

A CETACEAN BIOPSY SYSTEM USING LIGHTWEIGHT PNEUMATIC DARTS, AND ITS EFFECT ON THE BEHAVIOR OF KILLER WHALES

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ABSTRACT

Lightweight untethered pneumatic darts were used to biopsy killer whales, *Orcinus orca*, for genetic and toxicological analysis. Samples of epidermal, dermal, and hypodermal tissue weighing approximately 0.5 g were obtained by 65% of the 91 darts fired during the study. Sufficient DNA for multiple analyses was extracted from the biopsies, which were also used for fatty acid and toxic contaminant analyses. Reactions such as momentary shakes or accelerations were observed after 81% of the dart hits and 53% of the misses. Aversion to the research vessel was assessed by reapproaching target whales after the sampling attempts. In 6% of the hits and 8% of the misses aversion to the research boat increased immediately following the attempt. No similar increases in aversion were seen when killer whales were reapproached one day to one year after being hit. The darts were also tested successfully on humpback whales, *Megaptera novaeangliae*. In view of the simplicity of the system, its effectiveness in acquiring multipurpose samples, and the apparently short-term disturbance it caused, it is recommended for future cetacean biopsy studies.

Key words: biopsy sampling, DNA, genetics, killer whale, *Orcinus orca*, toxicology.

DNA analysis has become a vital tool in studies of cetacean phylogeny, population structure, and social organization (*e.g.*, Amos *et al.* 1993, Baker *et al.* 1993). Similarly, toxicological analysis is a major component of many cetacean

studies, particularly those focusing on the conservation of coastal or riverine species (*e.g.*, Muir *et al.* 1990). Tissue samples for these analyses have been obtained opportunistically from stranded whales (Calambokidis *et al.* 1990), from those killed in whaling operations (*e.g.*, Amos *et al.* 1993), and from the sloughed skin of living animals (Whitehead *et al.* 1990). Samples of skin and subdermal tissue have also been taken from free-ranging whales using biopsy-sampling devices fitted to projectiles (*e.g.*, Mathews *et al.* 1988). Sampling with projectiles has distinct advantages over other techniques: it provides fresh, uncontaminated tissue non-lethally; it can be used on species that strand rarely and do not slough skin; and it can be used to obtain samples from specific individuals known from photoidentification studies. The last factor is particularly useful when testing hypotheses about social systems using molecular analysis, or where it is desirable to control for age or sex in contaminant studies.

Most systems for sampling cetaceans use longbows or crossbows to fire bolts fitted with punch tips (*e.g.*, Lambertsen 1987, Mathews *et al.* 1988, Palsbøll *et al.* 1991). The tips excise plugs of skin and retain them with internal barbs. In biopsy studies using bows to sample sperm and baleen whales, the percentage of shots hitting the target animal ranged between 42% and 81%, and 61%–90% of hits obtained skin samples (Whitehead *et al.* 1990, Brown *et al.* 1991, Weinrich *et al.* 1991, Clapham and Mattila 1993, Brown *et al.* 1994). In a study using a large-bore pneumatic gun to biopsy humpback whales, Lambertsen *et al.* (1994) reported hits-per-shot and samples-per-hit rates of 95% and 88%, respectively.

Most studies that have assessed the responses of cetaceans to biopsy sampling procedures have focused on immediate reactions visible at the surface, such as tail slashes, and on short-term behavioral modifications, such as changes in swimming speed (*e.g.*, Weinrich *et al.* 1992). These data have served as indicators of the relative invasiveness of different procedures, and of the relative effects of biopsy sampling on individuals of different ages and sexes. While useful for these purposes, they do not directly measure the absolute disturbance wrought by biopsy sampling. We simply do not know whether subtle alterations in breathing pattern or swimming speed are indicative of annoyance or of fear, nor do we know the extent to which immediate or short-term responses map onto longer-term changes in behavior. No studies have examined whether whales become more evasive of boats after they are biopsied. Weinrich *et al.* (1991) found that humpback whales used the same geographic areas before and after biopsies were taken; however, no other studies have examined the effect of tissue sampling on the long-term movements of whales.

Here we describe our use of lightweight, untethered, pressure-propelled darts to obtain biopsy samples from individually identified killer whales (*Orcinus orca*). To our knowledge, this type of system has not been previously used on cetaceans. We describe the factors affecting our success in obtaining samples, the histology of the samples acquired, the whales' immediate responses to darting, and whether short- or long-term avoidance of the research boat resulted from the biopsy procedures. Finally, we discuss the applicability of the sampling method to future studies requiring tissue samples from free-ranging cetaceans.

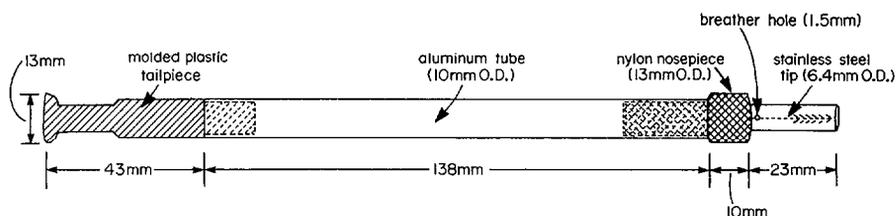


Figure 1. Assembled pneumatic dart, showing inserts of tailpiece and nosepiece into body tube and location of barbed broach. (O.D. = outside diameter.)

METHODS

Darting system—The darting system was modified from a system originally developed for terrestrial mammals (Karesh *et al.* 1987). A diagram of an assembled dart is presented in Figure 1. The tip consists of a stainless steel tube with a honed internal bevel at one end and an external thread at the other. The tip screws into a nylon nosepiece that acts as a stop to limit the depth of penetration. The nosepiece is pressed and glued into the dart body, a thin-walled tube of tempered, silicone-coated aluminium. A tailpiece of red polyethylene is pressed and glued into the other end of the body (tips and nosepieces custom machined, bodies and tailpieces supplied by Pseudart Inc., P.O. Box 1415, Williamsport, PA 17703, U.S.A.). The dart floats upright with the tail projecting above the surface for maximum visibility. The tip is fitted with a barbed dental broach (coarse type, 22-mm shaft length, fitted to a plastic handle, supplied by Canadian Dental Supply Ltd, 3900 North Fraser Way, Burnaby, B.C., Canada V5J 5H6), as shown in Figure 2. The plastic handle of the broach matches the inner diameter of the tip and is pressed into place using the tool shown in Figure 3. The tool is also used to remove the sample from the tip by pressing the broach through the bevelled end of the tube. The total weight of an assembled dart is approximately 12 g.

The darts were fired from a variable-power dart projector (Pseudart Model 191). The projector uses blank .22-cal. charges (Omark type CCI 4C22) to pressurize a sealed chamber. Gas flows from this chamber through a power-adjusting valve to a rifled barrel containing the dart. At the three power ranges

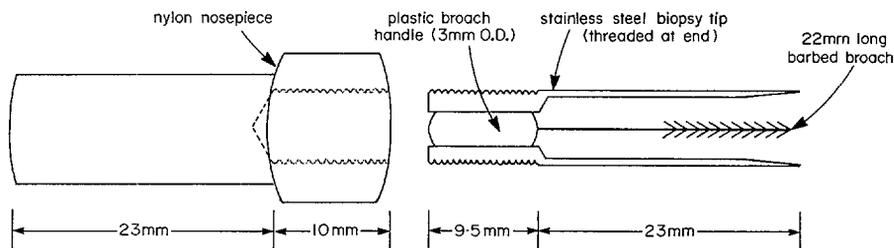


Figure 2. Detail of dart nosepiece and tip, showing barbed broach pressed into place.

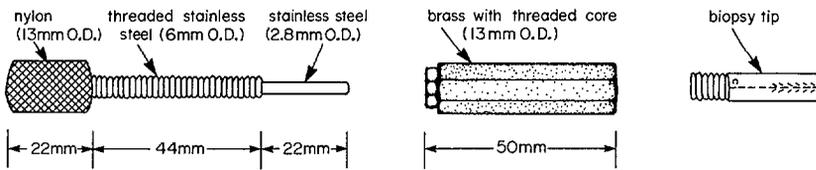


Figure 3. Broach installation/extraction tool. The dart tip threads into right side of brass body. The nylon-handled shaft threads into left side and presses the barbed broach into place in the tip, or, by further advancement, pushes it through and out of the tip.

used during the study (settings '1', '2', and '3'), the dart velocities measured with a projectile chronograph 10 m from the rifle were 42, 52, and 74 m/sec, respectively. The striking energies of darts fired at these ranges were calculated to be 10.6, 16.2, and 32.8 joules. We achieved an accuracy of 10 cm at a range of 15 m nine times out of ten when firing the darts on land.

Decontamination and sterilization—Prior to each use we fitted a new dental broach into the dart tip, screwed it to the dart body, and flooded the assembled dart with acetone and then hexane. This procedure ensured that the dart surfaces were sterile and free of substances that might contaminate the sample. Before loading the dart into the rifle, we swabbed the barrel with an acetone-soaked wad of cotton cloth to remove oil or other contaminants. After extracting a tissue sample from the dart, we washed the dart body, tip, and extraction tool with soap and boiling water, rinsed them thoroughly, and allowed them to air dry.

Sample processing and preservation—After extracting the sample from the dart tip, we placed it in a glass dish, pulled the dental broach from the tissue, and sliced the sample transversely between the dermis and hypodermis. We preserved the outer portion for DNA extraction in 20% dimethylsulphoxide in a saturated salt solution (Amos and Hoelzel 1991), and stored it at 4°C. We placed the inner portion of the sample in a decontaminated glass vial and stored it at -20°C for fatty acid and contaminant analyses.

We reserved a single biopsy sample for histological examination and placed it in Davidson's solution immediately after removal from the biopsy tip. It was subsequently imbedded in paraffin wax, sectioned to 5- μ thickness, and stained with Harris' hematoxylin and eosin Y following standard histological procedures.

Killer whale biopsy sampling—Biopsy sampling was conducted in 1992–1993 along the coast of British Columbia, principally in the region extending from 50°40'N to 53°00'N. Killer whales from three putative populations were sampled: *residents*, which eat fish and live in groups of 10–50; *transients*, which feed on marine mammals and live in smaller groups; and *offshores*, a recently discovered population with unknown diet and social structure (Ford *et al.* 1994). Most of the sampling attempts were conducted from an 8-m planing vessel with a cabin-top steering station for the driver and a sheltered cockpit aft that provided a stable platform for the darter. Several darting attempts were also made from a 5-m inflatable boat powered by a 30-hp outboard motor, with the driver in the stern and the darter seated forward.

Killer whales were located by visually searching areas of known concentration and by listening for vocalizations with a directional hydrophone. Sighting reports from other mariners helped to focus searches. When found, whales were approached to 20 m on a gradually converging course. Individuals were identified visually, by reference to Bigg *et al.* (1987) and to an unpublished catalog of recent photographs (G. M. Ellis, unpublished data). If the whales had not been previously seen that year, we took identification photographs of each individual present before beginning the biopsy sampling. We then selected a target individual based on a previously-established priority list and waited, if necessary, for it to begin swimming and surfacing in a predictable and consistent manner. When this occurred, we paralleled the whale's course and approached to an estimated 8–20 m, while noting (1) its activity (categorized according to Barrett-Lennard *et al.*, in press); (2) its position in relation to other group members; and (3) whether it adjusted its speed, course, or breathing pattern to move away from the boat as we approached. When in position alongside the whale, the darter aimed at a point on the back approximately 30 cm below the trailing edge of the dorsal fin and fired, usually when the whale arched its body just prior to diving. The dart, which usually bounced off the whale's skin after a strike, was retrieved from the water using a long-handled net. The whale was filmed prior to and during the darting attempt using a high resolution video camera.

Assessing disturbance—Notes were made on standardized data sheets regarding the estimated distance to the target whale at the time of darting and on the type and extent of the reactions of the whale to both hits and misses. Additional details were added upon reviewing the video footage. Visible changes in the behavior of other killer whales in the vicinity were also noted, and any changes in the position of the target animal with respect to other group members were recorded. Within 15 min of retrieving the dart from the water, we slowly reapproached the targeted whale, recording its breathing pattern, position in the group, and movements relative to the boat. The whale was approached to the same distance as in the darting attempt, unless it consistently avoided the boat. After 1–3 such approaches, we slowly moved away. If we sighted the whale again in the year following the darting attempt, we reapproached it in a similar way, while noting its behavior and inspecting the site of the dart wound with binoculars.

Humpback whale trials—To determine the effectiveness of the system in acquiring samples from larger whales and to obtain tissue for toxicological analysis, we carried out a limited series of trials on humpback whales. We photographed the tail fluke of each whale prior to darting to ensure that we did not sample the same individual more than once. We then maneuvered alongside the whale and fired at a point approximately 50 cm below the dorsal fin from a range of 15–25 m.

Statistical analysis—The data were analyzed with the SAS JMP statistical package, using logistic regression (Trexler and Travis 1993) and log-likelihood ratio contingency analysis (Zar 1984). Power analysis of one non-significant result was conducted using methods presented in Cohen (1988).

RESULTS

Killer whale sampling attempts—We fired 91 darts in sampling attempts during 34 encounters with killer whales. The target whale was hit and skin and blubber samples were obtained in 59 of the attempts (65%). In 13 of the attempts (14%) the whale was hit but the dart was lost, empty, or contained only small fragments of epidermis ($n = 4$, $n = 4$, and $n = 5$, respectively). Whales were missed on 19 attempts (21%). Non-targeted whales were never hit. The percentage of darts fired that hit the animal and the percentage of hits that yielded samples were 60% and 72%, respectively, for the first 30 attempts, 87% and 90% for the second 30 attempts, and 90% and 93% for the final 31 attempts. Logistic regressions of the effect of attempt number on (1) the probability of a dart hitting and (2) the probability of a hit yielding a sample were both significant ($\chi^2_1 = 15.39$, $P < 0.001$; $\chi^2_1 = 3.84$, $P = 0.050$, respectively).

We attribute much of the improvement in hit rate and sampling rate to the increasing experience of the darter and vessel driver. Incremental equipment modifications throughout the study also contributed to the efficiency of the system. For example, the .22-cal. charges that we used initially varied unpredictably in power; our hit rate improved when we found a supplier of more consistent charges, after attempt number 15. Similarly, a dart sank and was lost in an early attempt when the tailpiece detached; we prevented this from reoccurring by gluing, as well as press-fitting, the tailpieces to the dart bodies.

Factors affecting darting success—Killer whales were most readily darted when travelling at moderate speed, because they surfaced in predictable locations and arched their backs well above the surface when breathing, presenting relatively large targets. Resident killer whales that were foraging for fish in open water were unpredictable in their movements, and we seldom attempted to biopsy them. However, when foraging for fish along cliffs and steep shorelines, residents moved in a more consistent way and could often be approached for darting. Resting killer whales tended to form tight groups and to consistently adjust their course so as to maintain distances of 25 m or more from the boat. Resting whales also showed little of their backs when surfacing, and we rarely attempted to approach or dart them.

We biopsy-sampled killer whales at estimated distances ranging from 5–25 m (mean 11.3 m). The probability of hitting a whale decreased as firing range increased (logistic regression $\chi^2_1 = 14.27$, $P < 0.001$). At a range of 10 m the probability of hitting a targeted whale was approximately 92%, but by 15 m it fell to 65%. The probability of a hit yielding a sample was independent of range (logistic regression $\chi^2_1 = 0.037$, $P = 0.847$).

Most animals that were not resting could be readily approached to a distance of approximately 15 m, but approximately 5% of the whales consistently adjusted their course and speed to remain farther from the boat, regardless of their activity state. Most of these wary individuals were females, and all were estimated to be over 35 yr of age (based on Bigg *et al.* 1987). Three were biopsy darted during the study, all at ranges over 20 m.

The probability that a dart would acquire and retain a skin sample was strongly influenced by the angle of impact. If the orientation of the whale was not perpendicular to the line of fire, or if the dart hit high on the back where the skin surface curved towards the dorsal ridge, the dart tended to glance off with no sample or with only a minute scraping of skin. Dart tips with both internal and external bevels were tested; the angle of impact was less critical with internal bevels. In general, the angle of impact was least critical when the darts were extremely sharp.

Acute angles of incidence also appeared to increase the probability that a dart would remain attached to the skin rather than bouncing free. This occurred three times during the study and in each case the dart was lost. Twice we were able to see that the dart had fallen out within several minutes of the attempt, and once we were unable to determine how long the dart remained attached due to fading light. There was no evidence that the whales were aware of the attached darts.

We altered the power setting of the dart gun depending on the range of the whale. Early in the study we used a setting of 3 for whales that were > 10 m from the boat, and a setting of 2 for closer whales. However, we noted subjectively that lower power/distance ratios caused milder immediate reactions by the whales and also increased the probability that a dart would acquire and retain a tissue sample. By the midpoint of the study, we used power settings of 1, 2, and 3 for estimated whale distances of < 10 m, 10–15 m, and > 15 m, respectively. The darts dropped in flight more with low than with high power settings, but we compensated for this by adjusting the rifle sights.

Immediate responses—The immediate behavioral responses to darting attempts consisted of shaking, usually detected by quivering of the dorsal fin, and accelerating. If the shake or acceleration was momentary, ceasing before the whale submerged, the response was categorized as slight. Slight responses were often barely perceptible to the observers and in some cases were only detected when reviewing the video film. Responses that continued as the whale submerged, or that were in evidence on subsequent surfacings, were categorized as strong.

The immediate responses of killer whales to biopsy darting attempts are presented in Table 1. In the resident population we detected no significant difference in the immediate responses of mature males, mature females, and juveniles (log-likelihood ratio contingency test, $G = 0.70$, $df = 4$, $P = 0.951$). Fewer transient than resident killer whales responded visibly to sampling attempts, however the difference was not supported statistically ($G = 4.88$, $df = 2$, $P = 0.087$; test power = 0.77). The sample size of offshore whales was too small for meaningful comparison with the other populations.

Of four strong immediate responses to hits that were observed (Table 1), two consisted of acceleration followed by an increased swim rate, and two consisted of pronounced shakes that continued as the whales submerged, but ended before they resurfaced. The single individual that responded strongly to a miss was evasive prior to the attempt and was known from photoidentification studies to be consistently difficult to approach.

Seven whales that were hit by a dart once but not biopsied were hit a second

Table 1. Immediate responses of killer whales to biopsy darting attempts and percentage of dart hits that obtained biopsies. The first four rows show the percentage of animals in each population/age/sex category that exhibited the indicated response, followed by the sample size, in parentheses. The final row shows the percentage of biopsies acquired per hit, followed by the number of biopsy attempts that succeeded in acquiring samples, in parentheses.

Immediate response level	Hits						Misses All populations
	Resident population			Transient population	Offshore population	All populations	
	Females over 14 yr	Males over 14 yr	Under 14 yr (both sexes)				
None visible	20 (3)	13 (5)	17 (2)	60 (3)	50 (1)	19 (4)	47 (9)
Slight	73 (11)	82 (31)	67 (8)	40 (2)	50 (1)	74 (53)	21 (4)
Strong	7 (1)	5 (2)	8 (1)			6 (4)	5 (1)
Other			8 (1) ^a			1 (1)	26 (5) ^b
Biopsies per hit	80 (12)	79 (30)	92 (11)	80 (4)	100 (2)	82 (59)	

^a Whale looked back along its side, towards the dart impact site.

^b Responses not documented.

time. All were successfully biopsied on the second attempt, and no whales were hit more than twice. Five of the seven had slight immediate responses to the darts on both the first and second hit. One had no response to the first dart but responded slightly to the second, and one reacted strongly to the first hit but only slightly to the second one.

Targeted whales that were swimming in formation with other whales were never seen to change their position with respect to other animals in their group immediately after darting attempts. On only one occasion was there evidence that non-targeted whales were influenced by the darting of a group member: two mature males gave distinct shakes when a female approximately 20 m from them was hit with a biopsy dart. The female was believed to be the mother of both males (based on Bigg *et al.* 1990).

Followup approaches—We attempted to reapproach whales that had been hit by biopsy darts after 68 of the sampling attempts. On 62 of these occasions the whale showed no tendency to change speed or course to avoid the boat and was readily approached to 10–20 m. Four whales that were easily approached before being struck by a dart avoided the boat when reapproached, and two were evasive both before and after the strike. Of the seven whales that were hit twice, six were readily approached after both the first and the second hits. The seventh was evasive after both attempts.

After periods of one day to one year, we resighted and reapproached 46 of the whales that had been hit, including those that became evasive immediately after darting. None of these resighted individuals revealed a tendency to avoid the boat when approached to 10–20 m. In several cases the dart scar was visible as a black dot on the white saddle patch; no evidence of wound infection was seen. Most whales were darted and resighted in the same general areas, and there was no evidence that whales changed their travel patterns in response to darting.

We attempted to reapproach 12 whales that had been missed within 15 min of the darting attempt. Seven were readily approachable, one became evasive after the miss, and four were evasive both before and after the darting attempt. During subsequent encounters we sighted and reapproached all but one of the whales that had been missed; no changes in approachability were noted.

Description and utility of killer whale biopsy samples—The tip of a dart that had obtained a sample was usually filled completely with tissue, with several millimeters of blubber extending from the open end. One sample of typical size weighed 0.50 g.

Examination of histological sections of the tissue sample preserved in Davidson's solution revealed three primary layers: the epidermis, dermis, and hypodermis. The epidermis consisted of heavily pigmented cuboidal cells originating in a basal papillary region. The cuboidal cells became more and more flattened towards the outer surface, and those at the surface appeared to be loosened and ready to slough away. Interdigitating invaginations and papillae formed the junction of the dermis and epidermis. The thickness of the epidermis, from the surface to the base of the papillae, was 4–6 mm. The dermis was composed of thick, interwoven bundles of collagen, and was well supplied with blood vessels.

Some fat was present in the dermis and became more prevalent as the dermis gave way to the hypodermis. Collagen fibers penetrated from the dermis into the hypodermis, creating a strong junction.

We extracted genomic DNA from 0.025 g subsamples of the epidermal and dermal tissue by proteinase K digestion, phenol-chloroform extraction, and alcohol precipitation, following procedures presented by Sambrook *et al.* 1989. DNA yields estimated by UV spectrophotometry (Sambrook *et al.* 1989) ranged from 20 to 210 μg (\bar{X} = 85 μg , n = 40), sufficient for multiple DNA analyses. A 20-mg portion of the hypodermis immediately underlying the dermis was provided to the Pacific Biological Station, Canadian Department of Fisheries and Oceans, where gas-liquid chromatography of derived metal esters of the constituent fatty acids was successfully used to provide fatty acid profiles (J. N. C. White, unpublished data). The remaining portions of the hypodermis were provided to the Institute of Ocean Sciences, Canadian Department of Fisheries and Oceans, for analysis of dioxans, furans, and P.C.B.'s. This analysis was conducted successfully using high resolution gas chromatography combined with high-resolution mass spectrometry (M. G. Ikonomou, unpublished data).

Results of biopsy trials on humpback whales—Humpback whales were hit with biopsy darts 13 times; of these, 11 acquired samples of skin and blubber weighing approximately 0.5 g and two glanced off the whales without acquiring samples. The immediate responses of the whales were similar to those reported by Brown *et al.* 1994; detailed descriptions will await further trials.

DISCUSSION

The system described here differs from previous cetacean biopsy systems in three principal ways. (1) It uses darts that are both smaller and lighter than previous biopsy projectiles. Based on their descriptions, the projectiles used in other systems weigh between 50 and 350 g, a minimum of four times the weight of the darts used here. (2) Barbed dental broaches are used to retain tissue samples, unlike the inward-facing prongs (*e.g.*, Winn *et al.* 1973), central hooks (*e.g.*, Brown *et al.* 1991), or hinged flaps (Aguilar and Nadal 1984) used previously. (3) The system uses a pneumatic rifle to propel untethered, floating darts, whereas previous rifle systems have used retrieval lines (Winn *et al.* 1973, Kasamatsu *et al.* 1991, Lambertsen *et al.* 1994).

A primary objective in designing the darts was to minimize the disturbance and wounding caused by the biopsy strikes. Evidence that the striking energy of biopsy projectiles can significantly affect the severity of wounds in belugas (*Delphinapterus leucas*) was presented by Patenaude and White (1995). We used a dart that was low in mass to minimize the energy transferred to the whale by biopsy strikes. The small surface area of the darts was intended to limit the influence of cross winds and air friction on the flight of the dart, so that predictable trajectories could be achieved at low firing velocities. Despite the weight and size reductions, the dart impact energy was sufficient to excise tissue and recoil free of the skin surface. We had no reason to think that still

smaller darts would not have been equally effective and recommend that further size reductions be tested in future studies.

The barbed broaches were lightweight and provided a convenient way for ejecting the samples from the biopsy tips. Also, because the broaches were sterile when installed and each was used only once, there was no concern that retention of tissue or other material on the barbs might contaminate subsequent samples (Karesh *et al.* 1987). However, because the darts occasionally hit without acquiring a sample, it would be useful to compare experimentally the effectiveness of broaches and central hook tissue retainers (*e.g.*, Brown *et al.* 1991).

The pneumatic rifle used in this study is smaller and simpler to handle than either a longbow or crossbow and, due to the rifling of the barrel, is probably inherently more accurate. Accuracy in biopsy darting is more important for delphinid cetaceans such as killer whales than it is for the baleen or sperm whales that have been the subjects of previous systematic efforts. As well as being relatively small targets, delphinids frequently travel in tight groups, and inaccurately fired darts could put non-targeted individuals at risk. The percentage of firings resulting in hits and the proportion of firings that acquired samples during the final two-thirds of the study, 89% and 81%, respectively, compared favorably with those of experienced researchers biopsy-sampling humpback whales with crossbows (75% and 68% for Brown *et al.* 1994; 81% and 71% for Weinrich *et al.* 1991). The highest biopsy sampling rates for humpback whales reported to date, 88% hits per firing and 83% samples per firing, were achieved in a study using a pneumatic gun to propel arrows (Lambertsen *et al.* 1994).

Tether lines were not used in this study because we were concerned that they would reduce the accuracy of the darts and might increase the disturbance they caused. Weinrich *et al.* (1991) and Whitehead *et al.* (1990) reported adverse reactions to tether lines that entangled the flukes or fell across the backs of humpback and sperm whales. We found that darts were easy to locate and would only recommend the use of tethers if sea conditions, or the nature of the research vessel, made the retrieval of floating darts difficult.

As in previous studies (Mathews 1986, Weinrich *et al.* 1991), the darting success rate increased progressively throughout the study. This improvement resulted from the increasing experience of the darter and the vessel operator and from refinements to the equipment made throughout the study. This trend continued beyond the trials described here. In a subsequent study using this system on killer whales, the authors acquired samples from 88% of the 42 darts fired and from 97% of the darts that hit (details of this later study are not reported here, as follow-up approaches were not made). It is recommended that researchers starting similar projects use experienced personnel when possible and begin with modest sampling objectives until the biopsy procedures are well practiced.

The immediate responses of killer whales to the darts appeared to be minor, since behavioral activities were not interrupted as a result of darting. In most cases there was no indication that darted whales became evasive of the research boat in either the long or short term. This may reflect either the mild nature of

the darting stimulus, or it may indicate failure of the whales to associate the pain of a biopsy dart with the presence of the boat. Most of the whales that reacted strongly to the dart were evasive prior to the attempt, suggesting that these individuals may have had negative experiences with boats in the past. Prior to the 1970s it was not uncommon for mariners to shoot at killer whales in British Columbia (G. M. Ellis, personal observation). Three of the four animals that reacted strongly to a dart and the single animal that reacted strongly to a miss were estimated to be more than 35 yr of age (Bigg *et al.* 1987).

Resting whales were relatively difficult to dart for reasons described above. Furthermore, based both on our previous experience photoidentifying killer whales and on our observations during the present study, we believe that killer whales are more easily disturbed when resting than when engaged in more energetic activities. We therefore recommend that killer whales not be darted while resting.

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