Sloughed skin: a method for the systematic collection of tissue samples from Baja California blue whales

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ABSTRACT

The frequency of occurrence of naturally sloughed skin was investigated to verify the feasibility of this method to study blue whale genetics off Baja California. Sloughed skin was recorded in 97% of 337 surfacing intervals with blue whales, *Balaenoptera musculus*, along the Baja California peninsula, Mexico. No significant difference ($P > 0.05$) was found in size of pieces of skin sloughed from whales in different habitats, sea surface temperatures or whether they were alone or in pairs. Samples were recoverable independent of gender and age and could be linked to individuals. While yield of extracted DNA was low (0-0.15μg/mg tissue), gender determination was successful in 55% of the samples assayed.

KEYWORDS: SAMPLING STRATEGY; GENETICS; BLUE WHALE

INTRODUCTION

Non-invasive genetic sampling methods are widely used in free-ranging animals to address questions that would be difficult to answer using conventional methods that require the catching or handling of animals (Taberlet *et al.*, 1999). In free-ranging cetacean populations, the non-invasive method of collecting sloughed skin has been used with sperm whales, *Physeter macrocephalus* (Amos *et al.*, 1992; Richard *et al.*, 1996) and humpback whales, *Megaptera novaeangliae* (Clapham *et al.*, 1993; Valsecchi *et al.*, 1998). These studies demonstrated that sloughed skin samples provided DNA of sufficient quality and quantity for successful genetic analysis. However, there are two important limitations. First, a bias was noted toward sampling animals engaged in active behaviour. Only 20% of sperm whales and 0% humpback whales sloughed skin while not engaged in aerial or social behaviours (Whitehead *et al.*, 1990; Clapham *et al.*, 1993). Second, it is difficult to link sloughed skin to individuals in the field. Of more than 1,800 sloughed skin samples from humpback whales in Hawaii, only 11% were linked to individuals in the field (Valsecchi *et al.*, 1998).

Skin sloughing by the blue whale, *Balaenoptera musculus* has been reported in the North Atlantic (Sears *et al.*, 1990) and off Baja California, Mexico (Gendron and Zavala-Hernández, 1995), where desquamation was primarily observed by females accompanied by a calf. This paper examines the feasibility of sampling naturally sloughed skin from blue whales off Baja California in different habitats (inshore *vs* offshore, high *vs* low sea surface temperature) and from individuals of different age and sex classes (females with calves *vs* solitary animals). Measures of the quantity of DNA extracted and the success rate of gender determination are presented.

METHODS

During 1996 and 1997, blue whales were observed in five different habitats at various seasons in the waters off Baja California (Fig. 1). During multiple surfacing intervals of each blue whale, both sides of the whale and the ventral side of the flukes were photographed for photo-identification (Sears *et al.*, 1990), and then the slicks, visible from ca. 200m, were examined for the presence or absence of sloughed skin.

Sloughed skin samples were collected with a 30×20cm net (mesh size: 500μm to 1mm) attached to a 2.5m handle within the top meter of water in the slick left along the whale track. Pieces of skin were removed from the dipnet using stainless steel tweezers and preserved in 20% dimethysulfoxide (DMSO) saturated with table salt (Amos and Hoelzel, 1991). If no DMSO was available, the skin was placed in saturated salt solution and kept cool (1 to 3 weeks) until transfer into the DMSO solution. The dipnet was cleaned with seawater after each sample was collected (see Clapham *et al.*, 1993) and the tweezers were wiped with clean tissue.

Total genomic DNA was isolated from 47 of the sloughed skin samples. DNA extraction was performed on 10-200mg of tissue using either a lithium chloride procedure (Gemmel, 1996) ($n = 17$ samples) or a Bio 101 FastDNA® commercial kit ($n = 30$ samples). All 47 samples were typed for gender using the polymerase chain reaction (PCR) for multiplex amplification of the sex chromosome specific regions ZFX/ZFY and SRY (Fain and LeMay, 1995). Fifteen samples were also typed using amplification of SRY (modified from Fain and LeMay, 1995). DNA concentration was estimated by UV absorbance in a Perkin-Elmer Lambda 3B spectrophotometer.

Sea surface temperature data for 1996-1997 were obtained from the US National Oceanic and Atmospheric Administration.

RESULTS

In contrast with the sloughing of skin from humpback and sperm whales, which was linked with aerial or social behaviour (Amos *et al.*, 1992; Clapham *et al.*, 1993; Valsecchi *et al.*, 1998), blue whales were found to naturally slough skin when sounding. Social or active behaviour at the surface was seldom observed in this study although underwater contact could have occurred between females and their calves or between pairs of individuals swimming in close proximity.

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The collected sloughed skin was light grey in colour and easily observed in the water. Most often, the pieces were detected as they moved up and down in a circular pattern caused by turbulence in the last slick produced by a sounding whale. Less often, smaller pieces were detected floating at the sea surface. Three to four minutes after the whale had dived, the skin tended to sink. For this reason, it is unlikely that skin observed in a whale's slick could have come from a different animal.

A total of 139 blue whales were followed to test for the presence/absence of sloughed skin in three coastal and two offshore areas (Fig. 1, Table 1). The frequency of occurrence of sloughed skin from blue whales was high as all individuals followed were seen to slough skin, except one. Across study sites, 81.8% to 100% of surfacing intervals revealed sloughed skin. Overall, sloughed skin was present in 328 of 337 surfacing intervals. Sampling sloughed skin from individual blue whales appears to be easier than obtaining photographs suitable for individual identification; of the 139 whales followed, 128 of 139 were sloughed-skin sampled and 111 of 139 were photo-identified (Table 1). In some surfacing intervals, sloughed skin was observed in the slicks but could not be recovered because the skin fragments were already too deep and/or good photographs could not be taken before the whale would have been lost.

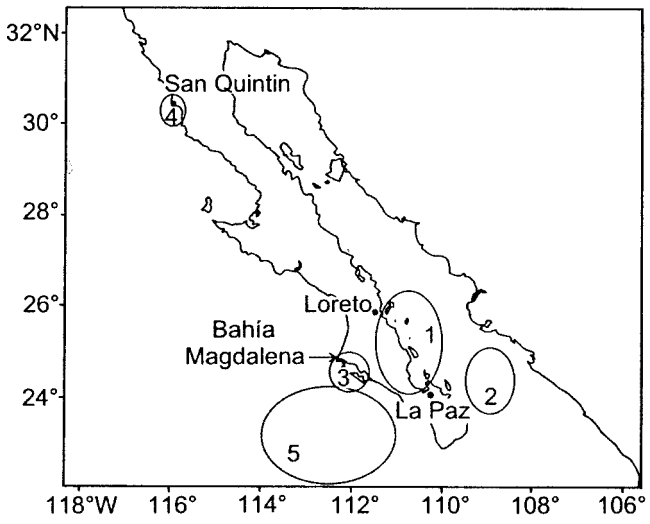

Fig. 1. Map of the study area showing the different habitats where blue whale sloughed skin was collected during 1996 and 1997: (1) coastal waters between Loreto and La Paz; (2) offshore area of southern Gulf of California; (3) coastal waters off Bahía Magdalena; (4) coastal waters off San Quintin and (5) offshore area along the southwestern coast of Baja California.

The size of the pieces of sloughed skin tissue collected varied from 5mm^2 to 50mm^2. The skin samples were classified qualitatively into two size categories: (1) skin of < 10mm^2 and (2) skin of > 10mm^2. No significant difference (Table 2) was found in the size of the pieces of sloughed skin collected from high (> 20°C) and low (< 20°C) sea surface temperatures ($\chi^2 = 2.10$, $P = 0.15$), from coastal or offshore habitats ($\chi^2 = 0.32$, $P = 0.57$) or from single individuals or females with a calf ($\chi^2 = 0.41$, $P = 0.52$). The contingency tables included expected average frequencies of at least 6.0 for $a = 0.05$ (Zar, 1996). These results indicate that sampling is not positively biased toward females with calves as was expected from the visible desquamation noted earlier (Gendron and Zavala-Hernández, 1995).

In contrast to humpback and sperm whales, which are thought to lose skin more frequently in tropical waters, blue whales off Baja California sloughed skin independently of season and sea surface temperature. However, a tendency for desquamation to decrease in cooler water was noted ($P = 0.15$). Off San Quintin (Fig. 1), where sea surface temperature ranged from 17-18°C, the lowest recorded in this study, sloughed skin was observed 81.8% of the time (9/11), less often than at other sites.

While the quantity of extracted DNA from the sloughed skin was low (mean = 0.05 μg/mg tissue; range = 0-0.15 μg/mg tissue) and there was less efficiency with the smaller pieces of tissue, extracted DNA was sufficient for gender determination in 55% (26/47) of the samples assayed. Preliminary gender results concur completely with field observations of known females ($n = 8$).

DISCUSSION

There are positive and negative aspects to sampling sloughed skin from Baja California blue whales. On the positive side, the sampling equipment needed is minimal and little skill, in calm sea conditions, is required. Samples are recoverable over 95% of the time and these samples can be linked to individual whales in the field (comparative analysis between dart-skin biopsy *vs* sloughed skin is currently underway). The systematic search for skin within slicks also raised our

Table 1

Blue whale sloughed skin study sites. Sample locations, habitat, date, sea surface temperature (SST), the number of blue whales studied, skin sampled, photo-identified, and number of cow-calf pairs at each site. Most animals were followed for at least one, and often for a series of, sequential surfacing intervals. The number of surfacing intervals in which sloughed skin was observed (+), the number of surfacing intervals in which no sloughed skin was observed (-) and the percent of surfacing intervals with sloughed skin.

Site	Habitat	Date	SST (°C)	No. blue whales studied	No. blue whales skin sampled	No. blue whales photo-ID'd	No. cow-calf pairs	No. surfacing intervals with sloughed skin (+)	No. surfacing intervals without sloughed skin (-)	% surfacing intervals with sloughed skin
1. Loreto-La Paz	Coastal	Feb.-Mar. 1996	20-21	30	30	30	2	135	1	99.3
1. Loreto-La Paz	Coastal	Feb.-Apr. 1997	19-20	61	56	43	5	103	4	96.3
2. Southern Gulf	Offshore	Mar. 1997	20-23	23	19	17	0	42	2	95.5
3. B. Magdalena	Coastal	Apr. 1996	19	12	12	10	2	18	0	100.0
4. San Quintin	Coastal	Jun. 1996	17-18	6	6	6	1	9	2	81.8
5. SW Baja coast	Offshore	Aug. 1996	25-28	2	1	1	1	3	0	100.0
5. SW Baja coast	Offshore	Oct. 1996	24-29	2	2	2	0	9	0	100.0
5. SW Baja coast	Offshore	Jun. 1997	20-26	3	2	2	0	9	0	100.0
Total				139	128	111	11	328	9	97.3

Table 2

Size of sloughed skin samples in relation to group category (single animal/cow-calf), habitat (coastal/offshore) and sea surface temperature (SST; high = >20º, low = <20ºC).

Samples	Size categories		Results
	<10mm^2	>10mm^2	
Single	31	60	$\chi^2 = 0.41$ ($P = 0.52$)
Cow-calf	6	8	
Coastal	37	68	$\chi^2 = 0.32$ ($P = 0.57$)
Offshore	7	17	
High SST	36	75	$\chi^2 = 2.10$ ($P = 0.15$)
Low SST	9	9	

chance of detecting and collecting faeces, which subsequently increased our data on prey species. On the negative side, we initially found it difficult to separately sample a female accompanied by a calf, when they both dived in close proximity. The pair formed one large slick and thus there was a high probability of mixing skin from both individuals. However, it was possible to sample most calves and females separately by waiting for the times when the calf surfaced alone before the female resurfaced. Another inconvenience was that searching for skin in the water requires time (three to four minutes, generally), which sometimes affected our ability to track the whale's heading while underwater. Finally, it must be stressed that the quantity of DNA that can be extracted from some pieces of sloughed skin is low and this may increase the rate of genotyping errors (Taberlet *et al.*, 1999). The cellular composition of the sloughed skin of blue whales may explain the low yield of DNA. The pieces of blue whale sloughed skin that we examined usually contained only cells derived from the external layer of the epidermis, which has the lowest concentration of nucleic acids (Gendron and Mesnick, 1999).

The collection of sloughed skin from surfacing individuals was found to be a successful method for systematically obtaining tissue samples for gender determination of individual blue whales off Baja California, where more active behaviour is rarely observed. The collection of sloughed skin may also be a useful method for obtaining skin samples from the other large whale species that do not perform aerial behaviour. Further investigation of the possible effect of temperature on desquamation frequency should be undertaken with larger sample sizes to see if this is an important factor. Similarly, water clarity might be an important factor to consider in regions where cloud cover might affect the ability to find the sloughed skin in the water.

There is a trade off, between the quantity of samples that can be obtained by the collection of sloughed skin and the quality of samples that can be obtained *via* directed biopsy darting. Combined use of both techniques may provide the most possible data with the least possible impact on the animals.

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