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Behavioral responses of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing

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Abstract

Information on reactions of delphinids to biopsy sampling and healing of associated biopsy wounds is limited. Results presented here report on the behavioral responses of free-ranging bottlenose dolphins (*Tursiops truncatus*) to remote biopsy sampling procedures, and provide information on stages of surgical biopsy wound repair. Biopsy samples of free-ranging dolphins were collected between February–May 1992 in Galveston Bay, Texas, using a sterilized corer-tipped bolt, launched from a crossbow. A total of 8 direct 'hits' were documented, 4 hits (50%) in which a tissue sample was obtained, 2 hits (25%) from which no sample was retrieved, and 2 hits (25%) in which the bolt struck the water (within approximately 30–60 cm) prior to striking the animal (no samples). Behavioral reactions were similar for all eight dolphins directly struck by a bolt, and were best characterized as startle responses. As part of a NMFS sponsored capture effort to assess a 1992 bottlenose dolphin die-off in Matagorda Bay, Texas, surgical biopsy samples were taken from 35 dolphins (Sweeney, 1992). Biopsies roughly 3–5 cm in diameter and 1 cm deep, were surgically removed. While surgical biopsy wounds were not identical to remote biopsy wounds, they were comparable. Sixteen of the 35 surgically biopsied individuals were photographically resighted between July 1992 and December 1993. Photographs of biopsies immediately after completion of surgical procedures and up to 476 days post-biopsy were classified into four stages of healing. Epidermis appeared to have covered wounds by 40–42 days post-biopsy, but in some cases possibly as early as 15–26 days. Repigmentation of epidermal tissue varied between individual dolphins, but in no cases occurred prior to 61 days post-biopsy. No indication of infection or related pathologies were detected from any of the biopsy wounds monitored during this study. Findings reported here suggest that when adequate care and caution are used, biopsy sampling of bottlenose dolphins is not likely to produce long-term behavioral alterations or result in physiological complications during wound healing.

Introduction

Molecular analysis of biopsy samples collected from cetaceans has proved to be both powerful and effective in providing the genetic data required to create realistic management and conservation policies (Hoelzel & Amos, 1988; Dover, 1991; Hoelzel, 1991; International Whaling Commission (IWC), 1991). While recent photoidentification and behavioral studies have significantly contributed to our understanding of cetacean biology and behavior (IWC, 1991), specific information on stock structure, genetic variability, and rate of genetic exchange among populations can only be assessed by genetic research (Baker *et al.*, 1990; Amos *et al.*, 1993; Amos & Dover, 1991; Dizon *et al.*, 1991; Hoelzel, 1991; IWC, 1991; Wada *et al.*, 1991).

The use of remote biopsy sampling for collection of genetic information is clearly an invasive biological technique, leading to some scientific and public opposition. Of particular concern is the ability to adequately monitor sample site wound healing, related shifts in normal home range patterns or site fidelities and overall changes in behavior (Brown *et al.*, 1991; IWC, 1991; Weinrich *et al.*, 1991).

Alternative approaches to tissue collection are available; however, each has limitations. Samples can be obtained from stranded cetaceans, but questions arise about how representative the location of a beach-cast animal is with respect to its natural range, and irregular subject availability may hinder acquisition of adequate sample sizes.

Temporary capture methods have been successfully used to acquire genetic samples (blood) from bottlenose dolphins (*Tursiops truncatus*) inhabiting shallow waters (Duffield & Wells, 1987; Sweeney, 1992). However, it is logistically more difficult to safely capture and release all but the smaller cetaceans occurring in deep water environments. While the collection of sloughed skin from cetaceans has proved effective in obtaining genetic samples (Whitehead *et al*., 1990; Amos *et al*., 1992; Milinkovitch *et al*., 1994), difficulty in linking tissue samples to specific individuals may be an insurmountable problem for studies on highly gregarious species. Therefore, in many cases, remote biopsy sampling may be the most feasible method for collecting genetic information from free-ranging cetaceans (IWC, 1991).

Several assessments focused on potential effects of remote biopsy sampling on the behavior of large whales have now been completed (Mathews, 1986; Whitehead *et al*., 1990; Brown *et al*., 1991; Weinrich *et al*., 1991, 1992; Clapham & Matilla, 1993; Brown *et al*., 1994; Lambertson *et al*., 1994). Reactions of humpback whales (*Megaptera novaeangliae*) were typically characterized by short-term behavioral changes, usually producing minimal disturbance (Weinrich *et al*., 1991, 1992; Clapham & Matilla, 1993; Brown *et al*., 1994; Lambertson *et al*., 1994). Responses of gray whales (*Eschrichtius robustus*) and north Atlantic right whales (*Eubalaena glacialis*) were generally minimal and short-lived (Mathews, 1986; Brown *et al*., 1991). Sperm whales (*Physeter macrocephalus*) reacted to biopsy darting with short-term startle responses (Whitehead *et al*., 1990). Opportunistic observations on the reactions of blue whales (*Balaenoptera musculus*), fin whales (*Balaenoptera physalus*), and sei whales (*Balaenoptera borealis*) also suggest limited behavioral responses (IWC, 1991).

Information on reactions of delphinids to biopsy sampling is more limited than that for large whales. Killer whales (*Orcinus orca*) in British Columbia reacted to sampling by a momentary 'shake' or acceleration at the surface, but did not perceptibly change activities, group formation, or travel direction after darting (Barrett-Lennard *et al*., 1996). Aguilar and Nadal (1984) reported no significant alterations in swimming patterns for striped dolphins (*Stenella coeruleoalba*) sampled with biopsy darts in the Caribbean. Cockcroft (1995, pers. comm.) collected biopsy samples using a crossbow from approximately 100 bottlenose dolphins and 4 hump-backed dolphins (*Sousa chinensis*) during a four-year-period off South Africa. In all cases, a detectable 'startle' response was observed, but no evidence of longer-term reactions was found, with biopsied dolphins often returning to the bow of the research vessel within a few minutes of darting. Additional accounts of behavioral responses of small dolphins have been described by Aguilar and Hohn (IWC, 1991). Aguilar reported that common dolphins (*Delphinus delphis*), striped dolphins, bottlenose dolphins, and Risso's dolphins (*Grampus griseus*) showed little or no reaction to biopsy samples taken with a hand-held pole, but demonstrated some reaction to a speargun technique, and a strong reaction to samples obtained by crossbow. Hohn (IWC, 1991) reported that *Stenella* spp. and bottlenose dolphins in the eastern Pacific showed either no reaction or temporary vessel avoidance to sampling with a hand-held pole. Finally, although not a biopsy sampling study, Würsig (unpublished data) found that bowriding dusky dolphins (*Lagenorhynchus obscurus*) reacted variably when small tags were hand-lanced into the blubber just below the dorsal fin. Of 24 individuals tagged, all flinched and curved away from the bow; 12 dolphins returned to ride the bow wave within 5 to 60 s while the other 12 were not known to ride again during the observation period; see Würsig (1982) for tag description.

On the basis of such reports, the International Whaling Commission (1991) concluded that biopsy sampling of individual cetaceans was not likely to produce any long-term deleterious effects. It was recommended, however, that biopsy studies be combined with ongoing photoidentification investigations to effectively monitor potential longitudinal effects on known individuals.

Research presented here reports on the behavioral responses of free-ranging bottlenose dolphins to remote biopsy sampling, and attempts to evaluate aspects of biopsy wound healing by use of photo-identification methods. The latter of these two objectives was achieved primarily by using data collected from bottlenose dolphins surgically biopsied during a 1992 National Marine Fisheries Service (NMFS) Southeast Fisheries Science Center (SEFSC) temporary capture effort in Matagorda Bay, Texas.

Methods

Remote biopsy sampling

Remote biopsy sampling of free-ranging bottlenose dolphins was conducted from small research vessels (<7 m) between February–May 1992 in Galveston Bay, Texas (29°20′N, 94°40′W). Samples were collected using a 7 mm diameter and 3 cm long corer-tipped bolt, launched from a 45 kg pull crossbow at approximately 15 m from the target animal. Prior to each sampling attempt the corer-tip was cleaned in hydrogen peroxide, sterilized, and dipped in a broad spectrum liquid antibiotic. To prevent the corer tip from penetrating too deeply, all but approximately 4 mm of the corer-tip shaft was sheathed in 2 cm diameter surgical rubber tubing which served as a stop and caused recoil. The corer-tip was beveled at the outer edge to aid penetration and was equipped with a single backward pointing internal barb for sample retention. Each biopsy sample consisted of two components, epidermal skin and blubber.

Behavioral reaction data were collected *ad-libitum* (Altmann, 1974) by onboard observers, and on Hi-8 mm videotape taken of the target animal immediately before and after biopsy sampling. Frame by frame video analysis and event coding were conducted by the senior author. Response levels were qualitatively coded following categories used by Mathews (1986) for gray whales. Categories included: (1) No Reaction—dolphin continued pre-biopsy behavior with no detectable change in conduct; (2) Low-level Reaction—dolphin modified behavior in a mild fashion; (3) Moderate Reaction—dolphin changed behavior in an observable but short-term manner; and (4) Strong Reaction—dolphin behavior dramatically modified. Only biopsy attempts in which sufficient visual and video documentation was obtained ($n=13$) were included in this report.

Surgical biopsy sampling

As part of a NMFS/SEFSC sponsored capture effort to assess the 1992 bottlenose dolphin die-off in Matagorda Bay, Texas (28°30′N, 97°20′W), surgical biopsy samples were taken from 35 free-ranging dolphins (Sweeney, 1992; G. Worthy, Texas Marine Mammal Stranding Network, 1994, pers. comm.). Diamond shaped biopsies roughly 3–5 cm in diameter and 1 cm deep, were surgically removed from approximately 10 cm behind and 10 cm to the left side of the posterior aspect of the dorsal fin, see Sweeney (1992) for surgical methods. Following surgical procedures, wounds were covered in Betadine soaked gauze until the dolphin was released. No additional antibiotics or other prophylactic treatments were administered. Each tissue sample consisted of two components, epidermal skin and blubber. All dolphins captured at this time also received dorsal fin roto-tags and ten dolphins were fitted with radio transmitters (Sweeney, 1992; Würsig & Lynn, 1995). In some cases, biopsy wound healing was compared with same-animal ratio-tag and roto-tag wound healing (Appendix 1). Tag attachment techniques are summarized in Würsig and Lynn, 1995.

Assessment of wound healing

Photoidentification was used for individual recognition and to document healing of remote and surgical biopsy wounds. Boat-based photoidentification methods and analysis techniques followed those described by Defran *et al*. (1990) and Würsig and Jefferson (1990). While remote biopsy wounds and surgical biopsy wounds were not identical, they were comparable. Surgical biopsies were considerably larger in area, but similar in penetration and location to samples collected from free-swimming dolphins. Thus, follow-up information from one remotely biopsied dolphin was combined with longitudinal photographic and observational data of 16 surgically biopsied dolphins to establish the database from which wound healing assessments were made.

Photographic data of recognizable individuals in Matagorda Bay were analyzed for the period of July 1992–December 1993. Slides of surgical biopsy wounds were examined with an 8 × loupe. Observations of the size, shape, and coloration of the wounds were recorded and changes in these parameters were compared longitudinally. Caliper measurements of various aspects of the wounds were derived from enlarged projected images. These relative measures were scaled to known size dorsal fin features obtained from capture photographs in which a vertical and horizontal measuring scale was displayed behind the fin. Wound healing stages were qualitatively classified as: Stage 0—'fresh wound'; Stage 1—'early wound healing'; Stage 2—'intermediate wound healing'; Stage 3—'late wound healing'; and Stage 4—'complete wound healing' (Table 1).

Results

Remote biopsy sampling

A total of 8 'hits' were documented, 4 hits (50%) in which a tissue sample was obtained, 2 hits (25%) from which no sample was retrieved, and 2 hits (25%) in which the bolt struck the water prior to striking the animal (no samples). Behavioral reactions were similar for all eight dolphins struck by the bolt, and were best characterized as Moderate Reaction startle responses. As a safety precaution, attempts to sample only from the dorsolateral flank were made. Strikes in this area caused an apparent stereotypic behavior sequence and response. As a target animal approached the surface, the rostrum generally broke the air/water interface first, followed in turn by the melon, blowhole, and dorsal fin. At this point, the target dolphin was usually struck by the biopsy bolt. In each case, the response of the target animal involved a rapid upward tail flick, resulting in the flukes breaking free of the water and producing an explosive splash. This reaction was accompanied by an increase in swim speed and a departure from its pre-biopsy course and location relative to the research vessel. While the basic reaction for each of the dolphins sampled was nearly identical, video analysis revealed that three dolphins hit by the bolt rolled laterally

Table 1. Qualitative stages of surgical biopsy wound healing

Stage	Days post-biopsy	n[1]	Pooled observations and descriptions for individual dolphins
0	0	2	Oval shaped wound, deeply pink to red in color, several mm deep. No other apparent discoloration.
1	8–18	4	Oval shaped wound, pinkish to white in color. Darker spot (5.2–5.6 mm diameter) at center of wound. Skin at edge of wound darker than surrounding normal skin (3.2–4.3 mm band). Wound surrounded by lighter gray halo fading into normal skin.
2	15–26	3	Pinkness absent. Oval shaped wound, white in color. Darker spot (4.7 mm diameter) at center of wound. Lighter gray halo surrounding wound in 5.1 mm band.
3	40–42	2	White spot. No other discoloration. No apparent epidermal depression.
4	⩾61–476	18	Normal or nearly normal pigmentation. Wound may be indented a few mm. Appearance varies from indistinguishable to slightly lighter or darker than surrounding normal skin.

[1]Number of individuals photographed in corresponding elapsed time period. Some individuals contributed data to more than 1 stage (see Appendix 1).

towards the point of impact on the skin, while one dolphin rolled laterally away from the point of impact. Five 'misses' were also recorded, 2 (40%) elicited Low-level Reactions in the form of the target animal diving and the school moving away from the research vessel at normal speed, while 3 misses (60%) produced No Reactions.

Photo-documentation of wound healing from dolphins sampled via crossbow in Galveston Bay was limited to one, a well-known individual named 'Octe'. Octe has been repeatedly photographed in the Galveston study area since 1987 (Fertl, 1994). On 28 February 1992, a biopsy sample was taken from Octe's upper dorsum posterior to the base of the dorsal fin. Nineteen days later on 19 March 1992, Octe was resighted during a routine survey of dolphins in Galveston Bay. The wound left by the biopsy corer was clearly visible, but appeared to be healing (Stage 2), with no sign of infection or pathological complications. Octe has subsequently been photographed numerous times in the Galveston study area (as recently as August 1995) and observation of the biopsy wound site clearly showed complete healing, a return to normal pigmentation, with only a slight epidermal depression remaining.

Surgical biopsy sampling

Sixteen of the 35 dolphins surgically biopsied in Matagorda Bay were photographically resighted between July 1992–December 1993. Resighting rates for these individuals ranged from 1–4, spanning 8–476 days (Appendix 1). A total of 27 post-biopsy photographs were of sufficient size and quality to allow for the assessment of wound healing. Observations from these 27 photographs, plus 2 photographs of 'fresh' biopsies immediately after completion of surgical procedures, are grouped into four stages of healing (Table 1). All 16 dolphins demonstrated some degree of wound healing between each photographic resighting, and no evidence of wound infection or repair complications were observed. Epidermis appeared to have covered wounds (as judged by the smooth appearance of the wounds and absence of any pinkish coloration) as early as Stage 2 but most commonly occurred by Stage 3. Repigmentation of epidermal tissue varied between individual dolphins, but in no cases occurred prior to Stage 4.

Dolphin FB517 (Appendix 1) provided the shortest documented progression from Stage 0 to Stage 4 healing, that of 61 days. FB517 was collected freshly dead (attributable to intestinal infarction, mortality unrelated to capture and biopsying) by the Texas Marine Mammal Stranding Network on 13 September 1993 in the Matagorda Bay region (D. Cowan, University of Texas Medical Branch, 1994, pers. comm.). Laboratory examination of the surgical biopsy wound showed complete healing and repigmentation with the exception of a slight 1–2 mm epidermal depression (see Appendix 1). The 61 day healing period for FB517 is merely an estimate of temporal stages of wound repair, it is possible that Stage 4 healing had been completed earlier but that the sensitivity of our photographic method was unable to detect it.

Discussion

Behavioral responses

Results from this study indicate that behavioral reactions of individual bottlenose dolphins to remote biopsy procedures were moderate and of limited duration. The most obvious and consistent reaction of biopsied dolphins was a startle response, similar to that observed for bottlenose and hump-backed dolphins (Cockcroft, 1995, pers. comm.), dusky dolphins (Würsig, unpublished data), killer whales (Barrett-Lennard *et al*., 1996), and sperm whales (Whitehead *et al*., 1990).

Startle responses of the type described in this study do not appear to be stimulus specific or uncommon for bottlenose dolphins. An illustrative example of startle behavior being non-stimulus specific comes from an observation during remote biopsy sampling efforts in Galveston Bay. In this case, four dolphins simultaneously surfaced near the research vessel; when the target animal (closest to the vessel) was struck by the biopsy bolt, it clearly startled as did its two closest surface affiliates. The target animal obviously responded to the stimulus of the bolt strike, while its two affiliates appeared to respond to an alternative stimulus, the reaction of their sampled affiliate. Startle reactions have been observed by the authors in captive bottlenose dolphins when they are 'spooked' by unexpected or novel stimuli. In free-ranging situations, vessel approaches, changes in vessel speed, and the shifting of outboard motors from neutral have all been noted to elicit startle responses. Dolphins also commonly appear to startle in response to one another when no apparent anthropogenic disturbance is present. Thus, the startle response observed during this study appears to be a relatively common and generalizable part of the bottlenose dolphin behavioral repertoire.

Shifts in range characteristics and behavior

Only one of the eight remotely biopsied dolphins was photographically resighted. This low resighting level suggests a possible abandonment of normal ranging patterns. However, Galveston Bay is characterized by both residential and transient dolphins and findings from this region over the past five years show limited site fidelity and low resighting levels to be typical for a majority of the $\sim$1500 dolphins identified (Henningsen, 1991; Bräger, 1992; Fertl, 1994; Weller, unpublished data). Bräger (1992) reported that of 1045 dolphins identified in Galveston Bay between 1990–1991, 72% (n=754) were never resighted. Therefore, we suggest that the low number of resightings for biopsied dolphins is a common sighting characteristic rather than actual shifts in range characteristics. It is also possible that as photographic data between 1992–1995 are analyzed, resighting rates for this subset of dolphins will increase. The single individual, Octe, we successfully resighted after biopsy sampling has been mostly residential to the study area over the past five years (Fertl, 1994; Weller, unpublished data). Octe has displayed no obvious signs of avoiding the sampling vessel, has remained within recognized pre-biopsy range limits and continues to associate with known affiliates.

Matagorda Bay is also populated by residential and transient dolphins (Gruber, 1981; Lynn, 1995; Würsig & Lynn, 1995). Sixteen of the 35 dolphins temporarily captured and surgically biopsied in July 1992 were regularly resighted through December 1993. Although this subset of dolphins was subjected to the relatively noxious and potentially traumatic situation of temporary capture, these individuals did not abandon their apparent home range (Appendix 1), and showed no detectable avoidance of the research vessel.

Wound healing

A major concern surrounding the use of remote biopsy sampling as a biological technique has to do with the adequate monitoring of sample site wound healing. Bottlenose dolphins worldwide are subjected to a variety of wound inducing stimuli, but appear to be generally quite capable of recovering from such injuries. For example, cookie cutter sharks (*Isistius brasiliensis*) often inflict wounds on bottlenose dolphins equivalent to or larger than either type of biopsy wound reported here (Jones, 1971; Norris *et al*., 1994), and many populations are subjected to attempted predation by larger sharks, often leaving massive crescent shaped and occasionally disfiguring wounds on their bodies (Corkeron *et al*., 1987; Cockcroft *et al*., 1989; Cockcroft, 1991). Field observations, stranding records, and necropsy reports for bottlenose dolphins in Texas coastal waters indicate that cookie cutter and other shark inflicted wounds heal in most cases without complications or incidence (Weller, unpublished data; Texas Marine Mammal Stranding Network, unpublished data). Finally, conspecific interactions, other marine mammals, and various anthropogenic activities such as boats, nets, and fishing gear have all been reported as sources of wounds for bottlenose dolphins, and several studies have monitored and documented the healing of such wounds (e.g. Lockyer & Morris, 1985; Morris & Lockyer, 1988; Lockyer & Morris, 1990; Bloom & Jager, 1994; Fertl, 1994). The successful healing of these larger traumas in nature is perhaps one of the strongest arguments for survivability following biopsy wounds. Descriptions of surgically induced wound healing in captive bottlenose dolphins (Bruce-Allen & Geraci, 1985) also closely parallel the various stages of wound repair reported here (see Table 1).

Photoidentification proved effective in the longitudinal monitoring of wound healing for residential dolphins but was of limited value for dolphins sighted only occasionally. Repeated resightings of Octe provided the novel opportunity to monitor the healing of a remote biopsy wound from a free-ranging bottlenose dolphin. Observations of Octe showed that within 19 days of the sampling event, the associated wound had substantially healed with no sign of infection.

The successful photographic monitoring of dolphins surgically biopsied provided a larger basis and more adequate sample size from which our wound healing assessments were derived. Remote biopsy wounds differed from surgical wounds in that unmeasurable levels of blunt trauma resulting from bolt strike were inflicted on target animals, a wounding variable clearly absent in the surgical method. However, while surgically induced biopsy wounds and remote biopsy wounds were not identical, we believe that the former served as an appropriate surrogate for the latter in terms of tissue removal. No indication of infection or related pathologies were detected from any of the biopsy wounds monitored during this study. Radio-tag wounds healed slower than the biopsy wounds but went through similar, albeit delayed, stages of repair (Appendix 1).

Conclusions

The results reported here suggest that when adequate care and caution are used, biopsy sampling of bottlenose dolphins is not likely to produce long-term behavioral alterations or result in pathological complications during wound healing. Biopsy sampling has proved to be an invaluable research tool in the study of cetacean biology, and will most certainly become a methodological staple in most field studies, particularly those involving threatened or endangered populations where genetic information is imperative for the creation of conservation strategies. While the behavioral reactions of cetaceans studied to date have been moderate and of limited duration, the technique is nonetheless biologically invasive. Therefore, efforts such as those of Patenaude and White (1995) to refine sampling procedures, reduce physiological trauma to the target animal and increase sample collection effectiveness are critically important.

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