

## OBTAINING SKIN SAMPLES FROM LIVING SPERM WHALES

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### ABSTRACT

Samples of sperm whale skin, useful for modern molecular analyses of DNA, can be obtained from living animals either by collecting skin sloughed naturally by the whales, or by using biopsy darts fired from crossbows or compound bows. Sloughed skin was found frequently in warm waters, and particular samples could often be linked to photographs which enabled individuals to be identified. However, sloughed skin seemed less available at higher latitudes. Two types of darts were found to collect skin but collected samples were very small ( $<4 \text{ mm}^2$ ) and insufficient for repeated DNA fingerprinting analyses. Sperm whales always reacted to darting by "startling" and showing changes of behavior over the next few minutes, but we found no indications of longer-term effects. In warm water studies, collection of sloughed skin seems to be generally effective, but for samples of sperm whale tissue at high latitudes modifications could probably be made to either of the darts in order to obtain larger-sized samples.

Key words: sperm whale, *Physeter*, skin, tissue sample, biopsy, sloughed skin.

New techniques in molecular biology allow important information on the identity, sex, relatedness, and stock identity of individual animals to be obtained from very small tissue samples (*e.g.*, Jeffreys *et al.* 1985a, 1985b, Burke and Bruford 1987, Hill 1987, Thomas *et al.* 1987, Mathews *et al.* 1988). Such samples can be obtained from unrestrained living whales using biopsy darts (Winn *et al.* 1973, Mathews 1986, Hoelzel and Amos 1988, Mathews *et al.* 1988) or by collecting skin sloughed by the whales. Here we report on some preliminary investigations of techniques for collecting skin samples from living sperm whales, *Physeter macrocephalus*.

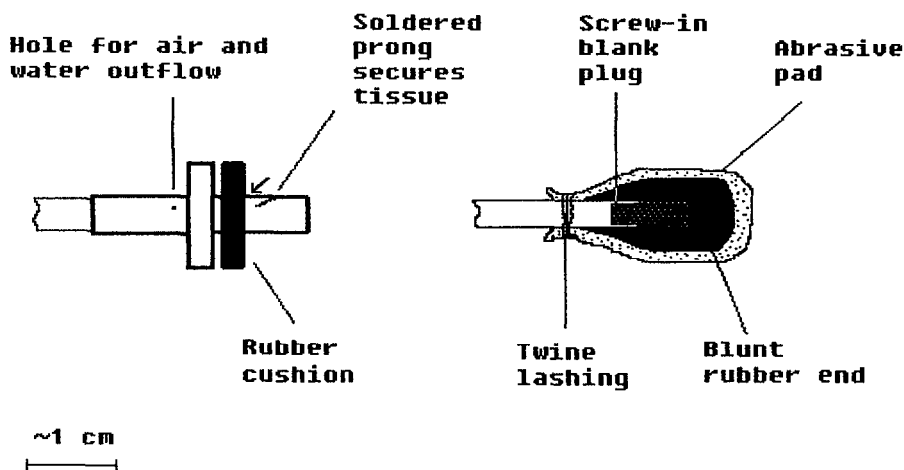


Figure 1. Standard-type biopsy (left) and glancing-type (right) darts. An arrow indicates the area in which skin was actually collected.

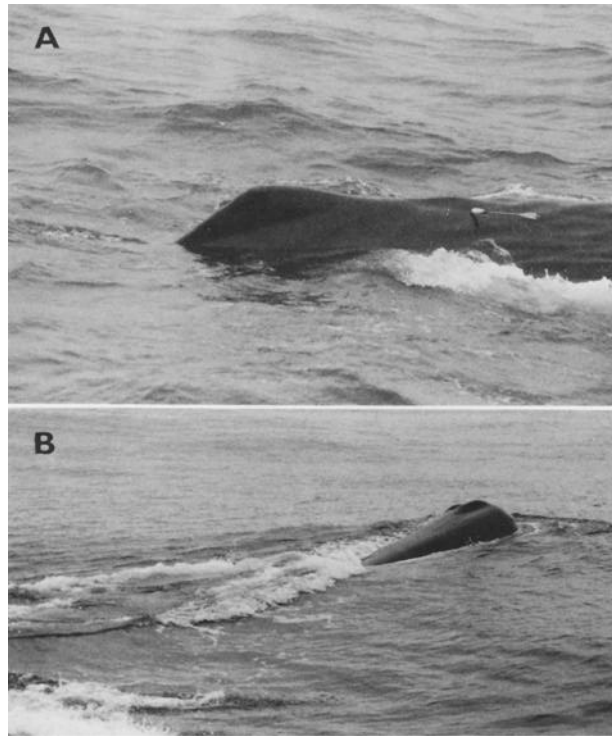
#### MATERIALS AND METHODS

Our research at sea was carried out from a 10-m auxiliary sailing yacht off Nova Scotia (44°N 59°W) during July and August 1988 (E.A.M. and H.W.), and off the Galápagos Islands, Ecuador, (0°S 91°W) during April and May 1989 (K.R.R. and H.W.), and from a 14-m auxiliary sailing yacht around the Azores Islands (39°N 28°W) during the summers of 1987 and 1988 (J.G.).

During our studies sloughed skin was seen in the wake of sperm whales. The skin was collected either using a dip net from the deck of the primary research vessel or from a dinghy, or, usually more effectively, by snorkellers. Restrictable DNA has been obtained from some of these samples of sloughed skin (W. Amos, personal communication).

During the 1988 study off Nova Scotia, we attempted to obtain skin samples using a standard biopsy dart. The dart, basically a hollow stainless steel cylinder with diameter 6 mm and depth to flange of 10 mm (Fig. 1), was that which was used successfully in obtaining skin and blubber samples from gray whales, *Eschrichtius robustus* (Mathews *et al.* 1988), and humpback whales, *Megaptera novaeangliae* (Mathews 1986). It was mounted at the tip of a bolt and fired from a Barnett Wildcat XL crossbow with a 50 lb draw. The dart was sterilized in alcohol before firing. The dart was retrieved using a fishing line and reel attached to the crossbow (Lambertson 1987). We approached the sperm whales from behind and attempted to position the archer, at the bow of the sloop, abreast and about 15 m from the region of the sperm whale's dorsal fin. The dart was aimed to hit the whale on its flank below the dorsal fin (Fig. 2A).

During the 1988 Azores study attempts were made to obtain skin samples using a new type of dart which was intended to glance off the sperm whale's body and scrape off a small amount of epidermis with an abrasive pad. (This design was prompted by observations of the nature of sperm whale skin made



*Figure 2.* (A) A standard-type biopsy dart in a sperm whale. (B) A sperm whale “startling” after a biopsy attempt.

by J.G. at Azorean whaling factories. The epidermis of these dead sperm whales is very soft. Local people often scraped off the epidermis with a blunt scraper and used the black paste as fishing “chum.”)

The dart consisted of a standard glassfibre arrow with four fletches (which can be used either way up and thus can be fired more quickly in succession) which was fitted with a blunt end covered with a rounded rubber head (Fig. 1). Nylon pan-scouring material (sterilized by boiling in a pressure cooker for about 15 min) was tied tightly over the rubber arrowhead. Arrows were fired from a compound bow (Barnett Safari) which had a 50 lb draw. No retrieval system was used. Arrows floated without any additional buoyancy and were retrieved with a dip-net or by swimmers after sampling attempts. This allowed several arrows to be fired in quick succession if necessary. Since whales were always approached from behind, the archer could aim at most of the exposed fore part of the whale’s body. Samples were taken from the upper part of the body, usually close to the dorsal fin. If a dart hit a whale its abrasive pad was removed and examined. Portions which had skin embedded were cut out and preserved. The rest of the pad was discarded.

During most collection attempts we monitored the behavior of the whales before, during, and after the darting. However, different behavioral data were collected off the Azores and Nova Scotia, so that, unfortunately, we cannot usually provide direct comparisons between the behavior of the whales during the two studies. All samples (sloughed skin and tissue collected with the biopsy or glancing-type dart) were preserved in saturated NaCl solution without buffers (Hoelzel and Amos 1988).

## RESULTS

*Sloughed skin*—The frequency and circumstances of occurrence of sloughed skin varied considerably between our study areas. In the tropical waters off the Galápagos Islands and around the temperate Azores Islands, sloughed skin was very frequently found after animals breached (leaped from the water), lobtailed (thrashed their flukes onto the water surface) or were seen in physical contact with one another during social situations, especially when more than about five animals were seen together at the surface. Except in the case of some lobtails, these samples cannot generally be linked to a single individual sperm whale, which are usually identified from photographs of their flukes (Arnborn 1987).

However, during studies off the Galápagos in April and May 1989, sloughed skin was found in approximately 20% of the slicks left at the surface after sperm whales raised their flukes for a nearly vertical deep dive. These samples could usually be linked to individually-identifiable fluke photographs taken during the preceding fluke-up, especially if the whale dived by itself. Success in finding sloughed skin from diving slicks varied considerably and depended greatly on water clarity, lighting conditions, and the roughness of the sea surface. Tests and observations showed that the sloughed skin sank. This indicated that the origin of the skin could only be attributed to individuals in the immediate vicinity of the collection site. For about 40% of the samples only one whale was seen near the collection site. The sinking of the sloughed skin also meant that for successful collection the vessel had to reach the slick very soon after the dive.

During the 1989 Galápagos field work 166 samples of sloughed skin were collected, 68 of which could be linked to one identifying fluke photograph, and 39 of which could be linked to either of two identifying fluke photographs. During the same period, a total of about 800 usable fluke identification photographs were taken. Sloughed skin was very rarely found from diving slicks off the Azores.

During studies of male sperm whales off Nova Scotia between 11 July and 4 August 1988 we observed virtually no social behavior (or breaches or lobtails) of the kind which frequently led to the collection of sloughed skin off the Azores and especially the Galápagos. From the deck of the slow-moving research vessel (*ca.* 1 km/h) we looked for sloughed skin in 56 slicks left at the surface by diving whales. Five slicks were examined by an observer wearing a mask and snorkel with her head in the water hanging over the side of a tethered inflatable dinghy. In a total of three slicks floating material which may have been sloughed

*Table 1.* Reaction of sperm whales to standard-type biopsy dart—Nova Scotia.

	Biopsy dart	
	Hit	Missed
Total	8	11
"Startled"	8	6
Immediate "deep" dive	4	2 <sup>a</sup>
Short shallow dive	4	3 <sup>a</sup>
Duration of short dive	30–105 sec	60–120 sec

<sup>a</sup> On one occasion the type of dive following the startle could not be determined because of fog.

skin was sighted, but we were never able to collect any of it using a dip net. In the other cases there was no indication of sloughed skin in the slicks.

*Biopsy darting—Nova Scotia*—We fired standard-type darts at whales 19 times. Of these, 11 missed and three hit but obtained no tissue from the whale. In the remaining five cases small pieces of epidermis were collected, but no skin/blubber cores of the type obtained by the same dart from humpback and gray whales were ever collected. Instead, small pieces (<4 mm<sup>2</sup>) of mostly epidermal, and sometimes dermal, tissue were wedged between the outside of the tip base and the rubber cushion of the flared backing (see Fig. 1).

*Glancing-type darting—Azores*—Skin collection was attempted with glancing-type darts on 14 occasions. Whales were hit on eight of these and four samples were obtained. The number of arrows fired was not always recorded, but records of the 11 occasions for which these data are available indicate that 31% of all arrows fired hit a whale. (The crew had no previous experience of archery which may largely explain this poor average.)

*Effects of biopsy darting on sperm whale behavior—Nova Scotia*—With one exception, the biopsy collection attempts off Nova Scotia occurred during surface periods between deep feeding dives of the sperm whales. Off Nova Scotia, these surface periods usually lasted 6–12 min during which time the whale moved slowly in a straight line at the surface while breathing (Mullins *et al.* 1988). The surface period ended with the whale lifting its flukes into the air at the start of a deep dive which lasted between 20–50 min (Mullins *et al.* 1988).

The most obvious and consistent reaction of the sperm whales to biopsy darting was that they "startled": suddenly flexing and turning their bodies and increasing their speed. Often the back was arched, and/or the head or flukes broke the surface (Fig. 2B). Startling was accompanied by a defecation on two occasions. After startling, the whale then either started a long dive (usually raising its flukes) or disappeared without raising flukes to reappear nearby 30–120 sec later (Table 1). After such a reappearance, the whale was often travelling significantly faster (4–7 km/h) than before startling (usually 3–5 km/h), but it generally slowed over the next 2–3 min.

On one occasion we successfully attempted to sample a whale which was consistently at or near the surface during a period of at least 105 min, never

raising its flukes but occasionally making shallow dives of 1–8 min. The biopsy was attempted 8 min after first sighting the whale, but apart from the startle and subsequent shallow dive, the behavior of the whale appeared unaffected by the darting during the following 97 min of observation.

In all eight cases in which the dart hit a whale, the whale startled. On two occasions the whale also startled as the vessel approached before darting was attempted. On 5 of the 11 occasions on which the dart missed the whale there was no visible reaction, but on the remaining 6 occasions the whale startled although the dart did not make contact (Table 1). The whales seemed to be responding to the sound of the dart hitting the water, but in one case the whale startled as the dart, which had overshot, was pulled across its back by the retrieval line.

There was no obvious change in the total time at the surface because of biopsy darting. The median time at the surface (between first appearance and flukes being raised at the start of a deep dive) was 7 min 30 sec when the dart hit, 7 min 15 sec when it missed, and 7 min 15 sec when no darting was attempted, and we were generally not as close to the whale as when darting was attempted. On four occasions (3 hits and 1 miss) we measured the rate of exhalations both before biopsy darting and after a shallow dive following the attempt. Twice the exhalation rate increased (3.53→4.02, 3.00→3.87 blows/min) and twice it decreased (3.09→2.37, 2.83→2.69 blows/min) following the darting attempt. In none of these cases was the exhalation rate significantly changed after the darting attempt (Chi-squared tests of number of exhalations per unit time,  $P > 0.05$ —the more sensitive Mann-Whitney  $U$  tests on exhalation intervals, used on the Azores data [see below], could not be applied as precise times of exhalations were not recorded off Nova Scotia).

Individual sperm whales can be identified from photographs of their flukes (Arnbom 1987). Reidentifications from one surfacing to the next allowed us to measure the dive times of individual animals. In three cases we measured the duration of the long deep dive immediately following a successful biopsy attempt, and could compare it with either previous or subsequent adjacent dive times: (1) a 38-min post-biopsy dive was followed by a 37-min dive; (2) a 33-min post-biopsy dive was followed by a dive of 38-min; and (3) a 28-min post-biopsy dive was preceded by two dives of 25 and 30 min respectively.

*Effects of glancing-type darting on sperm whale behavior—Azores*—Whales sampled off the Azores appeared to be feeding and performing a dive cycle similar to that described for the Nova Scotian animals. A startle response was observed on each of the occasions on which a whale was hit and also on some of the occasions when an arrow missed but hit the water close to the whale. On one occasion the whale defecated after startling. On another occasion, following a hit, the whale rolled onto its back with its head slightly raised and showed its lower jaw, slightly agape above the water but without snapping it. The snapping of the lower jaw above the surface is believed to be an agonistic behavior and has been observed by open-boat whalers both during attacks on whaling vessels and during fights between male sperm whales (Caldwell *et al.* 1966). This may have been a mild form of such behavior.



Whales typically made shallow dives of 30–310 sec (mean 130 sec) duration after the startle response but always returned to the surface to continue blowing for some time before fluking up to make a long dive. On one occasion the whale fluked up immediately after being hit but returned to the surface after 60 sec and continued blowing. On another occasion a whale startled and made a shallow dive after a sampling attempt in which it was not hit. The whale could be seen underwater by the lookout in the crow's nest and the boat followed it at a range of about 30 m. As we followed in its wake a large number of bleached squid of around 30 cm length were observed in the water and several of these were collected. Their bleached and semi-digested condition and the abundance of large nematodes lodged in their bodies indicated that they had been regurgitated by the whale which we had been following. Sperm whales are thought to regurgitate squid beaks and other hard parts of their prey regularly. Clarke (1980) has estimated that they should regurgitate this material every 2.1–2.5 d (based on the observed number of beaks in stomachs and the assumed daily ration). If this is so, an observation of vomiting might not be indicative of severe disturbance. However, as far as we are aware, regurgitation has never before been observed from sperm whales which were not being hunted. This is surprising in the light of the many hundreds of hours of observations made in recent years off Sri Lanka, the Galápagos, the Azores and Nova Scotia.

For seven sampling attempts off the Azores, exhalation rates and some other behavior were recorded using a hand held computer (Psion Organiser) as an event recorder. Analysis comparing exhalation rates before sampling attempts to those after whales had returned to the surface after startling and making shallow dives are summarized in Table 2. On four of these occasions exhalation rates were decreased significantly following sampling attempts (Mann-Whitney *U* tests on intervals between exhalations).

#### DISCUSSION

Our preliminary investigations show that both sloughed skin and biopsy darts can be used to obtain restrictable DNA from sperm whales. Which technique is best employed in a particular study will depend on a number of factors.

The availability of sloughed skin seemed low in the study off Nova Scotia (sea surface temperature during study 14–18°C) but was adequate for some purposes in the temperate Azores (18–23°C) and very satisfactory off the Galápagos on the equator (19–26°C). From observations of humpback whales off Hawaii and Alaska and of gray whales in northern latitudes it appears that baleen whales may generally slough epidermal tissue more frequently in warm rather than cold waters (E.A.M., personal observation). The same appears to be true of sperm whales, although the differences in availability that we have found between our study areas may also be partially due to the generally clearer waters and calmer conditions of the lower latitude studies. These effects may also be compounded by the less social and demonstrative behavior (such as breaches) observed from male sperm whales at high latitudes.

Although collection of sloughed skin appears to be an almost passive tech-

*Table 2.* Reaction of sperm whales to glancing-type dart—Azores. Tests for significant changes in respiration rates are Mann-Whitney *U* tests.

Date	Change in respiration rate (significance)	Comments
12 July 1988	decrease ( $P = 0.002$ )	3 darts fired; 1 hit; strong reaction.
12 July 1988	decrease ( $P = 0.02$ )	1 dart fired; 1 hit; startle; defecation.
12 July 1988	decrease (NS)	2 darts fired; no hit; slightly startled.
12 July 1988	increase (NS)	1 dart fired; 1 hit; startle then fluked.
12 July 1988	decrease ( $P = 0.003$ )	3 darts fired; 2 hits; startle; rolled on back and brought lower jaw above surface.
12 July 1988	decrease ( $P = 0.043$ )	1 dart fired; no hit; slight reaction without shallow dive.
19 July 1988	no change (NS)	1 dart fired; no hit; slight reaction.

nique, the movement of the research vessel into a slick left at the surface by a diving whale, and the subsequent entry of snorkellers into the water, could potentially be disturbing to the whale. No reactions to skin collection were observed at the surface (such as a sudden resurfacing), and our subjective impression is that dive times were not changed by attempts to collect sloughed skin from the slicks of diving sperm whales. The diving sperm whale would be about 70 m below the surface at the end of the minute or so it takes for our research vessels to enter the slick (Papastavrou *et al.* 1989).

For many applications it will be important to link samples to photographs which identify individuals. Both skin collection techniques allow such linkages on some, but not all, occasions. During the dive of a sperm whale immediately after biopsy with a standard-type dart the flukes usually have the wrong orientation for a perpendicular photograph to be taken from the sampling vessel. This is not a problem with the glancing-type dart since the vessel is directly behind the whale. However, if several whales are in the vicinity, a shallow dive following the startle may compromise certain identity of the biopsied animal with either dart type so that a subsequent fluke photograph cannot definitely be linked to the sample. In these cases, photographs of identifying marks or scars in the dorsal fin area can sometimes be used to link samples with particular fluke photographs. Similarly sloughed skin cannot be linked to a particular fluke photograph if several individuals are in close proximity, or have recently been in physical contact with one another.

In our observations sperm whales always reacted to successful darting, but the reaction was not restricted to occasions when the dart hit the whale. The whales appeared to react to a sudden disturbance whether it was a dart hitting them or entering the water near them, or the vessel approaching closely. If about to dive anyway, they would dive at this time; if not, they would make a short shallow dive for 30–310 sec and resurface to continue breathing. Our data suggest that the whale's normal behavior was interrupted for periods of up to a few minutes by the darting procedure but that there was no detectable change



in behavior due to darting over periods longer than this. It should be stressed that our sample sizes are small and our measures of behavior crude. We certainly cannot rule out longer-term effects on the whales caused by the darting process, but we did not detect any. Darting, especially with the standard-type dart, carries the potential risk of permanently injuring the whale if it should be hit in a particularly delicate area, especially the eye. However, sperm whales are sufficiently large that all such areas are several metres from the target of the biopsy dart near the dorsal fin, and almost always beneath the water surface. Therefore, we believe that the risk of permanent injury from such a mistake is virtually zero.

The standard-type biopsy darts that we used were designed to retrieve a skin and blubber core from living whales. Such samples are needed if the tissue is to be cultured. However, the darts did not retrieve blubber from sperm whales, probably because sperm whale skin is tougher and thicker than that of baleen whales. We suspect that, instead of shredding to leave a tapered "tail" of blubber, the tissue was held in place by the tougher dermal/blubber interface. Alternatively the tip may not have penetrated far enough into the blubber so there was no weaker breakoff interface.

Although we did not retrieve any cores of tissue, we did collect skin from around the base of the tip and it was clear that the dart had penetrated the skin. In one case we noted that the internal backward-facing prong (Fig. 1) had a small piece of dermis attached to it; after another attempt this soldered stainless steel prong was broken off—probably by the force of the tissue against the exiting tip. The impact of the arrow apparently forced the gap between the rubber backing and the base of the metal tip to widen and subsequently pinch and hold small pieces of epidermis against the outer circumference of the tip base.

A standard-type biopsy tip that was narrower (perhaps 3–4 mm), longer (up to 15 mm), and had a better internal mechanism for clasping the core of tissue, might ensure that a skin/blubber biopsy was retrieved and not left attached to the whale. Since larger samples are needed if the tissue is to be cultured, but not for the new analyses of DNA, a thinner and longer tip would probably be adequate for DNA fingerprinting analysis, but would reduce the likelihood of establishing viable cell cultures if these were desired.

There are several important advantages in a glancing-type of dart, such as that used in the Azores study. One difficulty when using the standard-type biopsy dart is in being able to hit a part of the whale which is close enough and perpendicular to the angle of flight to allow clean penetration. This is not a problem with a dart designed to glance off the whale. A particular advantage of a glancing dart with sperm whales is that it allows the whale to be approached from behind—the easiest angle of approach and the required orientation needed for useful identifying fluke photographs. From behind, the archer has a relatively large target (especially in the vertical plane, which is the most difficult to sight accurately). It is also likely that the risk of causing an infection is lessened with a dart which does not cut into the skin. The reduced accuracy required with

this dart allows the use of a compound or long bow, for which successive arrows can be fired at a faster rate. Furthermore conventional bows are considered by some to be safer for other crew members than crossbows.

Although the glancing-type darts did collect some material on half of the occasions on which they hit a whale, these samples have not proved sufficient for conducting repeated DNA fingerprint analyses using current techniques. Only a very small amount of material was removed from the whale in the first place and retrieving this material from the interstices of the abrasive mat has proved problematical introducing further losses at this stage. The final yield was sufficient for only a single fingerprint analysis using current standard techniques (W. Amos, personal communication). The performance of the dart may be improved by using a coarser form of abrasive material and by extending the time it is in contact with the skin. This latter might be achieved by mounting the abrasive material on a flexible rubber neck which could bend when the arrow hit the whale and maintain contact with the skin. It was assumed that a dart which inflicted only a glancing blow would cause less short-term disturbance than the standard type. This does not seem to have been the case.

None of the techniques which we have tried currently provide samples suitable for tissue culture. The sloughed skin samples that we collected were usually large enough for a number of DNA fingerprinting analyses. The samples from biopsy darts, as well as the pieces of sloughed skin, are sufficient for any analysis in which the polymerase chain reaction can be used for enzymic amplification of minute quantities of DNA (W. Amos, personal communication).

Legal, practical, or ethical factors may place restrictions on darting of either type, but will rarely restrict collection of sloughed skin (unless concerns are raised about the effects of rapidly approaching the diving slicks). The ease with which sloughed skin can be collected off the Azores and the Galápagos, and the disturbance caused by darting, means that it is unlikely that we will continue to use darts in either study. However, perhaps especially in high latitude studies, it may prove profitable for others to develop and use improved versions of either the standard-type biopsy dart, or glancing-type dart.

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