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A BIOPSY SYSTEM FOR SMALL CETACEANS: DARTING SUCCESS AND WOUND HEALING IN *TURSIOPS* SPP.

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ABSTRACT

Together with PAXARMS (NZ), we developed a biopsy system for small cetaceans and tested it on four populations of bottlenose dolphins (*Tursiops* spp.). The system consists of a modified 0.22 caliber rifle, and biopsy darts made out of polycarbonate with stainless steel biopsy tips. Animals were darted at a range of 2–15 m while travelling parallel to the vessel. Overall sampling success for obtaining biopsy samples when an animal was struck ranged from 96.6% to 100% in the four populations. However, hit rate varied for the four different populations. We did not observe a significant difference in strength of the reac-

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tion to the darting procedure when an animal was hit or missed, both among and within populations. Data from one population showed no significant difference in the reaction to biopsy sampling by four different age-sex classes. The only factor that had a significant influence on darting success was the hit location. Furthermore, we observed a significant positive correlation between the size of the sample obtained and the reaction to biopsy sampling. Biopsy samples were sufficient for microsatellite and d-loop analysis in 95.8% and for genetic sexing in 99% of all cases. In animals that we observed on a daily basis, wounds were healed after approximately 23 d.

Key words: biopsy sampling, bottlenose dolphin, cetaceans, darting, wound healing, *Tursiops* sp.

Biopsy samples from free-ranging cetaceans can be used for a variety of research questions including estimation of population genetic parameters, description of mating structure, and the analysis of pollutants in the blubber. Over the past decade, a large number of studies using genetic techniques have enhanced our understanding of cetacean phylogeography and evolutionary relationships. For all of these studies, tissue samples were needed to obtain genetic data. Biopsy samples have been obtained using crossbows (Lambertsen 1987, Weinrich *et al.* 1991, Palsbøll *et al.* 1991, Weinrich *et al.* 1992) or rifles (Barrett-Lennard *et al.* 1996). Recently, less-invasive techniques such as sloughed skin (Amos *et al.* 1992, Valsecchi *et al.* 1998) or skin swabs (Milinkovitch 1994, Harlin *et al.* 1999) have also been used for genetic studies.

The appropriate sampling technique for genetic studies is usually determined by the marker system used. For example, early genetic studies on population structure in cetaceans were usually based on allozymes (Danielsdottir *et al.* 1991) or chromosomes (Duffield and Wells 1991). Both methods usually require larger quantities of tissue or blood. However, PCR-based marker systems such as microsatellites or mitochondrial DNA (mtDNA) may be performed on minute amounts of tissue (Valsecchi *et al.* 1997), but there are drawbacks such as the possibility of allelic dropouts due to low DNA concentration (Gagneux *et al.* 1997). Secondly, for studies of social structure (Amos 1993, Richard *et al.* 1996, Clapham and Palsbøll 1997), it is crucial to determine from which particular animal the biopsy sample originates. It is then desirable to employ darting techniques because matching a sample to an individual is easier using this technique.

Biopsy sampling raises concerns about the short- and long-term effects on the individuals. It is especially important to minimize invasiveness while behavioral data are being collected, to avoid biasing results. Efforts have been made to devise low-impact biopsy methods and to assess their performance (Weinrich *et al.* 1991, Clapham and Mattila 1993, Brown *et al.* 1994, Patenaude and White 1995). Most of these studies involved large baleen whales. For small cetaceans, there are limited data (Aguilar and Nadal 1984, IWC 1991, Weller *et al.* 1997). The death of a common dolphin (*Delphinus delphis*) following biopsy sampling (Bearzi 2000), highlights the need for a safe and efficient biopsy procedure.

Most of the darting systems published to date are for use in large cetaceans (Lambertsen 1987, Palsbøll *et al.* 1991, Weinrich *et al.* 1991, Brown *et al.* 1994). These methods use crossbows with high dart velocities, large bolts and biopsy heads, and thus might pose a risk to smaller cetaceans. During pilot studies by ourselves and Nick Gales, we employed a darting system very similar to

that described in Barrett-Lennard *et al.* (1996). The dart design differed in the choice of the material of the dart body, which was Tasmanian oak instead of tempered aluminum. Instead of a dental broach for sample retention, we used barbs that were pressed into the biopsy head or alternatively a stainless steel hook. This design had problems of durability (darts tended to break on impact), effect on the animals (due to the small flange area, the darts had the tendency to stay attached to the animals without bouncing free), and success of tissue sampling (most samples were very small or minute). Hence, we decided in collaboration with PAXARMS (37 Kowhai Street, Timaru, New Zealand, e-mail: paxarms@es.co.nz) to design darts tailored for use in small cetaceans which would minimize disturbance and long-term effects to the darted animal. Here we present the results of field trials of our biopsy system at four different locations. We collected detailed data on behavioral responses of the darted dolphins, the suitability of samples for microsatellite and mtDNA analysis, and wound healing associated with the biopsy sampling for dolphins of Shark Bay.

METHODS

The PAXARMS Biopsy System

The PAXARMS biopsy system uses a modified 0.22 caliber rifle with a detachable barrel and a valve to adjust pressure in the chamber. PAXARMS biopsy darts have a hollow polycarbonate body and a steel biopsy tip that is beveled inwards (Fig. 1a). For sample retention, three evenly distributed small triangular shaped barbs are located 2 mm from the leading edge of the tip. The tip is welded into a metal flange that acts as a stop and screws into the body of the dart (Fig. 1b). The body is a thin-walled molded tube made out of bright red polycarbonate. An internal wall just below the thread for the metal flange prevents flooding of the body (Fig. 1a). An internal thread at the tail end of the body is used to screw in a small piece of polycarbonate that acts as a safety partition in case the tail flies off on impact (Fig. 1a and Results). The polycarbonate tail piece is also screwed in the tail end on top of the partition, with an o-ring to form a watertight seal. A barb-resetting tool (Fig. 1c) is used to reset the barbs after each successful hit. Its tip fits the inner diameter of the cutting tip and has an internal bevel at one end and a handle on the other. The tool is inserted into the unscrewed steel tip from the backside and pushed forward to reset the barbs. The dart is positively buoyant and floats in an upright position. The total weight of an assembled dart is approximately 21.5 g. We fired the darts using blank charges (PAXARMS). A valve fitted to the chamber on one side of the rifle acts as a control for the pressure propelling the dart. To facilitate aiming, we fitted the rifle with a Pro-Point red-dot laser sight (Tasco). This made aiming faster and more reliable compared to open sights.

Dart Preparation and Sterilization

Prior to each sampling day, we disassembled the darts, checked the plastic parts for visible cracks, and cleaned all parts individually. Wearing latex gloves during all handling, we wiped all polycarbonate parts with 80% ethanol. To remove tissue residues, we scrubbed the biopsy heads using a toothbrush and then

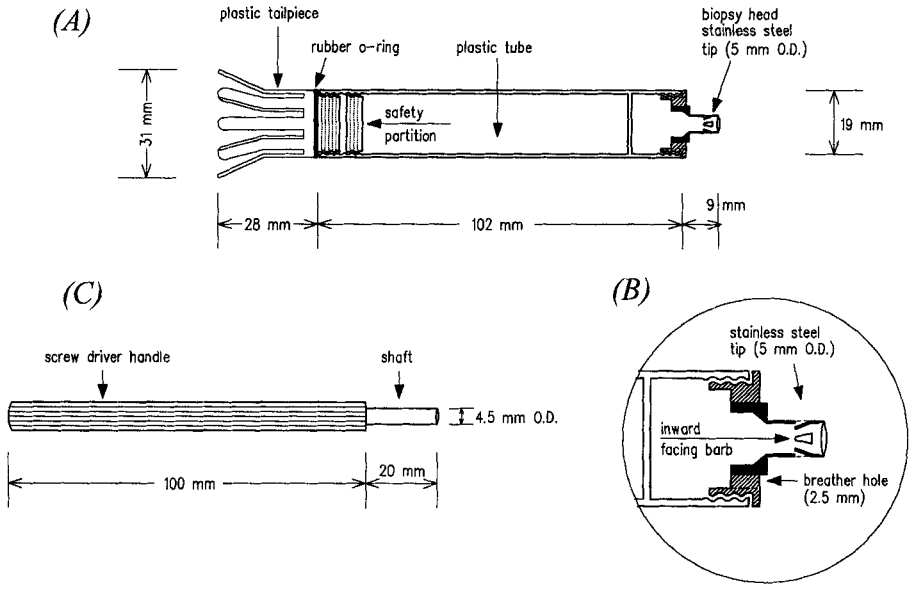

Figure 1. Biopsy dart. (A) Assembled biopsy dart showing inserts of biopsy tip, safety partition, and plastic tail. (B) Detailed drawing of biopsy tip. (C) Barb resetting tool. O. D. = outer diameter.

boiled the heads for 20 min in distilled water. After boiling, we resharpened the darts, using a high-speed steel center drill by applying the center drill forcefully on the tip and moving it clockwise and anticlockwise in a steady motion. We then smoothed the edge of the tip using a diamond file. Finally, we flamed the biopsy head twice after dipping it in 100% ethanol and applied a broad-spectrum antibiotic. The darts were then stored until use in a clean plastic container.

Biopsy Sampling Procedure

In all locations, we attempted to dart dolphins only when they were travelling at slow to moderate speed (0.5–1 m/sec) parallel to the vessel at a distance of 4–10 m. During travelling, dolphins dive and move in predictable patterns, and in good sea conditions, could be observed before breaking the surface. We did not attempt to dart during other behaviors, such as socializing and foraging, when the dolphins were usually unpredictable in their movements and, hence, it was difficult to identify and dart a particular animal. Also, resting dolphins were not darted because they had usually very little area exposed. To avoid harassment, we moved on to a new group if we had not obtained a sample after 10 min. We did not dart juveniles under three years of age (estimated by date of birth if known, or by their overall body length being smaller than 50% of an adult).

Darting took place only in reasonable wind conditions (up to Beaufort 4) and during times of adequate light (*i.e.,* only between 1 h after sunrise and 1 h before sunset). During a typical darting attempt, the darter stood in front of the console, with another investigator right behind the darter taking identifica-

tion photos of dorsal fins. A third person drove the boat and a fourth person was responsible for retrieving darts and collecting data. We fired a dart only after an animal was positively identified or an adequate identification photograph had been taken. When the target animal reached its highest point during surfacing, we aimed about 10 cm lateral to the base of the dorsal fin. Immediately after darting, the dart was retrieved using a long-haul net, while the darter and the photographer started the behavioral observations.

Assessing Behavioral Responses to Biopsy Sampling

Using *ad libitum* sampling methods (Altmann 1974), we documented the response to the darting procedure by following each targeted dolphin for five minutes after a shot was taken. We classified its reactions into one of five categories: (0) no visible reaction, dolphin continued prebiopsy behavior; (I) "startle" response, dolphin moved away (flinch) but stayed in the immediate vicinity of the boat; (II) splashing during moving away and/or tail slap, with or without return to the boat; (III) single leap or porpoise; and (IV) multiple leaps and porpoises. We considered categories 0–II as "mild reaction" categories. We also noted where the dart had hit the animal, relative to the dorsal fin (either on or at the base of the fin, or at least 5 cm lateral to the base of the fin), the size of the sample taken (classified by weight as described in results), and the angle of impact (right angles, or oblique from above, below, behind, or in front). If we darted an animal that was unknown at the time of darting, we also noted the age group and sex if possible.

Biopsy Sampling in Different Regions

In Shark Bay adult dolphins grow up to 2.1 m in length. From 1997 to 1999, the senior author sampled 303 different individuals in East Shark Bay (approximately 25°30′S, 113°30′E). Darting took place from a 4.5-m aluminum center-console boat with a tri-hull design for additional stability. Biopsy methods for sampling in Jervis Bay (approximately 35°07′S, 150°42′E) and Port Stephens (approximately 32°40′S, 152°05′E) were similar to those in Shark Bay. Bottlenose dolphins in Jervis Bay and Port Stephens have been identified as the *aduncus* type (Möller and Beheregaray 2001), although they are larger than the dolphins in Shark Bay (up to 2.6 m in length). The 5.6-m boat we used was an aluminum center-console. The darting set up was the same as in Shark Bay. At Patos Lagoon on the Brazilian coast (approximately 32°07′S, 52°05′W), we used a small aluminum boat. Here, adult animals grow up to 3.7 m in body length. Sampling in all other sites than Shark Bay was conducted by LMM.

Assessment of Wound Healing of Shark Bay Animals

We assessed wound healing on subsequent infrequent encounters with animals that had previously been darted. In cases where the animals came very close to the research vessel or were bowriding, we took photographs of the wounds using a 300-mm zoom lens. Four of the darted animals are part of the provisioning program at Monkey Mia (Connor *et al.* 1992) and visit the beach daily. For these animals we were able to document the healing process from close proximity. Due to relatively high forces on impact, we were concerned that damage to the underlying soft tissue might occur. Therefore, we also noted bruising or swelling of

wounds or their immediate surroundings. We considered a wound to be healed when it was covered with new epidermis, or to be infected when it remained red or pink for more than 20 d.

Validation of Genetic Markers

We extracted the sample from the tip by unscrewing the biopsy head from the body and, using sterilized forceps, we pushed the sample into a 3-ml cryovial filled with a saturated NaCl/20% dimethyl sulphoxide solution (Amos and Hoelzel 1991). We also removed possible tissue residues and added them to the sample. After capping the tubes and sealing with Parafilm, we stored them at −20°C until further processing in the laboratory. We used sterile equipment to cut the sample into small pieces and extracted the DNA using standard procedures (Davis *et al.* 1986). If present, we used about 0.3 g of the subcutaneous layer of the skin. If there was no such layer, we used either the epidermis or, as a third preference, blubber. Genomic DNA was amplified for ten microsatellite loci—MK3, MK5, MK6, MK8, MK9 (Krützen *et al.* 2001); EV1, EV14, EV37, EV94 (Valsecchi and Amos 1996); and D08 (Shinohara *et al.* 1997). We also amplified a 365 bp fragment of the d-loop, using primers dlp1.5 (Baker et al. 1993) and dlp3R (5′-GGTTGCTGGTTTCACGC-3′; developed by MK). We also genetically sexed the animals using sex-chromosome specific primers (Gilson *et al.* 1998).

Statistical Analysis

To test for response to the darting procedure, we examined a number of possible correlates of the procedure. First, if the response is to aspects of the procedure other than the dart-strike, then there should be no difference in response between cases where the dolphin was hit or missed. To test for differences in response to "hits" and "misses" overall and by region, we used a Friedman rank sum test in S-PLUS 4.5 (MathSoft Inc.). Secondly, for the Shark Bay population, we used chi-square contingency analysis to compare behavioral responses to the darting procedure in relation to age, sex, and group composition, and to compare the size of a sample taken with the intensity of the response. We also employed contingency analysis to test whether the size of the sample or the strength of the reaction to the darting procedure were influenced by the side on which the animal was hit, the angle of the hit, or the hit point. Contingency chi-squares were performed using SPSS 10.0 (SPSS Inc.). Significance level for all statistical analyses was $\alpha = 0.05$.

RESULTS

Overall Sampling Success

All data presented were obtained with the biopsy system as described in Methods and Figure 1; data obtained during the development of the system are presented only in the section "Development of Dart Design." We took 550 shots combined over all four sampling locations. Overall, an animal was hit in 374 cases (68.0%), and missed in 176 (32.0%) (Table 1). However, hit rates were different for all four locations: we had the highest success rate in Shark Bay

(75.2%), followed by Port Stephens (53.5%), Jervis Bay (52.4%), and Patos Lagoon (35.3%). For all four locations combined, 94.1% of all hits and 98.0% of all misses fell in the "mild reaction" categories 0–II.

Overall, we could not observe a significant difference in the response to the darting procedure when animals were hit or missed ($\chi^2 = 1.8$, $P = 0.18$, 1 df). There were also no significant differences in the response to the darting procedure among the four regions for both hits and misses ($\chi^2 = 3.84$, $P = 0.28$, 3 df, and $\chi^2 = 6.85$, $P = 0.08$, 3 df, respectively) (Table 1).

Biopsy Sampling in Shark Bay, Western Australia

A total of 414 attempts were made during the three years of this study. Of these shots, 314 (75.8%) hit, and we missed on 100 attempts (24.2%). A nontargeted dolphin was hit only once when the dolphins were in a very tight group. We biopsied dolphins at estimated distances of 2–10 m (4.6 ± 1.2 m, $n = 408$), and obtained a tissue sample in 303 (96.5%) of 314 strikes. Eleven hits could be divided in three special subcategories. In five of the eleven remaining hits, darts became attached to the animal; in three other cases, the tail piece came off on impact and the dart sank before we could retrieve it. This problem was remedied after the 1997 field season by adding the second partition (Fig. 1a). In the remaining three cases, the biopsy tip was empty when we retrieved it. We categorized the size of the 303 samples as follows: 37 samples (12.2%) were "small" (only small amounts of blubber, weighing less than 0.2 g), 39 samples (12.9%) were "medium" (blubber and parts of subcutaneous layer of skin, weighing between 0.21 and 0.5 g), and we obtained 227 (74.9%) "large" samples (large amount of blubber, subcutaneous layer of skin and epidermis, weighing more than 0.5 g).

We recorded the response for 302 dolphins that were hit and for 88 that were missed (Table 1). We did not include the reaction in the analysis when it was uncertain whether an animal was actually hit without obtaining a sample, or the animal was missed. When an animal was hit, 93.0% of all reactions fell in categories 0–II, and 97.7% in the same categories when we missed (Table 1).

In the five cases where a dart stuck after a biopsy attempt, we followed the animals until the dart was no longer attached. In all instances, the dart dangled while the animal was moving through the water, indicating that only the small tip became embedded and not parts of the dart body. Darts stayed attached from 2 min to 66 min (27.2 ± 28.0 min). Three of these animals reacted mildly to the darting attempt: two showed reaction I, one showed reaction II, and the darts were released after 46 min, 66 min, and 2 min, respectively. One animal reacted strongly (reaction III), but calmed down after one leap, and the dart was attached less than 3 min. The other animal reacted very strongly (reaction IV), leaping three times within the first minute after darting, and then calmed down; the dart stayed attached less than 19 min. In the two cases where we were able to retrieve the dart, the sample sizes were small.

The distribution of the different reaction categories for the animals that we were able to categorize into five age/sex classes is shown in Table 2. Due to small sample size, we pooled reaction classes III and IV (strong reactions). The different age-sex classes showed no significant differences in the reaction to darting ($\chi^2 = 10.29$, $P = 0.59$, 12 df, $n = 286$). We also tested whether the size of the sample taken had an influence on the strength of the reaction to the darting

Table 1. Reaction of dolphins from four different populations to darting. Reaction as described in text. SB = Shark Bay, JB = Jervis Bay, PS = Port Stephens, PL = Patos Lagoon. Numbers given in parentheses are %.

	Hit					Miss				
Reaction	SB	JB	PS	PL	Total	SB	JB	PS	PL	Total
0	0	9 (40.9)	3 (8.1)	3 (25.0)	15 (4.0)	12 (13.6)	8 (44.4)	7 (29.2)	13 (59.1)	40 (26.3)
I	179 (59.3)	11 (50.0)	27 (73.0)	1 (8.3)	218 (58.5)	64 (72.7)	5 (27.8)	15 (62.5)	2 (9.1)	86 (56.6)
II	102 (33.7)	2 (9.1)	7 (18.9)	7 (58.3)	118 (31.6)	10 (11.4)	5 (27.8)	2 (8.3)	6 (27.3)	23 (15.1)
III	8 (2.6)	0	0	0	8 (2.1)	0	0	0	0	0
IV	13 (4.3)	0	0	1 (8.3)	14 (3.8)	2 (2.3)	0	0	1 (4.5)	3 (2.0)
Total	302	22	37	12	373	88	18	24	22	152
Not noted	1	—	—	—		12	2	10	—	

Table 2. Individual responses to darting for five different animal classes in Shark Bay. Reactions I to IV as described in text. Numbers given in parentheses are %. Age data from J. Mann (unpublished).

Age-sex class	Reaction				Total
	I	II	III	IV	
Adult males ≥10 yr	61 (55.5)	42 (38.3)	4 (3.6)	3 (2.7)	110
Adult females ≥10 yr	53 (58.9)	31 (34.4)	1 (1.1)	5 (5.6)	90
Juvenile males 3–9 yr	19 (67.8)	8 (28.6)	1 (3.6)	0	28
Juvenile females 3–9 yr	22 (61.1)	9 (25.0)	1 (2.8)	4 (11.1)	36
Mothers accompanied by their calves	12 (54.5)	9 (40.9)	0	1 (4.6)	22

procedure (Table 3). There was a positive association between size of sample obtained and strength of the reaction ($\chi^2 = 14.70$, $P = 0.02$, 6 df, $n = 302$).

Several factors might influence the size of a sample or the strength of the reaction. We did not observe a significant difference in the size of the biopsy sample or the reaction to the darting when an animal was hit on one particular side of the body ($\chi^2 = 4.35$, $P = 0.11$, 2 df, $n = 292$ and $\chi^2 = 0.14$, $P = 0.94$, 2 df, $n = 291$, respectively). Furthermore, the angle of impact did not have a significant influence on sample size or reaction ($\chi^2 = 4.68$, $P = 0.10$, 2 df, $n = 284$ and $\chi^2 = 2.32$, $P = 0.31$, 2 df, $n = 283$, respectively). However, biopsy samples were significantly larger when an animal was hit below the dorsal fin (5 cm or more) compared to close to or on the fin ($\chi^2 = 79.14$, $P < 0.01$, 2 df, $n = 294$), but there was no significant difference in the strength of the reaction ($\chi^2 = 0.22$, $P = 0.90$, 2 df, $n = 293$).

Biopsy Sampling in Eastern Australia and Brazil

In Jervis Bay, estimated distances for biopsy sampling ranged from 2 to 15 m (7.4 ± 3.4 m, $n = 36$). Two samples were small (9.1%), two samples were medium (9.1%), and 18 were large (81.8%). For both hits and misses, all reactions fell only in categories 0–II (Table 1). In Port Stephens the estimated distance for biopsying was 2–9 m (5.2 ± 1.7 m, $n = 66$); in one case we hit the animal but lost the dart. The samples fell in the following size categories: four small samples (12.5%), two medium (6.3%), and 26 large (81.2%). Similar to Jervis Bay, all reactions we recorded for both hits and misses fell only in categories 0–II (Table 1). In Patos Lagoon, the estimated distance for darting was 4–10 m (6.9 ± 2.1 m, $n = 33$). In comparison to the other study sites, most of the samples were smaller: three samples were small (25%), five medium (41.7%), and four were large (33.3%). When animals were hit, 91.7% of all reactions were 0–II, and 95.5% fell in the same categories when we missed (Table 1).

Assessment of Wound Healing of Shark Bay Animals

The mean number of days until wounds on darted dolphins were observed to be healed was 47.5 ± 24.2 d ($n = 25$). However, this is likely an overestimate

Table 3. Size of biopsy samples obtained at Shark Bay, with corresponding reactions. Reaction and sample size as described in text. Numbers given in parentheses are %.

	Size of sample			
Reaction	small	medium	large	Total
I	25 (69.4)	20 (51.3)	134 (59.1)	179
II	8 (22.3)	17 (43.6)	77 (33.9)	102
III	3 (8.3)	2 (5.1)	3 (1.3)	8
IV	0	0	13 (5.7)	13

of wound healing time, since we were not able to observe the animals on a daily basis. For the four provisioned animals that we could monitor daily, the mean number of days until the wound was healed was 23.3 ± 5.6 d (n = 4).

The wounds or their immediate surroundings appeared to be swollen in 12 out of 43 (27.9%) of the animals. In those cases, swelling still seemed to be present between 12 and 32 d postbiopsy (20.2 ± 6.2 d). Signs of wound infection were not observed in any case. Repigmentation could be observed in four cases. This process was visible starting as early as 36 d postbiopsy (52.5 ± 23.4 d, n = 4). When checked about one year after darting, no scars were visible on the four provisioned animals at Monkey Mia in Shark Bay.

Typical progress of wound healing is exemplified by one provisioned animal that was observed daily. We hit the animal on the left side of the body just lateral to the dorsal fin, at an acute angle slightly from behind. The reaction to the darting procedure was "I" and the sample size was "large." Initially we could observe only a sickle-shaped black mark that probably originated from the edge of the flange, and a dark spot in the center where the sample had been taken. Four days postbiopsy, the area in the center had turned white with the sickle-shaped mark barely visible. Nine days after the darting, the center where the sample was taken appeared red and slightly indented. Eighteen days postbiopsy, tissue was growing towards the center from the edge of the wound and completely covered the wound after 25 d.

Validation of Genetic Markers

For validation of genetic markers we used only samples from Shark Bay. We were able to amplify all microsatellite loci and the d-loop in 302 cases (95.8% of all samples). Repeated amplification and scoring of low-yield samples confirmed that there were no allelic dropouts even in small samples (data not shown). In 310 cases (99.0%), we could genetically determine the sex of the animals.

Development of Dart Design

For ethical reasons, we attempted to minimize the impact during developmental stages. Firstly, we tried various designs of the system on a carcass of a common dolphin. In these trials, we always acquired a sample even if we used a head without inward facing barbs for sample retention; however, trials of those darts on the target species in Shark Bay were never successful (*i.e.,* we never obtained a sample when an animal was hit). Secondly, in field trials, we tried indi-

vidual designs of the cutting head only on a limited number of occasions (up to five hits). If we were not satisfied with the results (for example, the particular design retained mainly small samples, or darts tended to stay attached for more than five seconds), we changed to a different design. Our approach to development makes it impossible to gain sufficient quantitative data for statistical comparison of the success of different designs. However, as a guide for researchers who need to adapt our current system for their own purposes, we feel it is important to provide indications of how subtle design differences might influence the success rate.

A number of factors appeared to influence darting success. We tried different designs for the steel tip and (through trial and error) found that the position and shape of the barbs and the length-diameter ratio of the cutting head affected darting success. If the tip was too long (>8 mm), the darts tended to become attached to the dolphins more often: in three out of five cases the dart stayed attached for more than five seconds. Similarly, if the heads were too wide in diameter (>8 mm) they did not retain the sample as well: in six out of 11 cases the sample size was small. We also tried using an internal barb or a dental broach instead of cutting barbs in the outside of the cutting head as shown in the current design. However, in five cases where we used an internal barb, we obtained only one large sample, and the dental broach broke in both trials on a dead common dolphin. The shape of the internal barbs also seemed important. Triangular barbs appeared to be more effective in retaining the sample than the rectangular barbs used previously. Additionally, triangular barbs could be reset more often than rectangular barbs (in one case, a rectangular barb broke off after being reset about 20 times, while this never happened with a triangular barb). Dart preparation also appeared to be crucial. When we used tips that had not been sharpened, the effectiveness of obtaining samples, as well as the size of the biopsy, seemed to be greatly reduced (see results from Patos Lagoon). Furthermore, we found that it is vital to use charges of high quality. We never observed an inconsistency in power when we used the PAXARMS charges, but charges from another manufacturer were inconsistent in power and led to unpredictable trajectories in four out of 11 cases, possibly because of shelf-time at retailers.

Discussion

Our criteria for an ethically sound darting system were to minimize the distress and wounding caused by the biopsy hits, and to design a system that efficiently and reliably collects biopsy samples sufficient for DNA microsatellite analysis and large enough for contaminant analysis without the necessity of resampling. Several features of our design make this system the best available for darting small cetaceans. The large diameter of the stop helps the dart to bounce free of an animal relatively easy without the need for retrieval systems, which can lead to entanglement problems. The cost-effective modular assembly of the darts allows parts to be exchanged readily to reduce the risk of injury by breaking of worn parts. In a different study using a narrow-barrel dart with a significantly different design, deep penetration has been implicated in the death of a common dolphin (Bearzi 2000). In this particular case, the dart penetrated 5 cm, possibly causing vertebra trauma. It appears that the dart penetrated beyond the

stop. Our design with the wide-barrel body makes deep penetration very unlikely.

Rifles have various advantages over crossbows or pistols. Most of all, they are inherently more accurate. This is crucial when one attempts to dart small delphinids that are frequently found in tight groups. Also, the target area on a dolphin is about 10 cm by 10 cm, so accuracy is crucial in order to avoid or minimize possible injuries. Additionally, the system presented here allows the fine adjustment of the velocity at which the dart leaves the barrel. Therefore, the system could be readily adjusted to different sized species or variation in darting distance due to behavioral preferences of the target animals. During the preparation of this manuscript, our system has also been successfully employed to obtain biopsy samples from Hector's dolphins (*Cephalorhynchus hectori*)[2] and humpback whales (*Megaptera novaeangliae*).[3]

Differences emerged when we compared the four sites. The hit rate was higher in Shark Bay compared to the other sites. The differences can be attributed to several factors. Firstly, the animals in Shark Bay usually came closer to the research vessel than in the other three sites, and usually stayed longer. Secondly, in Shark Bay, unlike the other sites, we could generally see the animals readily before they broke the surface, which gave sufficient time for adequate aiming. The waters in Jervis Bay, Port Stephens, and especially Patos Lagoon are somewhat murky, which made aiming more difficult. Thirdly, darting was carried out by different personnel, which may have contributed to differences in hit rate.

Probably the most important finding is that the animals reacted equally to the darting procedure regardless of being hit or missed; this indicates that the reaction is probably mainly caused by the unexpected disturbance, rather than the biopsy. This finding did not differ significantly among the four regions. Additionally, our system seems to have the same effect on animals irrespective of sex, size, age, or population, making it particularly useful for darting small cetaceans in typical coastal habitats. Our biopsy method produces mainly mild short-term reactions, which is consistent with previous studies using other methods (*e.g.,* Weinrich *et al.* 1992, Clapham and Mattila 1993, Brown *et al.* 1994, Barrett-Lennard *et al.* 1996, Weller *et al.* 1997). Although not systematically studied by us, it appears that the targeted animals have not altered their long-term behavior since they are still easily approachable for systematic surveys and individual follows.[4]

We found a significant association between the size of the tissue sample obtained and the strength of the reaction. Higher impact or deeper penetration of the dart may also have caused a stronger reaction of the animal. Patenaude and White (1995) showed that there is a significant relationship between force of impact and severity of wounds in belugas (*Delphinapterus leucas*). The only parameter that had a significant influence on the size of the sample was the hit point: sample size was significantly smaller when we hit an animal at the base of the dorsal fin or at the fin. We attribute this to the presence of cartilage and lack of under-

[2] Personal communication from Franz Pichler, School of Biological Science, University of Auckland, Private Bag 92019, Auckland, New Zealand, 5 June 2000.

[3] Personal communication from Kirsty Russell, School of Biological Science, University of Auckland, Private Bag 92019, Auckland, New Zealand, 31 May 2001.

[4] Personal communication from Janet Mann, Department of Psychology and Department of Biology, Georgetown University, Washington, DC 20057, U.S.A., 22 June 2001.

lying soft tissue in the dorsal fin, since striking the dorsal fin also resulted in dart damage (breaking, $n = 3$; or distortion at the tip, $n = 4$). Similar to our results, Barrett-Lennard *et al.* (1996) found that the probability of acquiring and retaining a sample was strongly influenced by the location where the dart hit the animal, but, in contrast to our finding, the angle of impact also had a significant influence on darting success.

Even the smaller samples were adequate for many purposes. Almost all biopsy samples were large enough for DNA analyses. Although not tested here, the size of samples seemed to be sufficient for toxicological analysis. Gauthier *et al.* (1997) showed in balaenopterid whales that small blubber samples weighing as little as 0.035 g are adequate to quantify low concentrations of organic contaminants. Hence a sample obtained with the system presented here could be used in such manner provided that the sample is stored appropriately.

Wounds produced by the darting system generally heal quickly and without complications. In a study of bottlenose dolphins (*Tursiops* sp.), Weller *et al.* (1997) showed that relatively large skin biopsies (about 3 × 4 cm) surgically removed from bottlenose dolphins healed rapidly and were covered by epidermis and partly repigmented after 56 d. Corkeron *et al.* (1987) reported that even large wounds inflicted by sharks may heal relatively quickly. The muscles underlying the target area are highly functional locomotive groups, and swelling of the wound or its immediate surrounding raises concern that pain and swelling in this area may affect cost and efficiency of travel. Close examination of the wounds of the provisioned animals showed that swelling had occurred in two animals and the affected area was a circle about twice the area of the dart hit location, indicating that the swelling affected only a relatively small area.

Biopsy sampling of cetaceans has become an invaluable tool for obtaining tissue samples of free-ranging animals for a range of different purposes. If adequate care is taken, our system allows reliable and safe biopsy sampling of small cetaceans. This system could benefit from future developments aimed at a decreased impact to avoid damage of underlying soft tissue. A system without a retrieval line needs to be operated at a relatively high pressure so that the darts bounce free and do not become attached. A tethered system can decrease impact, but there is a relatively high risk that the animals will become entangled, especially for small cetaceans.

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LITERATURE CITED

AGUILAR, A., AND J. NADAL. 1984. Obtención de biopsia hipodérmicas de cetáceos en libertad. Investigatión Pesquera 48:23–29.

ALTMANN, J. 1974. Observational study of behaviour: Sampling methods. Behaviour 79:227–267.

AMOS, W. 1993. Use of molecular probes to analyse pilot whale pod structure: Two novel analytical approaches. Symposia of the Zoological Society London 66:33–48.

AMOS, W., AND A. R. HOELZEL. 1991. Long-term preservation of whale skin for DNA analysis. Pages 99–103 *in* A. R. Hoelzel and G. P. Donovan, eds. Genetic ecology of whales and dolphins. The International Whaling Commission, Cambridge.

AMOS, W., H. WHITEHEAD, M. FERRARI, D. A. GLOCKNER-FERRARI, R. PAYNE AND J. GORDON. 1992. Restrictable DNA from sloughed skin; its potential for use in population analysis. Marine Mammal Science 8:275–283.

BAKER, C. S., M. T. GILBERT, M. T. WEINRICH, R. LAMBERTSEN, J. CALAMBOKIDIS, B. MCARDLE, G. K. CHAMBERS AND S. J. O'BRIEN. 1993. Population characteristics of DNA fingerprints in humpback whales (*Megaptera novaeangliae*). Journal of Heredity 84:281–290.

BARRETT-LENNARD, L. G., T. G. SMITH AND G. M. ELLIS. 1996. A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. Marine Mammal Science 12:14–27.

BEARZI, G. 2000. First report of a common dolphin (*Delphinus delphis*) death following penetration of a biopsy dart. Journal of Cetacean Research and Management 2:217–221.

BROWN, M. R., P. J. CORKERON, P. T. HALE, K. W. SCHULTZ AND M. M. BRYDEN. 1994. Behavioural responses of east Australian humpback whales *Megaptera novaeangliae* to biopsy sampling. Marine Mammal Science 10:391–400.

CLAPHAM, P. J., AND D. K. MATTILA. 1993. Reactions of humpback whales to skin biopsy sampling on a West Indies breeding ground. Marine Mammal Science 9:382–391.

CLAPHAM, P. J., AND P. J. PALSBØLL. 1997. Molecular analysis of paternity shows promiscuous mating in female humpback whales (*Megaptera novaeangliae*, Borowski). Proceedings of the Royal Society of London-Series B: Biological Sciences 264:95–98.

CONNOR, R. C., R. A. SMOLKER AND A. F. RICHARDS. 1992. Two levels of alliance formation among male bottlenose dolphins (*Tursiops* sp.). Proceedings of the National Academy of Sciences, USA 89:987–990.

CORKERON, P. J., R. J. MORRIS AND M. M. BRYDEN. 1987. A note on healing on large wounds in bottlenose dolphins, *Tursiops truncatus*. Aquatic Mammals 13:96–98.

DANIELSDOTTIR, A. K., E. J. DUKE, P. JOYCE AND A. ARNASON. 1991. Preliminary studies on genetic variation at enzyme loci in fin whales (*Baleanoptera physalus*) and sei whales (*Baleanoptera borealis*) from the north Atlantic. Pages 115–124 *in* A. R. Hoelzel and G. P. Donovan, eds. Genetic ecology of whales and dolphins. The International Whaling Commission, Cambridge.

DAVIS, L. G., M. D. DIBNER AND J. F. BATTEY. 1986. Basic methods in molecular biology. Elsevier Science Publishing Co., New York, Amsterdam, London.

DUFFIELD, D. A., AND R. S. WELLS. 1991. The combined application of chromosome, protein and molecular data for the investigation of social unit structure and dynamics in *Tursiops truncatus*. Pages 155–169 *in* A. R. Hoelzel and G. P. Donovan, eds. Genetic ecology of whales and dolphins. The International Whaling Commission, Cambridge.

GAGNEUX, P., C. BOESCH AND D. S. WOODRUFF. 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Molecular Ecology 6:861–868.

GAUTHIER, J. M., C. D. METCALFE AND R. SEARS. 1997. Validation of the blubber biopsy technique for monitoring of organochlorine contaminants in balaenopterid whales. Marine Environmental Research 43:157–179.

GILSON, A., M. SYVANEN, K. LEVINE AND J. BANKS. 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 84:159–169.

HARLIN, A. D., B. WÜRSIG, C. S. BAKER AND T. M. MARKOWITZ. 1999. Skin swabbing for genetic analysis: Application to dusky dolphins (*Lagenorhynchus obscurus*). Marine Mammal Science 15:409–425.

INTERNATIONAL WHALING COMMISSION. 1991. Report of the ad-hoc working group on the effect of biopsy sampling on individual cetaceans. Pages 23–27 *in* A. R. Hoelzel and G. P. Donovan, eds. Genetic ecology of whales and dolphins. The International Whaling Commission, Cambridge.

KRÜTZEN, M., E. VALSECCHI, R. C. CONNOR AND W. B. SHERWIN. 2001. Characterisation of microsatellites in *Tursiops aduncus*. Molecular Ecology Notes 1:170–172.

LAMBERTSEN, R. H. 1987. A biopsy system for large whales and its use for cytogenetics. Journal of Mammalogy 68:443–445.

MILINKOVITCH, M. 1994. Exfoliated cells as the most accessible DNA source for captive whales and dolphins. Marine Mammal Science 10:125–128.

MÖLLER, L. M., AND L. B. BEHEREGARAY. 2001. Coastal bottlenose dolphins from southeastern Australia are *Tursiops aduncus* according to sequences of the mitochondrial DNA control region. Marine Mammal Science 17:249–263.

PALSBØLL, P. J., F. LARSEN AND E. S. HANSEN. 1991. Sampling of skin biopsies from free-ranging large cetaceans in west Greenland: Development of new biopsy tips and bolt designs. Pages 71–89 *in* A. R. Hoelzel and G. P. Donovan, eds. Genetic ecology of whales and dolphins. The International Whaling Commission, Cambridge.

PATENAUDE, N. J., AND B. N. WHITE. 1995. Skin biopsy sampling of beluga whale carcasses: Assessment of biopsy darting factors for minimal wounding and effective sample retrieval. Marine Mammal Science 11:163–171.

RICHARD, K. R., M. C. DILLON, H. WHITEHEAD AND J. M. WRIGHT. 1996. Patterns of kinship in groups of free-living sperm whales (*Physeter macrocephalus*) revealed by multiple molecular genetic analyses. Proceedings of the National Academy of Sciences, USA 93:8792–8795.

SHINOHARA, M., X. DOMINGO-ROURA AND O. TAKENAKA. 1997. Microsatellites in the bottlenose dolphin *Tursiops truncatus*. Molecular Ecology 6:695–696.

VALSECCHI, E., AND W. AMOS. 1996. Microsatellite markers for the study of cetacean populations. Molecular Ecology 5:151–156.

VALSECCHI, E., P. PALSBØLL, P. HALE, D. GLOCKNER-FERRARI, M. FERRARI, P. J. CLAPHAM, F. LARSEN, D. MATTILA, R. SEARS, J. SIGURJONSSON, M. BROWN, P. CORKERON AND W. AMOS. 1997. Microsatellite genetic distances between oceanic populations of the humpback whale (*Megaptera novaeangliae*). Molecular Biology and Evolution 14:355–362.

VALSECCHI, E., D. GLOCKNER-FERRARI, M. FERRARI AND W. AMOS. 1998. Molecular analysis of the efficiency of sloughed skin sampling in whale population genetics. Molecular Ecology 7:1419–1422.

WEINRICH, M. T., R. H. LAMBERTSEN, C. S. BAKER, M. R. SCHILLING AND C. R. BELT. 1991. Behavioural responses of humpback whales (*Megaptera novaeangliae*) in the southern gulf of Maine to biopsy sampling. Pages 91–97 *in* A. R. Hoelzel and G. P. Donovan, eds. Genetic ecology of whales and dolphins. The International Whaling Commission, Cambridge.

WEINRICH, M. T., R. H. LAMBERTSON, C. R. BELT, M. R. SCHILLING, H. J. IKEN AND S. E. SYRJALA. 1992. Behavioural reactions of humpback whales *Megaptera novaeangliae* to biopsy procedures. Fishery Bulletin, U.S. 90:588–598.

WELLER, D. W., V. G. COCKROFT, B. WÜRSIG, S. K. LYNN AND D. FERTL. 1997. Behavioural responses of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing. Aquatic Mammals 23:49–58.

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