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A BIOPSY POLE SYSTEM FOR BOW-RIDING DOLPHINS: SAMPLING SUCCESS, BEHAVIORAL RESPONSES, AND TEST FOR SAMPLING BIAS

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The collection of biopsy samples from free-ranging cetaceans has proven useful for addressing questions regarding population and social structure (*e.g.*, Baker *et al.* 1990), evolutionary relationships (*e.g.*, LeDuc *et al.* 1999), feeding ecology (*e.g.*, Walker *et al.* 1999), and contaminant levels (*e.g.*, Fossi *et al.* 2000). In the past, modified crossbows and rifles have been used to sample both large and small cetaceans (*e.g.*, Weinrich *et al.* 1991, Barrett-Lennard *et al.* 1996, Krützen *et al.* 2002). These systems have been shown to elicit only short-term behavioral responses by sampled animals, and no physiological complications have been reported during wound healing (*e.g.*, Weller *et al.* 1997, Krützen *et al.* 2002). The International Whaling Commission has deemed these methods acceptable because there is no evidence of long-term detrimental effects to sampled individuals or populations (International Whaling Commission 1991). However, such techniques are not without risk. For example, the use of a crossbow has led to the death of a common dolphin (*Delphinus delphis*) in the central Mediterranean Sea (Bearzi 2000). Less invasive techniques to obtain tissue samples from free-ranging small cetaceans are desirable, and other methods developed for this purpose include skin swabbing (Harlin *et al.* 1999) and fecal sampling

(Parsons *et al.* 1999). When selecting a sampling technique, the conservation status of the species and target population, as well as the potential behavioral response of the animals to sampling, should be considered. It is also important to assess if the research question can be answered with the amount of tissue obtained with a specific technique.

One problem with less invasive techniques with regard to genetic studies, such as when assessing population genetic structure, is that DNA may not be of sufficient quality and quantity to carry out the required analyses. Thus, biopsy sampling is often preferred for molecular genetic studies (Parsons *et al.* 2003). Biopsy techniques usually result in samples with sufficient DNA for repeated, multimarker genetic analyses, but obtaining the samples can be time consuming and difficult. For example, when samples of several individuals within one school are required, valuable time may be lost between each sampling attempt while retrieving the dart or arrow from a remote biopsy system.

Here, we present an alternative biopsy sampling technique to the commonly used remote biopsy sampling, the sampling of free-ranging, bow-riding dolphins using a biopsy pole. We investigate sampling success and behavioral responses by dolphins to the procedure, test for sex bias in sampling, and test for a difference in sampling success and behavioral responses when using different types of boats. Biopsy samples were taken from bottlenose dolphins (*Tursiops* spp.) and short-beaked common dolphins (*Delphinus delphis*) in coastal waters of Australia between 2003 and 2005. Coastal bottlenose dolphins from New South Wales (NSW), southeastern Australia, have previously been confirmed to belong to the Indo-Pacific species *Tursiops aduncus* (Möller and Beheregaray 2001), but the taxonomic status of those in southern Australia remains unclear (see Charlton *et al.*, in press). This biopsy sampling is part of a broader program addressing questions of taxonomic status and population genetic structure of bottlenose and common dolphins in Australia, aiming to define management units for conservation purposes. Biopsy samples of both bottlenose and common dolphins were collected in two areas of NSW and two areas of South Australia (SA). In SA, common dolphins were opportunistically sampled during transect surveys conducted as part of a research program by the South Australian Research and Development Institute (SARDI).

The biopsy pole is made of three extendable sleeves that enable length to be adjusted from a minimum of 1.4 m to a maximum of 3.5 m (Fig. 1). The distal part of the pole and the headpiece contain matching threads. A rubber o-ring sits between the end of the pole and the headpiece. The biopsy tip is inserted into the opening of the headpiece, and the head is partially screwed onto the pole. When tightening the headpiece firmly, the o-ring is flattened, reducing its inner diameter and holding the biopsy tip in place. To exchange the biopsy tip, the headpiece is unscrewed approximately two full turns (720°) so that pressure on the o-ring is reduced. Biopsy tips are hollow cylinders that have an incision and "lip" (or barb) that holds the sample in place. Water that flows into the hollow pole through the biopsy tip purges via a hole in the pole's shaft. The hole is oriented astern to allow purging and avoid backflow down the shaft, which prevents the sample from flushing out of the biopsy tip.

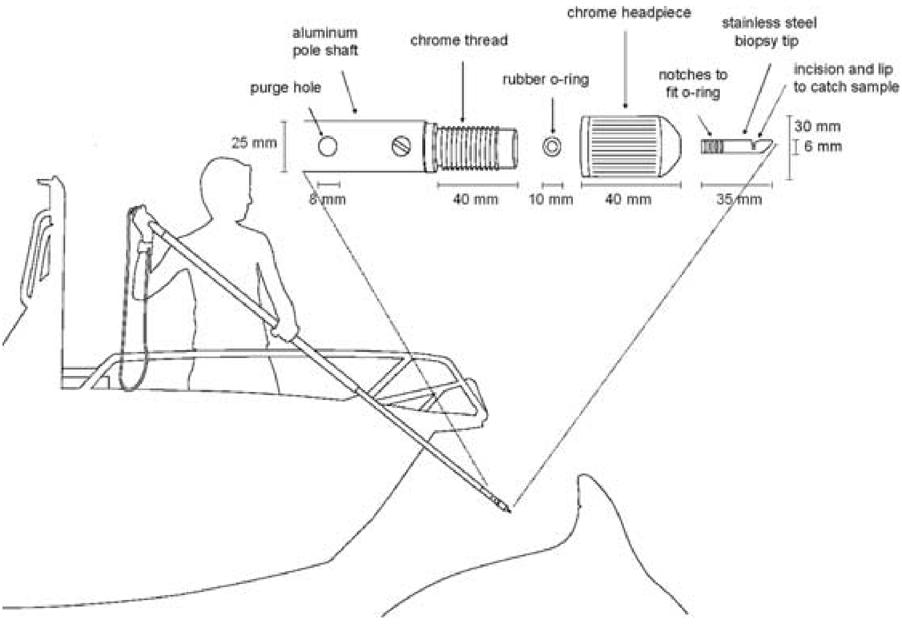

Figure 1. Lateral schematic of technique and detail of distal components of the biopsy pole system including biopsy tip.

For the collection of biopsy samples, we used five different boats of various length, hull type, and engine power, including (1) a 5.6 m aluminum powerboat with a single 90 hp outboard engine, (2) a 6 m aluminum powerboat with twin 115 hp outboard engines, (3) a 16 m vessel with twin 600 hp inboard engines, (4) an 18 m motor-sailing vessel with a 115 hp inboard engine, and (v) a 25 m vessel with a 425 hp inboard engine.

Bow-riding dolphins were sampled when they were close to the water surface (<1-m deep) and occasionally when they surfaced. Sampling was not attempted on calves. Depending on the depth of the dolphin and the speed of the boat, the biopsy pole was either dropped or thrown lightly toward the animal, aiming at the body areas lateral to the base of the dorsal fin. The pole was held at an angle of between 60° and 90° to the water surface, depending on the sampling boat. A constant speed of 7–8 kn was maintained during sampling from the largest boat (while on SARDI transects). Speeds of 2–6 kn were maintained when sampling from all other boats. After a successful sampling attempt, the sampler visually scanned the bow-riding dolphins for the presence of biopsy marks before the next attempt, in order to prevent repeated sampling of individuals. Natural marks such as pigmentation, scars, and fin notches were also used to identify individual animals and to avoid resampling. A second person on the sampling boat recorded the reaction of sampled dolphins and noted if and when individuals returned to the bow after being sampled.

Biopsy samples were approximately 5 mm in diameter and 1 cm in length, including skin and blubber tissues. Samples were stored in 100% ethanol or in salt saturated

20% dimethylsulfoxide (DMSO) and frozen at –20 °C. We extracted total DNA from tissues following a salting-out method modified from Sunnucks and Hales (1996). We then determined the sex of each sampled dolphin by amplifying fragments of the genes ZFX and SRY (Gilson *et al*. 1998) using the polymerase chain reaction conditions described in Möller *et al*. (2001). In addition, samples were used to amplify 12 nuclear microsatellite DNA markers and two fragments of the mitochondrial DNA.

The behavioral response of individuals was recorded whether the sampling attempt was successful or unsuccessful. Behavioral responses were split into five categories, following a protocol modified from Krützen *et al*. (2002). The response categories differed from the original protocol as follows: 0 for no noticeable reaction and the individual continues to bow-ride; 1 for a flinch, but the individual continues to bow-ride; 2 for an individual that accelerates under water and leaves the bow; 3 for an individual that accelerates and leaves the bow followed by a single leap/porpoise; and 4 for an individual that accelerates and leaves the bow followed by multiple leaps and/or porpoises.

Statistical analyses were conducted either using all individuals combined or using four categories based on dolphin species and sampling location: (1) *Tursiops* in NSW, (2) *Tursiops* in SA, (3) *Delphinus* in NSW, and (4) *Delphinus* in SA. Initially, we tested for overall differences in behavioral responses to "hits" (animal was biopsied) and "misses" (pole missed the animal), regardless of sampling location or species. We then used the species–location categories to test for differences in behavioral responses. We tested for sex bias in sampling for each category and also over the entire data set, then combined the categories to test for overall differences in behavioral responses between the sexes. We also investigated, across the entire data set, whether boat type had an effect on the rate of hits and misses. Finally, we tested for differences in behavioral responses when sampling from the different boats, again with categories combined. In order to compensate for small sample sizes in tests that involved behavioral responses, categories 0–1 and 2–4 were combined. For all statistical tests, chi-square contingency analyses ($\alpha = 0.05$) were performed in SigmaStat 3.0 (Systat Software Inc., Point Richmond, CA) or EcStat, an add-in for Excel (Microsoft, WA).

In 195 sampling attempts, 144 biopsy samples were obtained (73.8% success rate). When divided into the four species–location categories, 87.5% of the sampling attempts on *Tursiops* in NSW, 62.5% on *Tursiops* in SA, 77.8% on *Delphinus* in NSW, and 72.5% on *Delphinus* in SA were successful. Behavioral responses were recorded for individuals in each species–location category (Table 1). In total, dolphin behavioral responses to 151 hits and 29 misses were recorded. In four sampling attempts, no biopsy sample was taken and it was unclear whether the animal was missed or hit. These cases were excluded from analyses. The behavioral responses to hits without a biopsy sample being retrieved were recorded on seven occasions. The most frequently observed behavioral response to hits with the biopsy pole was 2, while the second most frequently observed response was 0 (Table 1). No individuals reacted with multiple leaps/porpoises (Category 4; Table 1). When the biopsy pole missed the individual, the most frequently observed behavioral response was also 2 (Table 1). Individuals did not show any other reaction to unsuccessful sampling attempts (Table 1). Two

Tursiops in NSW, five *Tursiops* in SA, and nine *Delphinus* in SA were observed returning to the bow within the first 2 min of being sampled.

No significant difference in response to hits and misses with the biopsy pole was detected with the four species–location categories combined ($\chi^2 = 0.37$, $P = 0.54$, 1 df). Behavioral responses of dolphins, now pooling hits and misses, did not differ significantly among species–location categories ($\chi^2 = 3.52$, $P = 0.32$, 3 df).

The sex of 139 of the 144 sampled dolphins was genetically determined. In five cases, the retrieved samples consisted of only small amounts of blubber and genetic sexing was unsuccessful. In addition, two common dolphins in SA were sampled twice. These double samples were identified through the microsatellite genotypes and the sexing analysis (the individuals showed identical genotypes at all microsatellite loci and were of the same sex). To detect if there was a sex bias in the collection of samples, we used 33 *Tursiops* samples from NSW, 28 *Tursiops* from SA, 21 *Delphinus* from NSW, and 55 *Delphinus* samples from SA. No significant difference from a 1:1 sex ratio was detected for sampled individuals in any of the four species–location categories (χ^2 ranged from 0.003 to 0.07, $P > 0.05$, and df = 1 for all categories), or when categories were combined ($\chi^2 = 0.01$, $P = 0.92$, df = 1). Behavioral responses did not differ significantly between males and females ($\chi^2 = 0.25$, $P = 0.62$, 1 df).

When using different boats for sampling, there was no significant difference in the rate of hits and misses with all species–location categories combined ($\chi^2 = 3.90$, $P = 0.42$, 4 df). However, behavioral responses were significantly different between the five boat types ($\chi^2 = 11.21$, $P = 0.024$, 4 df). Sampling from the two smallest boats generally resulted in stronger behavioral responses, falling exclusively in categories 2 ($n = 26$) and 3 ($n = 7$), and sampling from the largest boat usually resulted in no noticeable response or weaker responses, in categories 0 ($n = 10$) and 1 ($n = 2$).

The results from this study show that the biopsy pole generally elicits mild behavioral responses in the dolphin species studied, whether individuals are hit or missed, and that the different species react similarly to the procedure. There is no sex bias in sampling, and the strength of response also appears to be independent of the dolphin's sex. A similar success rate was attained using different boat types, suggesting

Table 1. Behavioral responses of dolphins to biopsy sampling with the pole system. Response categories: (0) no noticeable reaction and the individual continues to bow-ride; (1) a flinch but individual continues to bow-ride; (2) individual accelerates under water and leaves the bow; (3) individual accelerates and leaves the bow followed by a single leap/porpoise; (4) individual accelerates and leaves the bow followed by multiple leaps/porpoises.

	Hit/miss				
Response	*Tursiops* in NSW	*Tursiops* in SA	*Delphinus* in NSW	*Delphinus* in SA	Total
0	3/1	1/0	3/0	6/3	13/4
1	0/0	1/0	0/0	1/0	2/0
2	31/4	25/14	18/2	51/5	125/25
3	1/0	7/0	1/0	2/0	11/0
4	0/0	0/0	0/0	0/0	0/0
Total	35/5	34/14	22/2	60/8	151/29

that sampling success is possibly not associated with particular boat features, such as height of the bow off the water surface, engine configuration, and capacity. However, behavioral responses do differ between boat types, with smaller boats resulting in stronger responses than larger ones. It is possible that the high proportion of slightly stronger reactions when using the smaller vessels in our study may have resulted from traveling at lower speed during sampling, but this hypothesis requires further testing. Choice of boat, therefore, should be taken into consideration in future pole-sampling studies. In addition, other potential bias in sampling success and behavioral responses that were not possible to test in our study, such as age bias, should also be considered.

The biopsy pole technique presented here is an efficient method for obtaining skin and blubber samples from bow-riding dolphins. The method is cost effective, easy to apply, rapid to deploy and re deploy, and may therefore be useful in situations where remote biopsy guns or crossbows are not practical. The pole can also be used in conjunction with remote biopsy sampling rather than substituting for it. For example, during sampling with a remote system, dolphins may approach the bow of the boat where they can be biopsied using the pole. Once the animals move away from the bow, the sampler may return to using the remote system. The use of both biopsy techniques may significantly reduce the duration of time spent with dolphins for sampling. In addition, when using the biopsy pole, it is not necessary to wait for the dolphins to surface because individuals can be biopsied when they are still underwater (<1-m deep). This provides the sampler with more opportunities to take a biopsy than using only a remote system. Sampling attempts can follow each other in rapid succession because less time is taken to exchange biopsy tips than to retrieve darts, especially when working from large, less maneuverable boats.

Although the pole technique is useful for acquiring samples for studies of population genetic structure and molecular taxonomy, it may be of more limited use when information on the identity of individuals is required, such as in studies of social structure (*e.g.*, alliance formation, Möller *et al.* 2001) and parentage (*e.g.*, Krützen *et al.* 2004). In addition, the usefulness of the biopsy pole method will greatly depend on the frequency with which dolphins ride the bow of vessels. Nonetheless, when dolphins do bow-ride and vessel speed allows for safe sampling, the research vessel does not necessarily need to leave its course or slow down. It can therefore be a suitable method for working from vessels that are undertaking other activities, such as line-transects.

In conclusion, biopsy sampling with the pole method presented here is an effective method for obtaining genetic samples with sufficient DNA for a multiple genetic marker approach, and can be applied in situations were biopsy guns and crossbows are not convenient, or in conjunction with these remote systems.

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