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Effects of Remote Biopsy Sampling on Long-Finned Pilot Whales (*Globicephala melas*) in Nova Scotia

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Abstract

A long-term photo-identification study on long-finned pilot whales (*Globicephala melas*) off Pleasant Bay, Nova Scotia, Canada, expanded to include remote biopsy sampling via crossbow in 2010 to 2012. The present study aims to investigate any negative effects biopsy sampling may have on the animals. During sampling, each shot was videotaped for later analysis. We ranked the reaction of the target individual on a standard scale, where 1 was no response, 2 was a low-level response, 3 was a moderate response, and 4 was a strong response. Additionally, in the 2012 field season, we recorded group behaviour before and after sampling and opportunistically observed wound healing. Short-term responses to sampling were mostly low level, with no strong responses observed. Sampling did not change group behaviour any more than was normally observed from non-biopsy vessels, and the pilot whales were regularly re-approached by vessels post-sampling without difficulty. Wounds were found to close as early as 4 d post-sampling and showed no evidence to indicate infection or other problems with healing. This study found no indication that remote biopsy sampling has detrimental effects on long-finned pilot whales in Nova Scotia and, thus, is a viable and ethical technique for obtaining samples from this population.

Key Words: remote biopsy sampling, long-finned pilot whale, *Globicephala melas*

Introduction

The collection of DNA from free-ranging cetaceans has been increasingly used to better understand and manage populations. One of the most commonly used methods is remotely deployed biopsy darts that retrieve small tissue samples containing skin and/or blubber. The method has provided samples that have been vital in studies of cetacean phylogeny, population biology, social structure, contaminant load, feeding ecology, and other areas of biology for more than 40 species (Noren & Mocklin, 2012). Although a variety of biopsy propulsion tools exist, the most regularly used are airguns and crossbows. Biopsy sampling is often used in tandem with photo-identification techniques, allowing for DNA to be assigned to particular individuals and, thus, allowing a means to combine the power of genetic analysis with individual-based observational or photographic data to learn about aspects of animal biology that neither approach can address independently (Lambertsen, 1987; Parsons et al., 2003; Frasier et al., 2007).

Concerns have been raised that biopsy sampling is unnecessarily invasive, leading many studies to formally assess and describe the effects of sampling (e.g., Weinrich et al., 1991; Barrett-Lennard et al., 1996; Krützen et al., 2002; Cantor et al., 2010). A wide range of studies have found the majority of reactions to biopsy sampling to be low or moderate such as a startled shake, a dive, a small tail flinch, or a twitch (Noren & Mocklin, 2012). Only a small percentage of responses have been characterized as strong or extreme; these rare instances have commonly been associated with unintended issues during sampling such as a biopsy dart remaining lodged rather than rebounding (Weinrich et al., 1991; Noren & Mocklin, 2012). Many studies have reported no indication of long-term impacts on cetaceans from biopsy sampling with respect to behaviour, social interactions, distribution, and/or vessel avoidance (Noren & Mocklin, 2012); however, the death of a common dolphin (*Delphinus delphis*) was reported following sampling that was attributed to vertebral stress resulting from the dolphin's unusually thin blubber layer (Bearzi, 2000). Extensive studies conducted on larger cetaceans have never recorded such events; common dolphins may therefore represent a threshold in size under which biopsy sampling should be used with particular care.

While there are several compilations of the effects of biopsy sampling, findings are both species- and population-specific, with further variation existing between individuals (Noren & Mocklin, 2012). For example, humpback whales (*Megaptera novaeangliae*) sampled in the western South Atlantic never responded strongly to sampling, showed no difference in response between a hit and a miss, and had a stronger response when in smaller groups (Cantor et al., 2010). In contrast, 3.3% of humpbacks sampled in Maine responded strongly; there was a stronger response observed when the whales were hit than missed; and group size was not found to influence reaction level (Weinrich et al., 1991). Such variation of results indicates that one cannot generalize the findings of the limited number of studies on biopsy sampling to encompass all cetaceans. The effects of this potentially harmful technique should therefore be re-examined with each new species and population sampled to ensure population-specific viability.

Long-finned pilot whales (*Globicephala melas*) that inhabit the waters off Pleasant Bay, Nova Scotia, Canada, in the summer have been studied for over a decade via photo-identification from whale-watching vessels (Ottensmeyer & Whitehead, 2003; Auger-Methe & Whitehead, 2007; Nemiroff & Whitehead, 2009; Senigaglia & Whitehead, 2012). The Pleasant Bay study has recently expanded to include remote biopsy sampling with the goals of determining sex, gaining a better understanding of genetic relatedness within the population through molecular genetic analysis, and examining foraging behaviour through stable isotope analysis. Currently, there is little information on how remote biopsy sampling affects long-finned pilot whales, but a previous study of a population in the Strait of Gibraltar suggests that the physical effects are minimal (Gimenez et al., 2011). The aim of the present study is to investigate the physical and behavioural effects of remote biopsy sampling on long-finned pilot whales in Pleasant Bay.

Methods

Field Research

Remote biopsy sampling of long-finned pilot whales took place in July and August of 2010 to 2012 along the northwest coast of Cape Breton Island, Nova Scotia, Canada. The research vessel, a semi-rigid 4.5-m inflatable zodiac, departed from Pleasant Bay harbour (46° 49' 58" N, 60° 47' 46" W) and travelled the coastline ranging from 40 km south to 30 km north of Pleasant Bay while remaining within approximately 8 km of shore. Trips were completed in mornings (0600 to 0930 h) and evenings (1900 to 2130 h) at times when no whale-watching vessels were operating but there was still sufficient light and a Beaufort Sea State no greater than 4. During the remainder of the day (0930 to 1900 h), photo-identification took place from a 13-m whale-watching vessel that travelled the same range as the sampling vessel.

When a group of pilot whales was sighted (as defined by a 200-m chain-rule), the research vessel approached it parallel to the animals' direction of movement. We noted whether any pilot whales within the group (1) had a uniquely marked dorsal fin that could be photo-identified and matched to the existing database of the Pleasant Bay pilot whales (Auger-Methe & Whitehead, 2007), and (2) had not previously been sampled. If no identifiable and nonsampled pilot whales were found in the group, we resumed the search until a different group was located. When a sampling candidate was chosen, the vessel travelled parallel to the individual, maintaining a separation of approximately 7 m. Once we acquired a sufficiently high-quality photograph of the target individual's dorsal fin, we deployed a sampling dart to retrieve a skin and blubber sample. The shooter aimed for the dorsal-lateral region directly below, and slightly posterior to, the dorsal fin. We did not attempt sampling when the behaviour of the animal was unpredictable (e.g., socializing) or when a clear shot of the targeted individual was not possible.

Until 11 August 2012, we used an Excalibur Vixen II crossbow with a draw weight of 68 kg; for the remainder of the 2012 field season, we used an Excalibur Apex with a draw weight of 40 kg. The switch to a less powerful crossbow was to reduce both the damage to the arrows and the force hitting the pilot whales. Sampling darts were obtained from Finn Larson (CETA-DART, Denmark), and a detailed design can be found in Palsbøll et al. (1991). Darts included a 2 cm × 0.7 cm sampling tip that contained three prongs to prevent the loss of samples and a hole for air escape. Behind the tip, a compressed foam stop collar functioned to prevent further penetration of the dart, causing the dart to rebound off the pilot whale and flotation of the dart for sample retrieval. Between sampling events, tips were cleaned with detergent and brushes, rinsed, and then soaked in ethanol for sterilization. Tips were individually wrapped in tinfoil so that they could be screwed onto the dart without touching the tip with fingers. The tinfoil cover was then removed just prior to a sampling attempt.

Sampling shots were videotaped with a Canon FS20 video camcorder, and we recorded the following data for each sample: date and time, sample number, names of the individuals on the vessel, name of vessel, location (latitude, longitude), Beaufort Sea State, location of hit on the targeted individual, behavioural reaction of the targeted individual, how many shots were attempted, whether skin and/or blubber were collected, whether a video was successfully taken, and the frame numbers of all photos that captured the target individual. In 2012, we expanded data collection to include the behavioural state of the group before and after sampling. Photographs of wounds from the biopsy darts were opportunistically taken in 2012 to investigate wound healing time.

We used scan samples to record predominant group behavioural state immediately before and after biopsy sampling. Behavioural states were categorized as resting, foraging, travelling, or socializing. Resting was assigned to a group when the majority of individuals were at the surface with little to no activity, moving slower than the idling speed of the boat (3.5 to 4 kts). A group was classified as foraging when individuals were moving in different directions at the surface and spending long periods of time diving. Travelling was characterized by the majority of individuals actively swimming in the same direction faster than the idling speed of the boat. Finally, we defined a group as socializing when the majority of individuals were actively interacting, which includes body rubbing and spy hopping (i.e., holding a position partially out of the water with rostrum and melon exposed).

We ranked the behavioural reaction of target individuals to a sampling shot from 1 to 4 with 1 being no response and 4 being a strong response (Table 1). The ranking system reflects that used by Noren & Mocklin (2012) who found this to be a sufficiently standard system across the majority of remote biopsy sampling studies. We added specific elements to the definitions to make the system more applicable to long-finned pilot whales (Table 1). To better capture the full range of reactions, we ranked intermediate responses as 1.5, 2.5, and 3.5 as has been done in other biopsy studies (Best et al., 2005). In the field, behavioural reactions were noted only when samples were successful; such field observations were at times unreliable due to the fast pace of the work. Therefore, we later reranked all behavioural reactions to sampling attempts based on closer examination of video recordings in order to (1) increase the reliability of our results and (2) incorporate behavioural reactions of both successful and unsuccessful shots. This resulted in the alteration of 17% of in-field rankings.

Analysis

We classified sampling shots as to whether the sampling dart missed the target individual, the dart struck the individual but no sample was collected, or the dart successfully collected a sample. We used Mann-Whitney U tests with a significance level of $\alpha = 0.05$ to test for a difference in the response of the pilot whales to biopsy attempts across the three success levels. A separate Mann-Whitney U test was used to test for a difference in response levels between the two crossbows used in 2012. In an effort to investigate potential bias in ranking individual responses, five volunteers with a range of experience with marine mammal behavioural analysis analysed a subset of 20 sampling videos and ranked the response of the pilot whales using the definitions seen in Table 1. We compared each set of rankings to those we assigned from the same videos using a Spearman's Rank Correlation Coefficient.

The proportion of times that group behaviour state changed following sampling was compared to the proportion of times that group behaviour state changed when sampling did not occur as observed from a whale-watching vessel during the collection of photo-identification data. A chi-squared test ($\alpha = 0.05$) tested the null hypothesis that rate of change of behavioural state was the same when sampling took place and during the whale watches.

Results

Individual Behavioural Response

Immediate individual behavioural responses (as determined by video analysis) ranked by five volunteers were strongly or very strongly correlated to the ranks assigned here ($r_s = 0.80, 0.87, 0.74, 0.78$, and 0.66, respectively).

In total, we attempted 138 sample collections from 2010 to 2012, resulting in the successful collection of 84 tissue samples: 24 in 2010, 8 in 2011, and 52 in 2012. Behavioural response analysis was not possible for two of the sampled individuals because the reactions were not recorded in the

Table 1. Rankings of responses of individual long-finned pilot whales (*Globicephala melas*) to remote biopsy sampling

Rank	Category	Definition
1	No response	Individual continues pre-biopsy behaviour
2	Low response	Brief, mild response (e.g., flinch, twitch, small tail swish with little to no white water)
3	Moderate response	More forceful, but not prolonged, response (e.g., tail slap, flipper slap, breach)
4	Strong response	Succession of forceful activities (e.g., multiple tail slaps or swishes, multiple flipper slaps, consecutive breaching)

field or with video (occurred once in 2010 and once in 2012). An additional five reactions were not recorded with video (occurred in 2010) and another three were video recorded but the behavioural response was out of frame (occurred in 2012); in these instances, response rankings were based on comments made on field data sheets.

In 2010-2012, 60 to 70% of behavioural reactions were ranked as 2 or lower when pilot whales were struck with a dart regardless of whether a sample was collected (median rank = 2) or not (median rank = 2). In contrast, 100% of responses to missed darts were ranked as 2 or lower (median rank = 1) (Figure 1). The response rankings differed significantly ($p < 0.0001$) when individuals were missed compared with when they were hit both when a sample was collected (z = 7.58) or a sample was not collected (z = -4.17). Pilot whale response to being struck was not significantly different when tissue was collected compared to when it was not (z = 0.79; $p = 0.21$) (Figure 1).

Although a greater percentage of response rankings showed no effect on individuals when the less powerful crossbow was used (27%) than when the more powerful crossbow was used (8%) in 2012, the response rankings did not differ significantly between the two deployment devices (z = 0.89; $p = 0.3734$). However, detecting such a difference is currently limited by the small sample size associated with the use of the less powerful crossbow.

Group Behavioural Response

The proportion of group behavioural state that changed following sampling did not differ significantly from changes that took place when sampling did not occur, whether the initial behavioural state was travelling ($X^2 = 2.49$; $p = 0.115$), resting ($X^2 = 1.93$; $p = 0.164$), or foraging ($X^2 = 1.91$; $p = 0.167$) (Figure 2).

Calf Response

We observed calves responding to the sampling of nearby individuals on three occasions, each of which were stronger responses than that of the target individual. In one instance, the target individual was successfully sampled and reacted with a brief tail swish (rank = 2). The calf swimming on the target individual's nonsampled side reacted with a more forceful tail swish (rank = 2.5) and vocalized briefly. On another occasion, the dart missed the target individual that had no reaction (rank = 1), landing in nearby water at which time a calf swimming beside a different adult reacted with a small tail flick (rank = 2). In addition, three tail slaps of a calf (rank = 4) followed the successful sampling of a target individual that showed no behavioural response (rank = 1). While the tail slaps were directionally toward the vessel, they did not take place until approximately 20 s post-darting; the calf did not appear to be in contact with the target individual; and social behaviour was noted in the group before and after

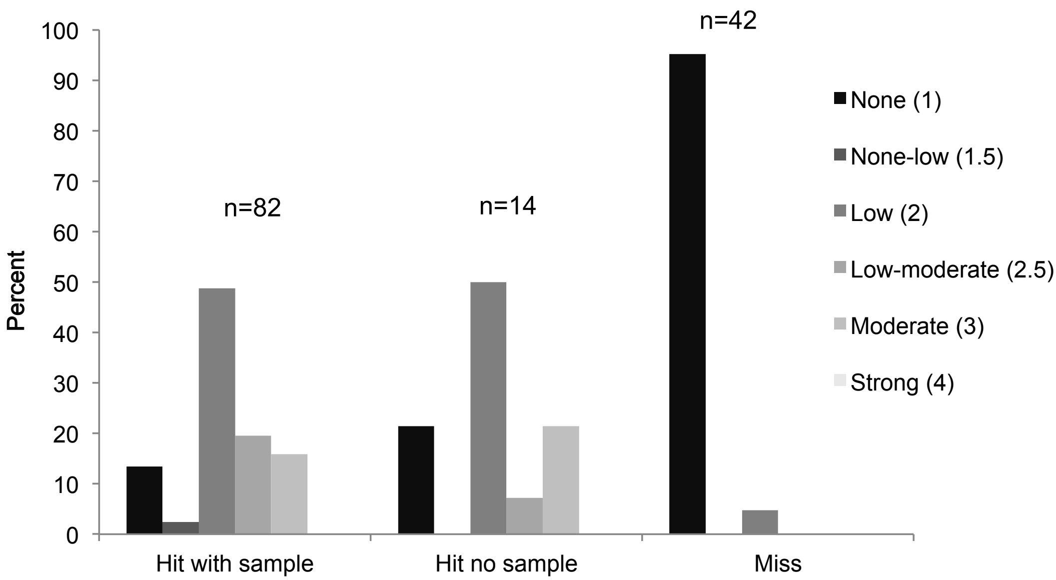

Figure 1. The percent of each level of behavioural response (ranked from 1 to 4) that occurred when long-finned pilot whales (*Globicephala melas*) were struck by a remotely deployed biopsy dart and a biopsy sample was collected (hit with sample); struck by a dart, but no sample was collected (hit no sample); and when the dart missed the target individual, landing in nearby water (miss) in July and August of 2010 to 2012

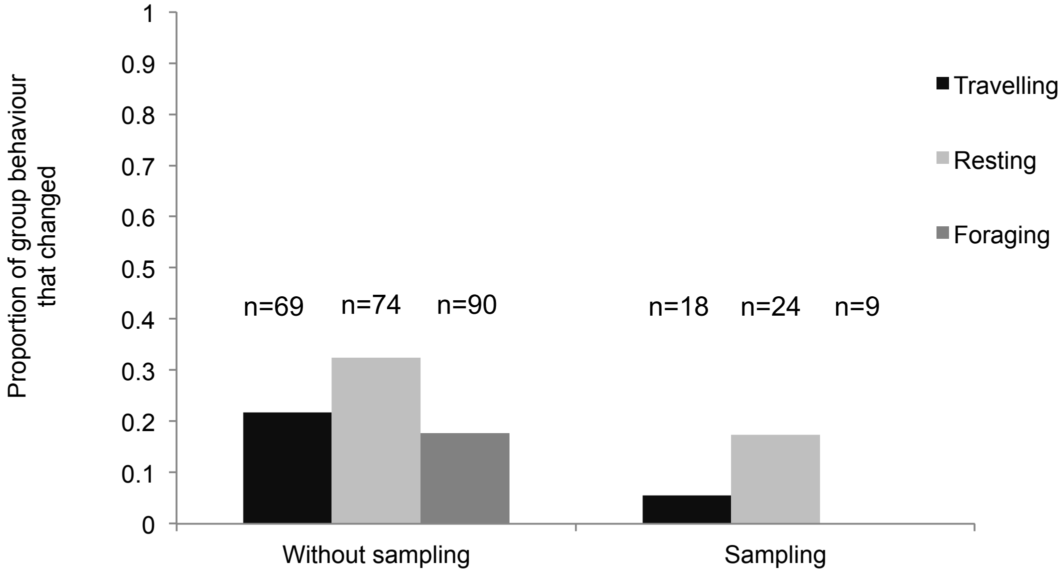

Figure 2. The proportion of group behaviour that changed when no sampling took place (every 5 min) and immediately following remote biopsy sampling in terms of travelling, resting, and foraging in July and August of 2012

sampling, making it difficult to determine if this was a reaction to sampling.

Wound Healing

Observations of wound healing were limited as only 11 post-sampling photos of wounds were collected, none of which were of the same individual. We observed wounds to close as early as 4 d post-sampling with the onset of scar tissue. After 9 d of healing, the majority of tissue taken had redeveloped; and by 17 d, only white scar tissue was left no larger than is typically seen on pilot whales (Figure 3). We noted minor bleeding of a wound on one occasion in early 2012 from an individual that reacted with a low-level response (rank = 2). In this instance, the sample was collected from the lower-mid region of the dorsal fin.

In 2012, 12 long-finned pilot whales that had been sampled in either 2010 or 2011 were photographed on their previously sampled side; no wounds were apparent on these individuals (Figure 4).

Boat Aversion and Resightings

On 10 occasions in 2012, we were able to re-approach a group of pilot whales within 10 min post-sampling and successfully sample a second individual. At least 23 sampled individuals were re-approached by vessels post-sampling in 2012 with no apparent stress or boat aversion observed: 12 individuals that had been sampled in the 2010 and 2011 field seasons, and 11 individuals that had been sampled within the 2012 season.

Resampling

Our goal was to use on-site assessments of individual IDs to ensure that no individuals were sampled more than once; however, after full analysis of the photo-identification data, there was evidence of resampling on one individual on three different occasions: once on 25 August 2010 on the right side (response rank = 2.5), once on 29 July 2012 on the right side (response rank = 2), and once on 30 July 2012 on the left side (response rank = 2). We found no other cases of resampling.

Discussion

Immediate reactions to sampling, if any, were brief and mostly low level. We observed no strong responses. These findings are similar to those from other studies of the effects of remote biopsy sampling on other cetacean species, including short-finned pilot whales (*Globicephala macrorhynchus*), bottlenose dolphins (*Tursiops* spp.), humpback dolphins (*Sousa chinensis*), and killer whales (*Orcinus orca*) (Barrett-Lennard et al., 1996; Jefferson & Hung, 2008; Oremus, 2008; Tezanos-Pinto & Baker, 2011).

Targeted individuals responded more strongly when they were hit than missed with a sampling dart as almost all missed individuals showed no reaction. While Weinrich et al. (1991) also found this when sampling humpback whales, a number of studies observed no difference in response between hit and missed individuals, leading the authors to suggest that the animals were showing

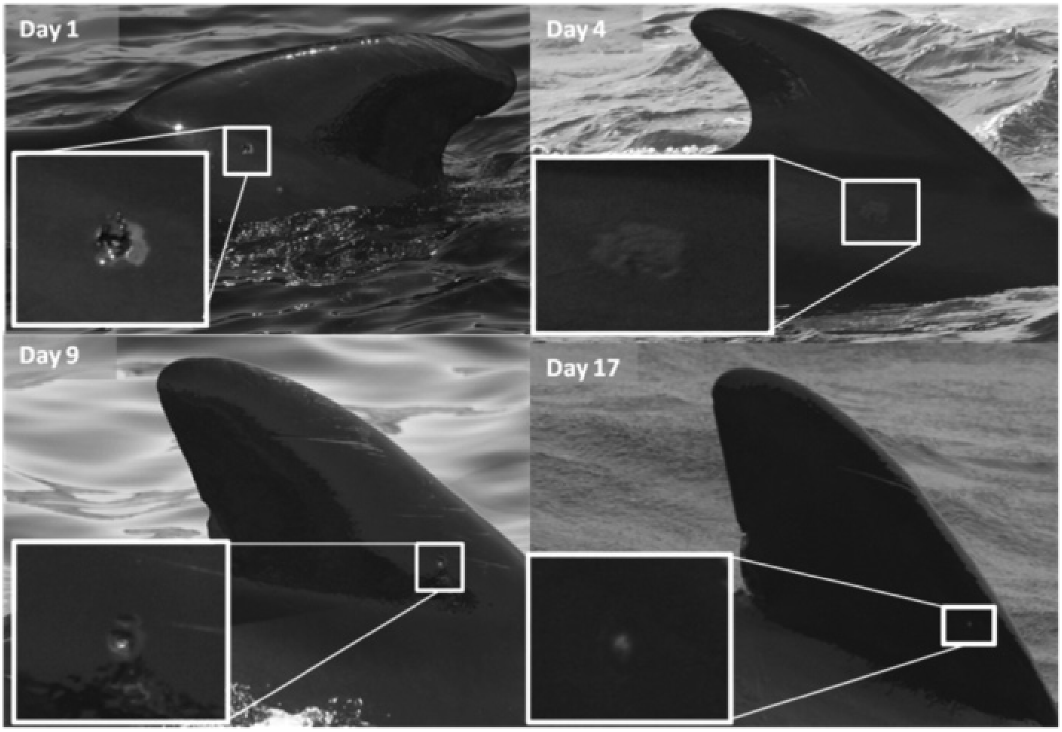

Figure 3. Dorsal fins and magnified images of wounds of long-finned pilot whales after 1, 4, 9, and 17 d of healing following remote biopsy sampling in 2012

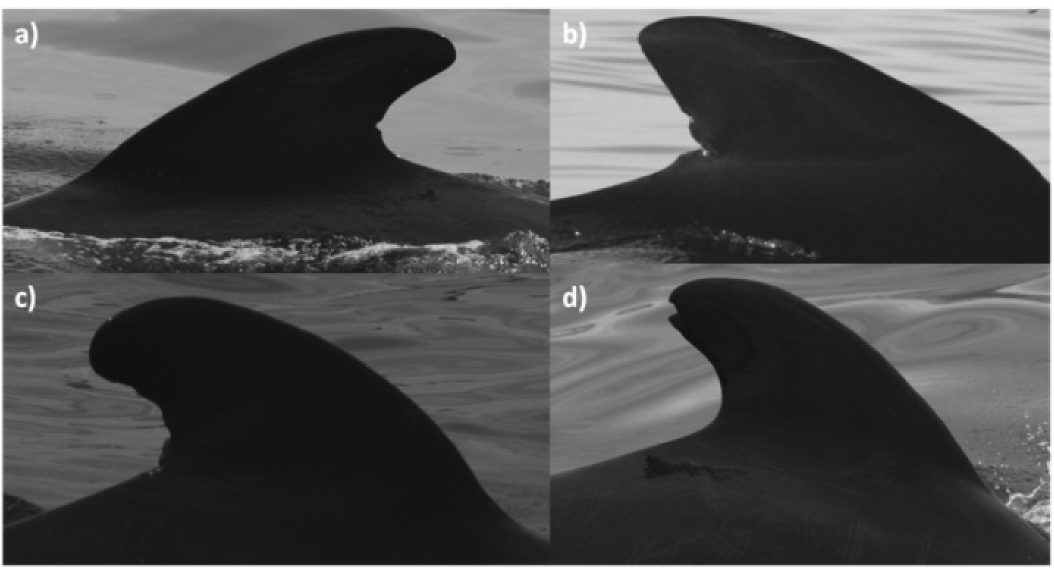

Figure 4. Photographs taken in 2012 of individuals sampled in 2010 (a and b) and 2011 (c and d) in which the images display the same side that the individual was sampled on 1 to 2 y prior; no wounds were apparent on these individuals.

a startle response, not a pain response, to the biopsy (Krützen et al., 2002; Bilgmann et al., 2007; Jefferson & Hung, 2008; Cantor et al., 2010; Kiszka et al., 2010; Tezanos-Pinto & Baker, 2011). The lack of response to misses we observed may be a reflection of the long-finned pilot whales residing in tightly knit groups commonly containing more than 15 individuals. They are likely accustomed to sudden splashes in the water by conspecifics, especially playful calves, and therefore do not have a surprise response when a dart hits the water. The behavioural reactions of pilot whales were not found to differ when they were struck by a dart, and a sample was successfully collected as compared to when they were struck but no sample was collected. This may suggest that they are reacting to the sudden impact and not to the removal of tissue.

Behavioural reactions of remote biopsy sampling by nontarget individuals have been reported by a number of studies, all of which found the occurrence to be rare (Barrett-Lennard et al., 1996; Weller et al., 1997; Gorgone et al., 2008; Kiszka et al., 2010). Similarly, we definitively observed reactions of nontarget individuals only twice; however, unlike previous studies, in both cases the nontarget individuals responding were calves. In one instance, the adult that the calf was swimming with responded to sample collection with a small tail swish that likely startled or even hit the calf, leading to a large tail swish and vocalization of the calf. On another occasion, a calf responded to a dart striking the water nearby. This was likely a startle response as it took place on a calm day at a time when the group of pilot whales was not particularly active. While such occurrences were rare and the reactions brief, these findings suggest that long-finned pilot whale calves might be more sensitive to sampling than adults, even when they are not targeted. Some agencies go as far as prohibiting the sampling of adults with young calves (Brown et al., 1994); however, no negative effects of biopsy sampling were found on southern right whale (*Eubalaena australis*) cow-calf pairs in terms of reproduction or calf survival (Best et al., 2005).

The healing of biopsy wounds appears to occur quickly and without complications such as swelling or infection. These conclusions, however, are limited by the opportunistic nature of our study. Even so, the findings are congruent with a more thorough study undertaken on long-finned pilot whale biopsy wounds in the Strait of Gibraltar that found wounds to have closed in only 4 d post-sampling, to be covered by new epidermis after 60 d, and to be repigmented in under a year (Gimenez et al., 2011). The repigmentation may explain why the wounds of individuals sampled in 2010 and 2011 were not observed in 2012. Similarly, in bottlenose dolphins, wounds heal within 23 d; and in humpback dolphins, wounds heal in less than 21 d (Krützen et al., 2002; Jefferson & Hung, 2008). Pilot whales are likely tolerant to minor trauma inflicted by a biopsy dart as they face equal or greater damage regularly from intraspecific aggression and other causes (International Whaling Commission [IWC], 1991). One study found long-finned pilot whales to possess an average of 19 different markings on their bodies, almost all of which heal so efficiently that they were deemed unsuitable for photo-identification techniques (Auger-Methe & Whitehead, 2007).

Indicators commonly used to assess impacts of remote biopsy sampling include change in behavioural state, the pilot whales' behaviour towards vessels post-sampling, and the frequency at which sampled individuals are resighted. Long-finned pilot whales did not show an increased rate of change in behavioural state when being biopsy sampled relative to when being followed by whale-watching boats; they showed no aversion to vessels immediately following sampling; and biopsied individuals were resighted hours, days, weeks, and years post-sampling. These findings are in accordance with other studies that concluded that there was no indication of long-term effects of biopsy sampling on cetaceans (Weinrich et al., 1991; Barrett-Lennard et al., 1996; Jefferson & Hung, 2008; Cantor et al., 2010; Kiszka et al., 2010; Tezanos-Pinto & Baker, 2011).

In summary, we successfully retrieved 84 tissue samples of long-finned pilot whales from 138 biopsy sampling attempts. Behavioural responses to biopsy sampling were brief, if any, and mostly low level. Pilot whales were re-approached by vessels regularly following sampling without noticeable aversion. Wounds were observed to heal quickly and without complication. While no evidence that would indicate long-term impacts of biopsy sampling has been found here, or in the available literature, long-term impacts are the most difficult to examine as they may be masked by other factors such as the effects of research vessels (Noren & Mocklin, 2012). We recommend that the effects of sampling be re-evaluated on the present population regularly to better assess any potential long-term impacts. Based on the present findings, remote biopsy sampling appears to be a viable and ethical technique to study the long-finned pilot whales that frequent the summer waters of Pleasant Bay, Nova Scotia. The method will potentially shed light on aspects of pilot whale society and biology that are currently poorly understood in free-swimming populations, including group social structure through sexing of individuals, gaining a better understanding of genetic relatedness within the population through molecular genetic analysis, and examining foraging behaviour through stable isotope analysis.

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