 0.22). The close agreement of the organismal and gametic recapture estimates provided strong evidence that this humpback whale wintering ground represents an autonomous population unit that is relatively closed to demographic and reproductive interchange. The small size of this breeding unit highlights the slow recovery of some populations following intensive 20th century commercial and illegal Soviet whaling.

INTRODUCTION

Although photo-identification has proven effective for estimating population abundance and describing migratory movement of humpback whales and other cetaceans (e.g. Hammond et al. 1990), there are known limitations of this method. These include the potential for changes in natural markings (tag loss), the lack of distinctive markings for some individuals and sex-specific patterns of behavior that result in heterogeneity of capture probability. The development of genetic markers for sex determination, individual identification and parentage assignment has provided information that can extend, and in some cases replace, photo-identification or tagging (e.g., Amos et al. 1993, Kohn et al. 1999, Palsboll et al. 1997, Pearse et al. 2001, Richards et al. 1996, Taberlet et al. 1997).

Despite the potential for improvement of capture-recapture estimates through microsatellite profiling and sex identification, a central question often remains unanswered; what population or population unit is being estimated? The genetic structure of humpback whale populations is complex (Baker & Palumbi 1995, Baker & Medrano 2002) reflecting long-distance migration from summer feeding grounds to winter breeding grounds (Clapham 1996). In the North Pacific and North Atlantic, humpbacks show considerable maternal fidelity to migratory destinations, resulting in a segregation of mtDNA haplotypes on regional feeding grounds (Baker et al. 1990, 1993, Palsboll et al. 1995, Larsen et al. 1996). Abundance estimates from these feeding grounds are assumed to reflect relatively discrete, maternally directed units, although the geographic boundaries between these units are not clearly defined and, in some areas, probably shift in response to changes in prey availability. In all oceans, humpbacks congregate during winter months in shallow coastal or insular waters of tropical latitudes (Mackintosh 1965). Although these geographically isolated wintering grounds are assumed to represent breeding units or 'stocks', the evidence that mating occurs in these regions is indirect and the degree of reproductive isolation has not been established.

Here we used the genotypes of individual males in the population to identify the likely paternity of calves. Our purpose was to evaluate the reproductive autonomy of this wintering ground by comparing the gametic recapture estimate to the organismal recapture estimate. The combined use of photo-identification and microsatellite genotyping for individual identification and paternity assignment, provided an improved understanding of the mating system of humpback whales and dynamics of recovery in this depleted population.

MATERIAL AND METHODS

Field 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 from photographs of the unique markings on the ventral surface of their tail flukes (photo-identification, Katona et al. 1979). Small

samples of skin tissue were collected using a crossbow and specially adapted bolt and dart (Lambertsen 1987) or a small scoop net for skimming water following an active surface behavior (Clapham et al. 1993). When possible, skin tissue collection was combined with photo-identification (Garrigue & Greaves 1999).

Photo-identification catalogue

The complete photo-identification catalogue includes 250 individual whales identified primarily by fluke photographs and a small number identified by dorsal fins or lateral markings (Garrigue and Greaves, 1999). For the purposes of the present study photographs taken prior to initiation of biopsy samples 1995; individuals identified only by dorsal fins or lateral markings (N=3); and, calves were deleted from the catalogue. To assure accuracy of individual identification (Friday et al. 2000, Perry et al. 1990), the catalogue was further evaluated and photographs of marginal quality were discarded.

Molecular analysis

A total of 321 humpback whales were sampled genetically in New Caledonia between 1995 and 2001 using skin tissue collected with a biopsy dart (n = 285) or sloughed naturally from individuals following surface behavior (n = 36). The samples were preserved in 70% ethanol and stored at –20° C. 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. A set of 11 fluorescently labeled microsatellite loci was tested and standardized with collaborators (Anderson et al. 2001, Medrano et al. 2001): three tetranucleotides (GATA28, GATA53, GATA417, Palsboll et al. 1997b), one trinucleotide (TAA 31, Palsboll et al. 1997b) and seven dinucleotides (464/465, Schloterrer et al. 1991, and EV1, EV14, EV21, EV37, EV94, EV104, Valsecchi & Amos 1996). Molecular identification of the sex was carried out using the SRY system and ZFX positive control described by Gilson and Syvanen (1998). An approximately 550 base pair (bp) fragment of the 5' end of the mitochondrial (mt) DNA control region (D-loop) was amplified using primers light strand Dlp1.5 (tPro whale, 5'-TCACCCAAAGCTGRARTTCTA-3') and heavy strand Dlp5 (5'-CCATCGWGATGTCTTATTTAAGRGGAA-3') following Baker et al. (1996).

The polymerase chain reaction (PCR) was carried out in 20µl volumes under the following conditions: 2µl PCRII buffer, 2µl MgCl2, (1mM), 0.4µl of each oligonucleotide primer (4mM), 0.2µl of dNTP (0.2mM), 0.1µl of Ampli*Taq* or *Taq* Gold DNA polymerase (0.5u) and 1µl of working DNA extract (approximately 50 ng). 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).

Intensity of PCR products was visualized with agarose gel electrophoresis and EtBr staining in preparation for ABI genotyping in two multiplexed sets of loci. The two multiplexed sets were designed to avoid overlap in size ranges for each flourescently labeled primer; 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) were analyzed 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 base pair (bp) in length. As internal controls and to further assist in binning of alleles, a standard set of whales was used in all gels to provide an 'allelic ladder'. The amplified mtDNA control region fragment was sequenced on an ABI 377 or modified ABI 373 automated sequencer (Applied Biosystems Inc.) using BigDye™ Dye Terminator Chemistry.

Probability of 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 further analysis. The probability of identity for the other 9 loci was calculated following Paetkau and Strobeck (1994). This represents the average probability that two unrelated animals share the same genotype by chance alone. The expected number of random matches calculated was adjusted for multiple comparisons following Palsboll (1999). The distribution of alleles were analyzed by sex for each locus.

Paternity assignment

Replicate samples of individuals and potential paternity of calves were identified using the computer program CERVUS 2.0 (Marshall et al. 1998, Marshall 2001). Genotypes differing by a single allele were reviewed by eye and repeated if there was evidence of ambiguous sizing or allelic dropout (Waits et al. 2001).

To evaluate the statistical confidence of a paternity, the computer program CERVUS 2.0 (Marshall 2001) was used to calculate the ratio of the likelihood of paternity for a particular male to the likelihood of paternity for a randomly selected male in the population given the frequency of alleles at each locus. This is expressed as the natural log of the combined likelihood ratios across all (unlinked) loci (i.e., the LOD score). Paternity is then assigned to the non-excluded male that presented the highest LOD score (Marshall et al. 1998, Taylor et al. 2000). The inference of paternity was evaluated by calculation of a Delta value in CERVUS 2.0. This is the difference between the LOD score

of the most-likely male and the next most-likely male in the sample. A critical value of Delta was determined by simulations of parental and offspring genotypes based on the observed allele frequencies and other parameters (Marshall et al. 1998). The critical values of Delta at a 95% confidence level were derived from overlap in the distribution of values for true fathers and most likely non-fathers generated in the simulations. Paternity was assigned when the LOD of the potential father was large relative to the LOD of alternative males. Then the significance of delta values found in the paternity inference was tested against the critical value. In all but one case, the assumed mothers and inferred fathers had a matching allele at each of the 9 loci (i.e., there was no exclusion of these individuals). Individual males that had a mismatch at a single locus were reviewed and individual genotypes were carefully compared to assure accurate sizing. In all cases, the mother of a calf was documented by behavioral observations and confirmed using both genotyping and mtDNA sequences (C. Garrigue, unpublished data).

Organismal capture-recapture estimates

Identification by genotyping and photographs was conducted independently and then compared. Population abundance was estimated using Chapman's modification of the Petersen two-sample model based on comparison of the 2001 samples to all previous years (Seber 1982). This estimate is considered to be comparable to the gametic recapture estimate discussed below. The coefficient of variation (CV) and the symmetrical 95% confidence intervals were calculated following Begon (1979). Two data matrices were used for the organismal estimates. In the photo-identification matrix the first identification of an individual by photograph constitutes the capture, and the followings photographic identification of this animal corresponds to the recaptures. In the genotype and sex matrix each genotype is treated as a mark and a recapture is recorded whenever an identical genotype is found in subsequent biopsy samples. The genotype matrix was partitioned based on molecular sexing, allowing sex-specific estimates of abundance (e.g., Palsboll et al. 1997a).

Gametic capture-recapture estimates

An alternate estimate of male abundance was derived by 'gametic' capture-recapture analysis based on paternity assignment (e.g., Pearse et al. 2001). The total number of males genotyped across the seven-year study formed the first capture sample and the total number of calves genotyped formed the second capture. The number of calves with assigned paternities formed the gametic recapture of the males. An individual male first genotyped as a calf was excluded from the first capture sample but no attempt was made to exclude other genotyped males based on judgments of size or minimum age. The gametic capture-recapture estimate was based on Chapman's correction to the Petersen two-capture model,

$$N = [(n_1+1)\ (n_2+1)/(m+1)]-1,$$

where n_1 is the total sample of non-calf males, n_2 is the total number of calves sampled and m is the total number of calves with inferred paternity.

The intent of our gametic recapture analysis was not to estimate the effective size of the male population (i.e., male N_e), but to provide a comparison to the sex-specific organismal population estimate. We assumed that an agreement between these two estimates would be evidence of reproductive autonomy for the New Caledonia wintering grounds, i.e., the proportion of fathers captured by the paternity analysis was consistent with the estimated size of the local male population. Alternatively, a gametic recapture estimate that was larger that the organismal recapture estimate would be evidence that male reproduction reflected a larger population, perhaps including males from other wintering grounds. A smaller gametic estimate would evidence that reproductive males were over-represented in the regional sample. The distribution of the paternal alleles of the calves with unknown father were compared to the distribution of the alleles of the males for each locus in order to detect the origin of these unknown fathers.

RESULTS

Individual identification and distribution of the alleles in the population

Comparisons of genotypes showed that the 321 samples were derived from 229 unique individuals (including calves), of which 175 were also photo-identified. The records used for the purposes of the capture-recapture estimation of abundance contained 210 individuals for the photo-identification catalogue and 213 individuals for the genotype catalogue.There was a significant bias towards males in the number of individuals genotyped (n = 213, 62% males) and in the number of photo-identified individuals of known sex (n = 175, 63% males). Although the sex ratios were similar in the two sets of records, comparisons suggest that not all individuals were equally available for the two methods. For example, 45 individuals were photo-identified but not biopsied and 54 individuals were biopsied but not photo-identified. In this last set, 30% of the animals were mother/calf pods.

The distribution of alleles between males and females have been compared for each locus using a Khi² test. No significant difference were found due to sex. So we cannot conclude that males and females come from different populations.

Organismal recapture estimates

The Chapman's correction of the Petersen two-sample model using all individuals sighted from 1995-2000 as one sample and individuals sighted in 2001 as the second sample gave an abundance estimates of the New Caledonian population ranged from 574 to 643 with a CV of 0.18. The sex-specific estimates based on genotyping were 382 males and 239 females (Table 1).

Maternity confirmation

A total of 38 cow/calf pairs were sighted in the seven-year study including at least three females sighted with calves in more than one year. Of this total, 16 cow/calf pairs were biopsied and genotyped. Two of these included replicate sightings of a cow (one cow sampled three times with three different calves). Assumed maternity of the cow/calf was confirmed by sequencing the first 440 bp of the mtDNA control region and by microsatellite genotyping (Table 3). The 16 cow/calf pairs had matching mtDNA haplotypes (n = 12 unique haplotypes) and shared one or more alleles at each of the nine microsatellite loci.

Paternity inference

Simulations conducted with CERVUS provided critical values of delta considering a number of candidate males of 382 as estimated from the field data and a proportion of candidate males sampled of 0.35. These values were 2.66 in the strict confidence level of 95% and 1.48 in the relaxed confidence level of 80%. From the total genotype sample of 133 males, a likely father was identified for 5 of the 16 calves (Table 2), two at 95% confidence and the three others at 80% confidence as judged by the delta values. Four of the five males presented paternal genotypes compatible with one of the cow/calf pairs at all nine loci and each match was supported by moderate to high LOD scores (range, 5.45 – 7.47). The genotype of the fifth candidate father was supported by a moderately high LOD score (4.2) but did not account for a non-maternal allele at one of the nine loci (EV37). At this locus, the candidate father was a homozygote suggesting the possibility of a null allele in the inferred father. Alternately, the calf's unique allele could be a mutation. None of the inferred fathers shared a mtDNA haplotype with its matching cow/calf pair, excluding the possibility that an older full sibling, maternal half-sibling or other maternal relative was mistakenly inferred to be the father of a calf.

Gametic recapture and distribution of the paternal alleles

Using Chapman's correction to the Petersen model with the non-calf sample of 133 males as n_1, the 16 cow/calf pairs as n_2 and the 5 inferred paternities as m, the gametic recapture estimate of male abundance was 379 (CV = 0.30). This was essentially identical to the organismal two-sample estimate based on male genotypes (N = 382, CV = 0.22).

The distribution of the paternal alleles of the calves with unknown father were compared to the distribution of the alleles of the males. Results of the Khi² test gave no significant differences. Thus there was not strong evidence to conclude that the unknown father of these calves came from another population than the one identified in New Caledonia.

DISCUSSION

Demographic closure

The combined genotyping and photo-identification records provided strong support for the assumption that the New Caledonia wintering grounds are a significant demographic unit for conservation and management. Demographic closure was suggested previously by collaborative comparisons of photo-identification catalogues from wintering grounds throughout the South Pacific (Garrigue et al. 2000 and 2002). Although these comparisons demonstrated some movement of individuals from New Caledonia to eastern Australia, New Zealand and Tonga, the rate of this between-region interchange was low in comparison to the rate of within-region annual return (Garrigue et al. 2002). The precision of photo-identification and genotype capture-recapture estimates presented here provides further evidence of demographic closure.

Reproductive autonomy

Our combined use of organismal and gametic recapture methods also provided the first direct evidence of reproductive autonomy for a humpback whale wintering grounds in the Southern Hemisphere. The number of inferred paternities (31%) found among the New Caledonia males was consistent with the estimated proportion of sampled males (35% based on two-sample model) and, consequently, the gametic recapture estimate was essentially identical to the sex-specific organismal estimate. This conformed to the prediction that calves were fathered primarily by males in the local New Caledonia population, not by males from a larger, undefined population that might include other regional wintering grounds (e.g., eastern Australia, Fiji or Tonga). Even the calves with unknown father may have been fathered by males of the population that have not yet been genetically identified.

By combining organismal genotype captures and paternal gametic recaptures, we sought an unbiased estimate of total males for comparison to the direct sex-specific estimates (genotype and photo-identification). The organismal

genotypes used were collected from all encountered groups and, we assumed, represented a random sample of the population (excluding only the known calves sampled during the study).

Confidence in our gametic-recapture estimate, and in turn our conclusion of reproductive autonomy for the New Caledonia wintering ground, depended on confidence in our paternity assignment. Several characteristics of our data and analysis indicated that our inferences were robust. First, the LOD score was high (> 4.2) and the probability of non-exclusion was low ($< 2 \times 10^{-4}$) for each of the five assignments. In a retrospective assessment of paternity inference using CERVUS, Slate et al. (2000) considered an LOD of > 3.0 sufficient to confirm paternity in red deer (*Cervus elaphus*) on the Isle of Rum. Second, the number of loci was relatively large and the heterozygosity of most loci was high. In a simulation of paternity inference in a population of 500 individuals, Nielsen et al. (2001) found that the probability of a correct assignment was high using as few as five loci with 10 alleles of equal frequency or 10 loci with four alleles of equal frequency. Our use of nine loci with an average nearly 10 alleles and average heterozygosity of 0.768 was intermediate between these two scenarios. Third, our experimental error for genotyping was reduced by the collection of corresponding photo-identification records and visual review of the automated size binning implemented in GENOTYPER. As a result, we accepted only assignments that matched at all nine loci in four out of five of the inferred paternities. In the assignment involving a single missing paternal allele, we concluded that the mismatch was likely due to a null allele in the father (an apparent homozygote) or a mutation in the calf (Slate et al., 2000). Finally, we used mtDNA haplotypes of cow/calf pairs to exclude the possibility that an older full sib of the calf or other maternal relative was not mistakenly inferred to be the father. In all five cases, the mtDNA of the inferred father did not match that of the cow/calf pair.

Together with the evidence of demographic closure and maternal fidelity to migratory destinations (e.g., Baker and Medrano 2002), the evidence of reproductive autonomy emphasizes the potential vulnerability of subdivided populations to local extirpation (Clapham et al. 1999). Nearly four decades have passed since humpback whales in Oceania reached their lowest numbers, probably following the intensive unreported Soviet hunt in Antarctic waters during the summer of 1961/62 (Mikhalev 2000). Under realistic rates of potential increase, extrapolation backwards from current estimates of abundance based on either photo-identification or genotyping would suggest that the New Caledonia population was perilously close to extinction at that time. Humpbacks from other wintering grounds in Oceania were also exposed to the intensive hunting on the feeding grounds but, by chance, might have differed in the number of survivors. Even small differences in such low numbers of individuals surviving could help explain the variability now observed in abundance and rates of increase among stocks in the Southern Hemisphere.

ACKNOWLEDGEMENTS

Surveys of humpback whales in New Caledonia were made possible by contributions of Les Editions Catherine Ledru, and the Provinces Sud, North and Isles, Inco S.A. Partial support for the 1999, 2000 and 2001 research seasons was funded by International Fund for Animal Welfare (IFAW). We thank Mike Donoghue from the Department of Conservation of New Zealand for initiating and managing this funding. We thank Jacqui Greaves, Dominique Breitenstein and Veronique Ducreux and all the voluntary workers that helped in the field. Field research was carried out by 'Opération Cétacés' and laboratory analysis at the Laboratory of Molecular Ecology and Evolution, University of Auckland. Support for laboratory analysis was provided by the University of Auckland Research Council (to C.S. Baker and S. Lavery), the Conservation Action Fund (to C.S. Baker and M. Poole), the International Fund for Animal Welfare (to C.S. Baker) and the Marsden Fund of New Zealand (to C.S. Baker).

REFERENCES

Amos B, Schlotterer C, Tautz D (1993) Social structure of pilot whales revealed by analytical DNA profiling. Science 30:670-672

Anderson MJ, Hinton G, Paton D, Baverstock PR (2001) A model for integration of microsatellite genotyping with photographic identification of humpback whales. Mem Queensl Mus 47:451-458

Baker CS, Palumbi SR, Lambertsen RH, Weinrich MT, Calambokidis J, O'Brien SJ (1990) Influence of seasonal migration on the distribution of mitochondrial DNA haplotypes in humpback whales. Nature 344:238-240

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 world-wide population structure in humpback whales. Proc Natl Acad Sci USA 90:8239-8243

Baker CS, Palumbi SR (1995) Population structure, molecular systematics and forensic identification of whales and dolphins. In: Avise J, Hamrick JL (eds) Conservation genetics: Case histories from nature. Chapman and Hall, New York, p. 10-49

Baker CS, Cipriano F, and Palumbi SR (1996) Molecular genetic identification of whale and dolphin products from commercial markets in Korea and Japan. Molec Ecol 5:671-685

Baker CS, Medrano-Gonzalez L (2002) World-wide distribution and diversity of humpback whale mitochondrial DNA lineages. In: Pfeiffer CJ (ed) Cell and molecular biology of marine mammals. Krieger Publishing Co., Inc, Melbourne, FL

Begon M (1979) Investigating animal abundance: capture-recapture for biologist. Baltimore: University Park Press

Clapham PJ, Young SB, Brownell-Jr. RL (1999) Baleen whales: conservation issues and status of the most endangered populations. Mammal Rev 29:35-60

Clapham PJ, Palsboll PJ and Mattila DK (1993) High-energy behaviors in humpback whales as a source of sloughed skin for molecular analysis. Mar Mamm Sci 9 (2):213-220

Friday NA, Smith TD, Stevick PT, Allen J (2000) Measurement of photographic quality and individual distinctiveness for the photographic identification of humpback whales, *Megaptera novaeangliae*. Mar Mamm Sci 16 (2):355-374

Garrigue C, Aguayo A, Amante-Helweg VLU, Baker CS, Caballero S, Clapham P, Constantine R, Denkinger J, Donoghue M, Florez-Gonzalez L, Greaves J, Hauser N, Olavarria C, Pairoa C, Peckham H, Poole M (2002) Movements of humpback whales in Oceania, South Pacific. J Cetacean Res Manage 4 (3):255-260

Garrigue C, Forestell P, Greaves J, Gill P, Naessig P, Baker CS, Patenaude N (2000) Migratory movement of humpback whales (*Megaptera novaeangliae*) between New Caledonia, East Australia and New Zealand. J Cetacean Res Manage 2:111-115

Garrigue C, Greaves J, Chambellant M (2001) Characteristics of the humpback whale population at New Caledonia (South West Pacific). Mem Queensl Mus 47:539-546

Garrigue C, Greaves J (1999) New Caledonia, a rendezvous for the humpback whale. Ledru C (ed), New Caledonia, 160 p

Gilson A, Syvanen M (1998) Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains and hair forensic samples from California. California Fish and Game 84 (4):159-169

Hammond PS, Mizroch SA, Donovan GP (1990) Individual recognition of cetaceans : Use of photo-identification and others techniques to estimate population parameters Rep int Whal Commn (Special Issue 12)

Katona S, Baxter B, Brazier O, Kraus S, Perkins J Whitehead H (1979) Identification of humpback whales by fluke photographs. In Winn HE, Olla BL (eds) Behaviour of marine animals, Vol 3 Plenum Press, New York, p 33-44

Kohn MH, York EC, Kamradt DA, Haught G, Sauvajot RM, Wayne RK (1999) Estimating population size by genotyping faeces. Proc R Soc Lond B 266:657-663

Lambertsen RH (1987) A biopsy system for large whales and its use for cytogenetics. J Mammal 68:443-445

Larsen AH, Sigurjonsson J, Oien N, Vikingsson G, Palsboll P (1996) Population genetic analysis of nuclear and mitochondrial loci in skin biopsies collected from central and northeastern North Atlantic humpback whales (*Megaptera novaeangliae*): population identity and migratory destinations. Proc R Soc Lond B 263:1611-1618.

Mackintosh NA (1965) The stocks of whales. Fishing News (Books) Ltd, London

Marshall TC, Slate S, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molec Ecol 7:639-655

Marshall T (2001) CERVUS 2.0 software, University of Edinburgh, UK

Medrano-Gonzalez L Baker CS, Robles-Saavedra MR, Murrell J, Vazquez-Cuevas MJ, Congdon BC, Straley JM, Calambokidis J, Urban-Ramirez J, Florez-Gonzalez L, Olavarria-Barrera C., Aguayo-Lobo A, Nolasco-Soto J, Juarez-Salas RA, Villavicencio-Llamosas K (2001) Transoceanic population genetic structure of humpback whales in the North and South Pacific. Mem Queensl Mus 47:554

Mikhalev YA (2000) Biological characteristics of humpbacks taken in Antarctic Area V by the whaling fleets *Slava* and *Sovietskaya Ukraina*. Unpublished report (SC/52/IA12) to the Scientific Committee of the International Whaling Commission. Available from the International Whaling Commission, The Red House, 135 Station Road, Impington, Cambridge, CB4 9NP, UK

Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. Molec Ecol 3:465-480

Palsboll PJ (1999) Genetic tagging: contemporary molecular ecology. Biol J Linn Soc 68:3-22

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

Palsboll PJ, Bérubé M, Larsen AH, Jorgensen H (1997b) Primers for the amplification of tri- and tetrame microsatellite loci in baleen whales. Molec Ecol 6:893-895

Palsboll PJ, Allen J, Berubé M, Clapham PJ, Feddersen TP, Hammond PS, Hudson RR, Jorgesen H, Katona S, Larsen AH, Larsen F, Lien J, Mattila DK, Sigurjonsson J, Sears R, Smith T, Sponer R, Stevick P, Oien N(1997a) Genetic tagging of humpback whales. Nature 388:767-769

Pearse DE, Eckerman CM, Janzen FJ, Avise C (2001) A genetic analogue of 'mark-recapture' methods for estimating population size: an approach based on molecular parentage assessments. Molec Ecol 10:2711-2718

Perry A, Baker CS, Herman LM (1990) Population characteristics of individually identified humpback whales in the central and eastern North Pacific: a summary and critique. Rep int Whal Commn (Special Issue 12):307-318

Richards K R, Whitehead H, Wright J M. (1996) Polymorphic microsatellites from sperm whales and their use in the genetic identification of individual from naturally sloughed skin. Molec Ecol 5:313-315

Sambrok J, Fritsch EF, Maniatis T (1989) Molecular cloning : a laboratory manual, 2nd edition. Cold Spring Harbor Laboratory Press. New York

Seber GAF (1982) The estimation of animal abundance and related parameters. 2nd edition. Charles Griffin & Co., London. 654 pp

Slate J, Marshall T, Pemberton J (2000) A retrospective assessment of the accuracy of the paternity inference program CERVUS. Molec Ecol 9:801-808

Taberlet P, Waits LP, Luikart G (1997) Non-invasive genetic tracking of the endangered Pyrenean brown bear population. Molec Ecol 6:869-876

Taylor AC, Cowan PE, Fricke BL, Cooper DW (2000) Genetic analysis of the mating system of the common brustail possum (*Trichosurus vulpecula*) in New Zealand. Molec Ecol 9 :869-879

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

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions, guidelines. Molec Ecol 10:249-256

Table 1. Abundance estimate of New Caledonia humpback whales using Chapman's correction of the Petersen two-sample model based on photo-identification and genotyping in 2001 compared to total for all previous years.

Matrix Used	Individual whales sampled prior to 2001	Individual sampled in 2001	Total resightings	Estimated abundance, (95% CI) and *CV*
Photo-identification	162	66	18	574 (373-774) *0.18*
Genotype	152	79	18	643 (412-874) *0.18*
Only female	59	27	6	239 (104-374) *0.29*
Only male	93	52	12	382 (220-544) *0.22*

Table 2. The probability of non exclusion, the LOD score and delta value, for each of the five mother/calf/father trios (* relaxed confidence level, ** strict confidence level).

Calf-Mother-Father	probability of non exclusion	LOD	Delta
NI9908-HNC180-HNC040	$1.83\ 10^{-5}$	7.47	1.66*
NI0143-NI0142-HNC187	$1.74\ 10^{-4}$	5.88	2.56*
NI0144-HNC247-NI0131	$4.85\ 10^{-5}$	7.02	4.06**
HNC171-HNC113-HNC088	$1.69\ 10^{-4}$	5.44	3.21**
NI0020-HNC197-HNC167	$2.01\ 10^{-4}$	4.20	1.66*

Table 3. Genetic profile of the five mother/calf/candidate father trios (F:female, M:male).

ID	Status	Sex	mtDNA	464	GATA417	TAA31	EV1A	EV37	EV21	EV94	EV104	GATA28
NI9908	Calf	F	NC9709	149/149	187/214	106/112	123/127	206/214	113/117	208/216	149/149	147/147
HNC180	Mother	F	NC9709	143/149	214/222	106/112	123/127	212/214	111/117	208/210	149/149	147/147
HNC040	Father	M	NC9601	143/149	187/214	106/109	123/127	202/206	113/117	212/216	149/149	147/147
NI0143	Calf	F	NC9505	139/149	195/199	88/103	123/127	194/204	109/111	212/216	147/149	147/187
NI0142	mother	F	NC9505	143/149	199/226	88/103	123/127	192/204	109/111	214/216	147/153	147/183
HNC187	father	M	NC0034	137/139	195/199	88/103	123/127	194/194	109/111	212/220	149/149	147/187
NI0144	calf	F	NC9608	139/141	222/226	97/106	123/123	192/194	109/111	212/218	149/149	147/179
HNC247	mother	F	NC9608	139/139	222/222	106/115	123/123	192/198	111/111	212/218	149/149	179/183
NI0131	father	M	NC9504	141/143	203/226	97/103	123/123	194/214	109/115	212/216	149/149	147/147
HNC171	calf	M	NC9713	137/139	199/207	97/100	123/127	198/202	109/111	212/214	149/149	175/187
HNC113	mother	F	NC9713	137/139	199/199	100/106	123/127	198/212	111/111	212/214	149/149	147/175
HNC088	father	M	NC9907	131/139	187/207	97/100	123/123	202/208	109/115	214/216	149/149	147/187
NI0020	calf	M	NC9805	139/139	199/195	97/103	123/127	198/218	109/111	206/208	149/149	147/155
HNC197	mother	F	NC9805	139/139	199/195	97/103	123/127	198/214	109/117	208/208	149/151	147/147
HNC167	father	M	NC9841	139/139	207/199	97/115	123/127	210/210	111/115	206/218	149/149	147/155

Figure 1. Map of New Caledonia and location of primary study site.