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Effects of Biopsy Sampling on Indo-Pacific Humpback Dolphins (*Sousa chinensis*) in a Polluted Coastal Environment

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Abstract

We conducted a biopsy sampling study of Indo-Pacific humpback dolphins (*Sousa chinensis*) in Hong Kong between October 2004 and January 2006. Humpback dolphins were sampled with a Barnett Ranger RX-150 crossbow. In total, 87 shots were taken at ranges of 8 to 28 m, and 36 tissue samples were collected. The hit rate was 56.3%, and the success rate was 41.4%. There was a better chance of hitting the dolphin with the dart when animals were closer to the shooter (all hits were at < 23 m distance). Humpback dolphin reactions to the procedure were mostly slight, with a few moderate reactions but no extreme ones. Humpback dolphins reacted similarly to hits and misses, and their reaction can best be characterized as a startle response. All reactions were short-term, and there was virtually no evidence of long-term impacts on behavior, social organization, or distribution patterns. Wounds appeared to heal well and were healed over with tissue in < 21 d. When conducted carefully by experienced persons, biopsy sampling of humpback dolphins can be done safely and effectively.

Key Words: sample collection, impacts, behavior, wound-healing, crossbow, Indo-Pacific humpback dolphins, *Sousa chinensis*

Introduction

Over the past several decades, biopsy sampling has become an important and widely-used technique for studying cetaceans in the wild. Early concerns over its potential to cause long-term disturbance and even physical harm to the study animals have largely been allayed by the results of recent studies, which document the short- and long-term reactions of animals to the procedure. In general, baleen whales and larger odontocetes have shown only minor, short-term reactions to biopsy darting (Brown et al., 1991; Clapham & Mattila, 1993; Barrett-Lennard et al., 1996; Gauthier & Sears, 1999; Hooker et al., 2001; Jahoda et al., 2003; Best et al., 2005). This is also generally true for smaller dolphin species, such as bottlenose dolphins (*Tursiops* spp.) (Weller et al., 1997; Krützen et al., 2002), although Parsons et al. (2003) reported a few cases of stronger short-term reactions. Only one report of a death resulting from biopsy sampling has been reported (in this case, a short-beaked common dolphin [*Delphinus delphis*]), despite many thousands of samples collected over many years (Bearzi, 2000). Wound-healing and the potential for injury from the impact of the dart have been less well-studied. Several studies on bottlenose dolphin populations have indicated that the wounds tend to heal-over with tissue in less than a month (reported ranges of 15 to 30 d; Weller et al., 1997; Krützen et al., 2002; Parsons et al., 2003).

A long-term research program on Indo-Pacific humpback dolphins (*Sousa chinensis*) in Hong Kong began in late 1995 and is now in its 13th year (see Jefferson, 2000; Jefferson & Hung, 2004). Enormous progress has been made in learning about the biology of this population of humpback dolphins, but some aspects have remained elusive. In particular, those aspects that rely on sampling of specimens (e.g., population demographics, life history, reproduction, and effects of contaminants) are still poorly known due to the fact that the vast majority of available specimens are strandings that are very badly decomposed (Jefferson et al., 2006). Since this information is so important to the conservation and management of the population, a new approach to the collection of specimen material was deemed necessary. Biopsy sampling was the approach of choice.

Initially, there were concerns about the safety of this technique in this situation because there were no detailed, published reports of biopsy sampling of humpback dolphins (*Sousa* spp.). Additionally, the humpback dolphins live in the Pearl River Estuary, the waters of which have heavy contaminant and organic loads (see Jefferson, 2000; Fu et al., 2003; Peng et al., 2005; Jefferson

et al., 2006). Hong Kong also has one of the highest human population densities on the planet, and sewage is pumped into coastal waters inhabited by the humpback dolphins with only minimal treatment. Therefore, a trial program to test the safety of biopsy sampling on these animals was conducted.

Materials and Methods

We initiated biopsy sampling on this population of humpback dolphins in October 2004. The trial was deemed successful (Jefferson, 2005), and a full-scale sampling program began in late 2005. Biopsy samples were collected through January 2007. Sampling was only attempted when the following conditions were met: (1) weather was good, with adequate visibility and relatively calm sea states of Beaufort 0 to 4; (2) dolphins were behaving in an approachable and predictable manner; (3) sampled individuals could be identified through the simultaneous collection of identification photos and/or video; (4) sampled individual(s) appeared in good health and were behaving normally; and (5) no calves were sampled (although we did sample from some groups with calves in them).

Dolphin groups were generally approached from behind and to the side to minimize disturbance. We maneuvered the vessel close to the dolphin group in the same manner as when taking dolphin identification photos (see Jefferson, 2000). The shooter (TAJ) was stationed on the bow of the vessel. For safety's sake, all other personnel were instructed to stay behind the shooter.

A Barnett Ranger RX-150 crossbow with a 68-kg draw weight was used. This crossbow shoots arrows at a speed of 69 m/s. A Crossman red dot sight was used to assist in aiming. Darts were ACC carbon fiber darts produced by Ceta-Dart (Copenhagen, Denmark), and tips were made at the Scripps Institution of Oceanography (SIO) machine shop (University of California–San Diego, San Diego, CA, USA). The 25-mm tips have a sharpened, beveled leading edge, which acts as a cutting surface, and there are three internal barbs to aid in sample retention. Biopsy tips were immersed in 10% ethanol prior to being attached to the dart to reduce the chances of cross-contamination of samples and of infection. The thoracic area just ahead of the dorsal fin was targeted.

Data sheets documenting environmental conditions, individual involved, dolphin responses, and types of sample collected were completed for each biopsy attempt, whether successful or not. Photographs and/or video documentation were collected for most biopsy attempts. Dolphin responses were classified into several different types, and these are summarized in Table 1.

Results

A total of 87 shots were taken between October 2004 and January 2007, including those from both the trial and main study periods. Shots were taken at estimated distances of 8 to 28 m from the target dolphin (mean = 16.7 ± SD 4.60 m, $n = 86$). Overall, there were 36 hits with sample, 13 hits with no sample, and 38 misses. This corresponds to a hit rate of 56.3% and a success rate (number of samples/number of shots) of 41.4%.

The reactions of the dolphins to biopsy shots were all interpreted to be relatively minor and short-term. No extreme reactions (e.g., breaches or radical changes in the general behavior of the dolphins) were observed. Most dolphins flinched, and some also exhibited a tail-swish or fluke-slap. They generally sped up and swam away from the vessel, but it was possible to approach several sampled individuals closely again within 3 to 5 min of sampling. Some dolphins showed no visible reaction to misses or to direct hits. The reactions of the dolphins were often indistinguishable for hits and misses.

Dolphin responses to the biopsy procedure were analyzed in detail. The results are shown in Figure 1. For hits, more than half of the cases resulted in a slight response (flinch or tail-swish), while the remainder were split between no response and a moderate response (fluke-slap). We used data from hits to test whether dolphins react differently to misses, and the results suggest that they do ($\chi^2 = 6.56$, df = 2, $p < 0.05$). This is

Table 1. Summary classification of dolphin responses to the hits and misses of the biopsy dart

Response	Description	Level of response
None	No obvious reaction is observed	None
Tail-swish	Dolphin makes a slight jerk of the tail, with little or no splash	Slight
Flinch	Dolphin makes a jerk of its entire body, with little or no splash	Slight
Fluke-slap	Dolphin slaps its flukes on water surface, with a splash	Moderate
Flipper-slap	Dolphin slaps its flipper on water surface, with a splash	Moderate
Breach	Dolphin leaps out of the water and falls back with a splash	Major

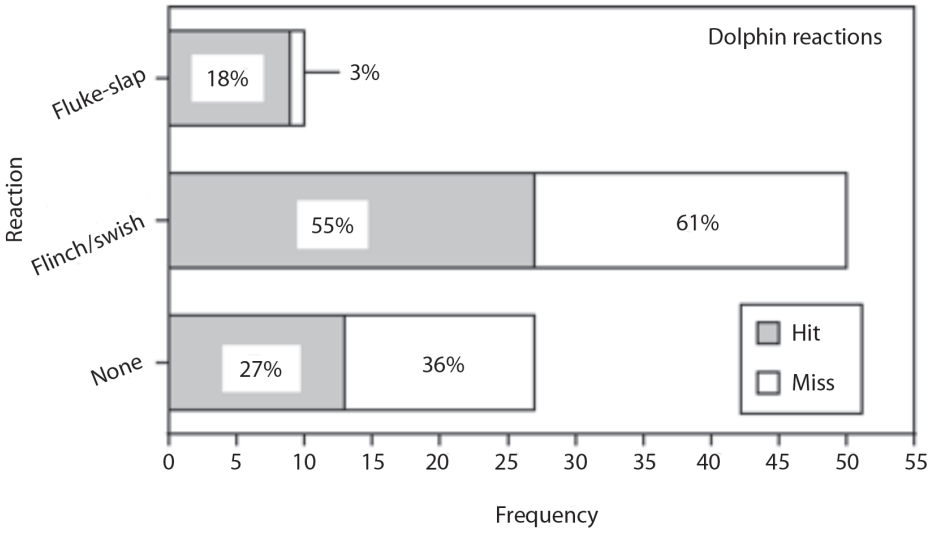

Figure 1. Bar graph showing the reactions of dolphins to hits and misses of the biopsy dart

largely due to a lower probability of a moderate reaction to misses.

We also investigated the effect of distance of the shooter on the dolphin's reaction to both hits and misses (Figure 2). For hits, there was no significant difference among distances for the three types of reactions (one-way ANOVA, F = 0.0701, df = 48, $p > 0.05$). For misses, after excluding the single moderate reaction (which was considered to be an outlier), there was no significant difference in distance for slight and no reactions (t-test, $t = 0.0703$, df = 34, $p > 0.05$).

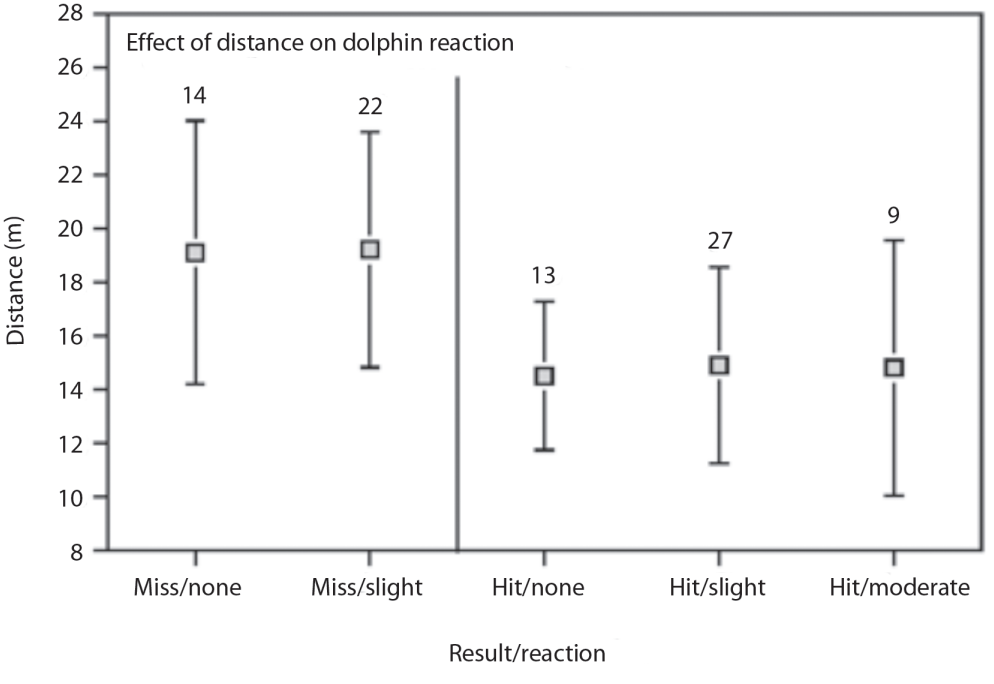

Figure 2. Graph showing average distance (± SD) of various responses in relation to hits and misses of the biopsy dart

Discussion

The above results show that Indo-Pacific humpback dolphins generally react in a broadly similar fashion to biopsy hits and misses (showing mainly a somewhat higher tendency for moderate reactions to hits than to misses) and that for both hits and misses, distance of the target dolphin has very little effect on its reaction to the biopsy dart. This suggests that the dolphin's response (in both hit and miss situations) is a startle response rather than a reaction to any pain or discomfort caused by the impact or penetration of the biopsy dart.

Examination of data from our long-term photo-identification database allowed us to make some useful conclusions regarding the progress of wound healing and the potential for long-term disturbance from the sampling procedure. Photographs taken through July 2007 were examined for this analysis.

Most individuals that were biopsied early in the study were resighted at least once (and many several times) and always appeared healthy and to be behaving normally. The only ones that were not resighted were either sampled most recently (providing little opportunity for resighting) or were individuals that were generally not frequently seen in the photo-ID database. This is consistent with the idea that the biopsy sampling procedure did not have adverse long-term impacts on the study animals.

Most of the known individuals that were sampled have been observed subsequently in Hong Kong waters, ranging from 1 to 814 d later. There were several situations in which it was possible to assess the status of the healing of the biopsy wound (Table 2). The wounds were only observed unhealed within a few days after the sampling, and all observations at 21 d or more after the sampling showed the wound to be completely healed over with tissue.

Individual EL01 was closely approached 15 min after being sampled (suggesting that it was not avoiding the research vessel even minutes after being hit with the biopsy dart), and the biopsy wound was clearly visible as a small black dot but with no apparent bleeding (Figure 3a). This animal was seen on 13 subsequent days since being sampled, in each case within the focal area of its normal range (see Hung & Jefferson, 2004). It was first observed the day after being sampled with NL32 (one of its associates on the day of sampling). It was next observed on 26 January 2005 (about 3 mo after sampling, again with NL32), and the biopsy wound area was completely healed over with tissue and was only visible as a slight depression (Figure 3b).

A particularly instructive case is that of WL26 (HKB15). This male dolphin was sampled on 8 November 2006 and was observed 2 d later, at which time the biopsy wound was clearly visible as a red dot (Figure 4a). When the dolphin was next observed, 21 d after sampling, the biopsy wound appeared to be completely healed over with tissue, and, in fact, it was almost impossible to locate the wound (Figure 4b). This is very similar to results found in Weller et al. (1997).

We can conclude the following from the above analyses and observations:

- Most dolphins respond to both hits and misses of the biopsy dart with a slight reaction, and many show no visible reaction at all (however, some respond to hits with a moderate short-term reaction).
- Within the target range, distance of the target dolphin has no significant effect on the dolphin's reaction to the biopsy procedure, suggesting it is a *startle* reflex.
- Biopsy wounds do not bleed significantly, suggesting that they do not penetrate deeper than the blubber layer (photos taken within minutes and days of sampling show the wounds as small dark dots, with no visible blood).

Table 2. Summary of wound healing progress for sampled individuals during the study

Biopsy date	Individual	Sample	Gender	Status of healing
28 Oct 2004	WL11	HKB1	Female	Wound healed 331 d later
28 Oct 2004	EL01	HKB2	Male	Wound healed 90 d later (small depression)
10 Nov 2004	EL07	HKB5	Male	Wound healed 43 d later
16 Feb 2006	NL181	HKB12	Male	Wound healed 214 d later
8 Nov 2006	WL26	HKB15	Male	Wound visible as small dot 2 d later; healed-over 21 d after sampling
9 Nov 2006	NL225	HKB18	Female	Wound healed 71 d later
29 Nov 2006	NL118	HKB20	Female	Wound visible as small scrape 3 d later
4 Dec 2006	SL37	HKB22	Male	Wound healed 42 d later
11 Dec 2006	NL128	HKB26	Male	Wound healed 35 d later

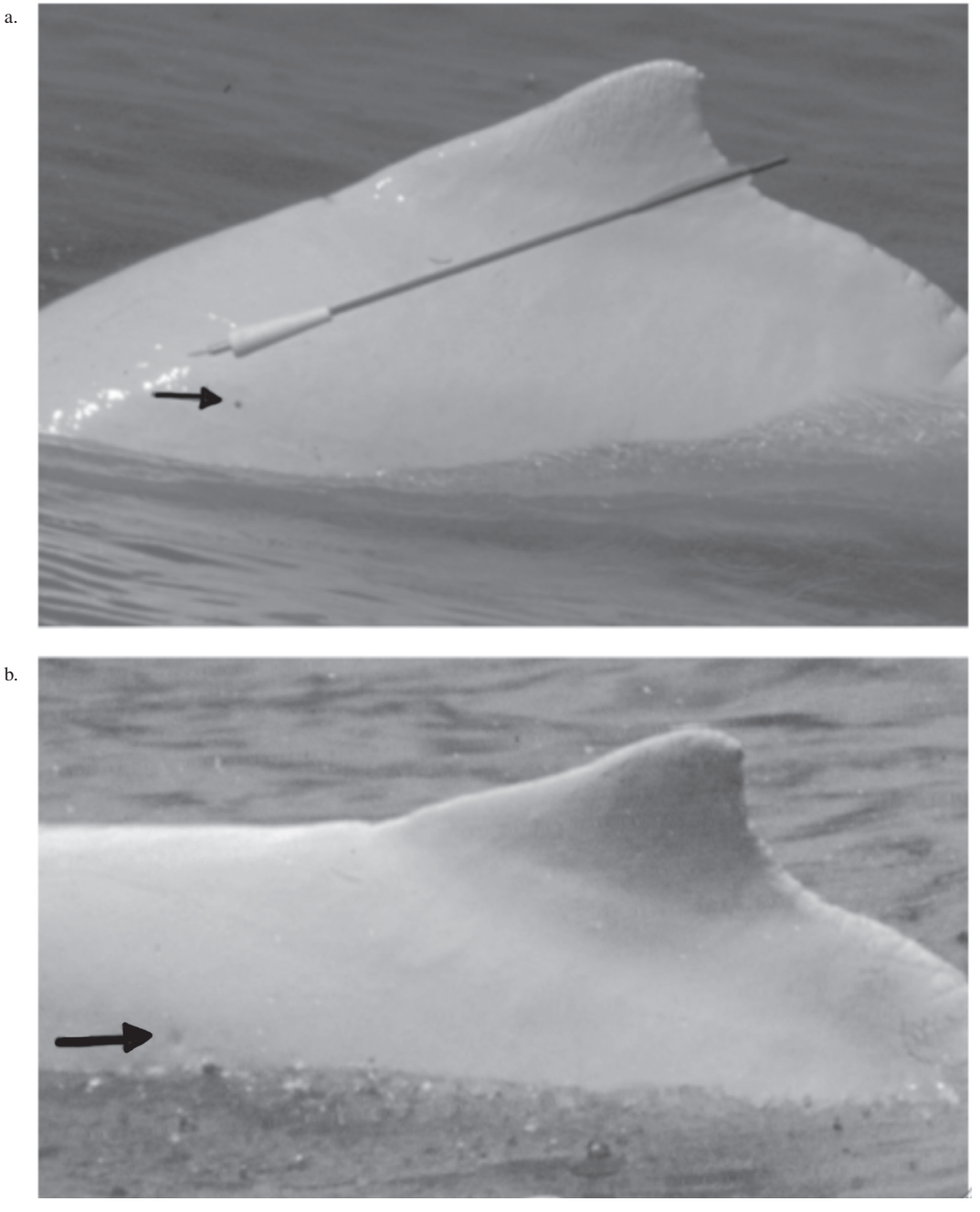

Figure 3. a. A biopsy sample being collected from individual EL01 on 28 October 2004; the biopsy wound can be seen as a small dot just below the float of the rebounding biopsy dart. b. The lower photo shows the same individual on 26 January 2005, 3 mo later; the biopsy wound is healed-over with tissue, and it is visible as a slight depression just above the waterline (indicated by the arrow).

- Biopsy wounds appear to be healed over with scar tissue in less than 21 d (perhaps much less), and the *scars* appear as white dots on a dark background or as a very slight depression on a white background (probably only visible with just the right lighting).

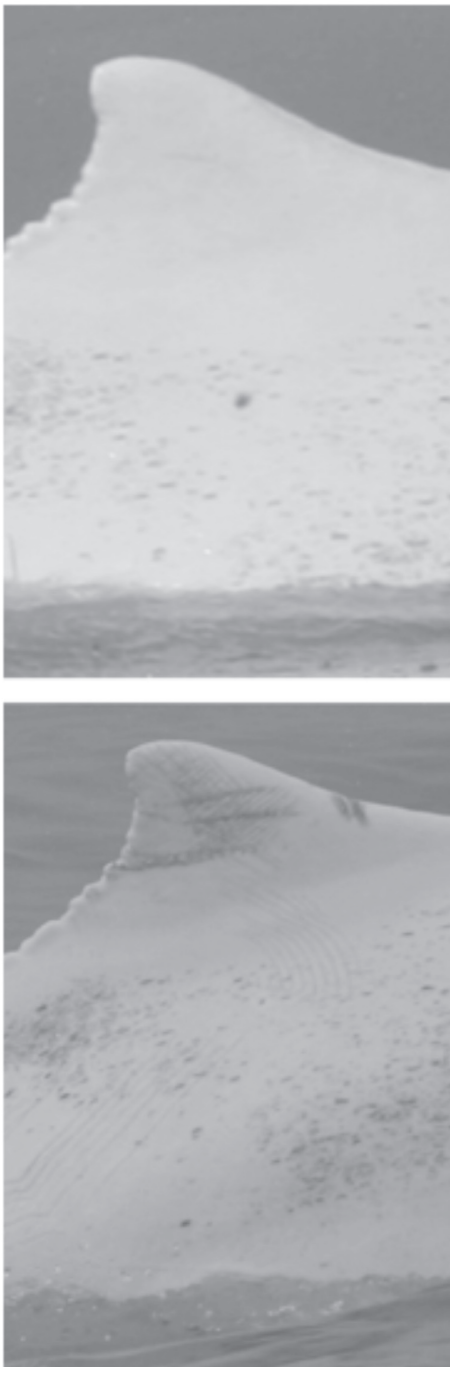

Figure 4. a. Individual WL26 on 10 November 2006, 2 d after being sampled—the biopsy wound is clearly visible as a dot on the side directly below the dorsal fin. b. The same individual 21 d after sampling on 29 November 2006—the biopsy wound appears to be completely healed-over and is barely visible in the photograph.

- There are no significant infections or other problems in the healing of the small wounds (based on long-term observations and photo-documentation).
- Dolphins do not appear to alter their behavior in any discernible way after the initial startle response of being hit by the dart (i.e., they can generally be closely approached again within a few minutes of sampling, and long-term monitoring shows that they continue to use the same areas as they had previously and appear to associate with many of the same individuals).

In conclusion, there is no evidence that the heavy pollution loads in Hong Kong's waters hindered healing of the wounds. In fact, much larger wounds on bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico were covered with tissue within 15 to 26 d in some cases (Weller et al., 1997). Indications more than 3 y after the initial sampling are that the sampled individuals in this study have not changed their behavior or area of occurrence as a result of the biopsy sampling. Although biopsy sampling is slightly invasive, it is by far the least risky and least harmful of the methods available for obtaining tissue from living dolphins. We will continue to monitor the dolphins' activities and the progress of wound healing with the data from our long-term research program.

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Literature Cited

Barrett-Lennard, L. G., Smith, T. G., & Ellis, G. M. (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.

Best, P. B., Reeb, D., Rew, M. B., Palsbøll, P. J., Schaeff, C., & Brandão, A. (2005). Biopsying southern right whales: Their reactions and effects on reproduction. *Journal of Wildlife Management*, *69*, 1171-1180.

Brown, M. W., Kraus, S. D., & Gaskin, D. E. (1991). Reaction of North Atlantic right whales (*Eubalaena glacialis*) to skin biopsy sampling for genetic and pollutant analysis. *Report of the International Whaling Commission, 13*(Special issue), 81-90.

Clapham, P. J., & Mattila, D. K. (1993). Reactions of humpback whales to skin biopsy sampling in the West Indies. *Marine Mammal Science*, *9*, 382-391.

Fu, J., Mai, B., Sheng, G., Zhang, G., Wang, X., Peng, P., et al. (2003). Persistent organic pollutants in environment of the Pearl River Delta, China: An overview. *Chemosphere*, *52*, 1411-1422.

Gauthier, J., & Sears, R. (1999). Behavioral response of four species of balaenopterid whales to biopsy sampling. *Marine Mammal Science*, *15*, 85-101.

Hooker, S. K., Baird, R. W., Al-Omari, S., Gowans, S., & Whitehead, H. (2001). Behavioral reactions of northern bottlenose whales (*Hyperoodon ampullatus*) to biopsy darting and tag attachment procedures. *Fishery Bulletin (U.S.)*, *99*, 303-308.

Hung, S. K., & Jefferson, T. A. (2004). Ranging patterns of Indo-Pacific humpback dolphins (*Sousa chinensis*) in the Pearl River Estuary, People's Republic of China. *Aquatic Mammals, 30*(1), 159-174.

Jahoda, M., LaFortuna, C. L., Biassoni, N., Almirante, C., Azzelino, A., Panigada, S., et al. (2003). Mediterranean fin whale's (*Balaenoptera physalus*) response to small vessels and biopsy sampling assessed through passive tracking and timing of respiration. *Marine Mammal Science*, *19*, 96-110.

Jefferson, T. A. (2000). Population biology of the Indo-Pacific hump-backed dolphin in Hong Kong waters. *Wildlife Monographs*, *144*. 65 pp.

Jefferson, T. A. (2005). *Biopsy sampling of humpback dolphins in Hong Kong, October-November 2004: Final report of the trial program*. Unpublished report submitted to the Hong Kong Dolphin Conservation Society and Agriculture, Fisheries and Conservation Department. 23 pp. [Available from the authors].

Jefferson, T. A., & Hung, S. K. (2004). A review of the status of the Indo-Pacific humpback dolphin (*Sousa chinensis*) in Chinese waters. *Aquatic Mammals*, *30*(1), 149-158.

Jefferson, T. A., Hung, S. K., & Lam, P. K. S. (2006). Strandings, mortality and morbidity of Indo-Pacific humpback dolphins in Hong Kong, with emphasis on the role of environmental contaminants. *Journal of Cetacean Research and Management, 8*, 181-193.

Krützen, M., Barre, L. M., Moller, L. M., Heithaus, M. R., Simms, C., & Sherwin, W. B. (2002). A biopsy system for small cetaceans: Darting success and wound healing in *Tursiops* spp. *Marine Mammal Science, 18*, 863-878.

Parsons, K. M., Durban, J. W., & Claridge, D. E. (2003). Comparing two alternative methods for sampling small cetaceans for molecular analysis. *Marine Mammal Science*, *19*, 224-231.

Peng, X., Zhang, G., Mai, B., Hu, J., Li, K., & Wang, Z. (2005). Tracing anthropogenic contamination in the Pearl River estuarine and marine environment of South China Sea using sterols and other organic molecular markers. *Marine Pollution Bulletin*, *50*, 856-865.

Weller, D. W., Cockcroft, V. G., Würsig, B., Lynn, S. K., & Fertl, D. (1997). Behavioral observations of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing. *Aquatic Mammals*, *23*(1), 49-58.