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A quantitative analysis of the response of short-finned pilot whales, *Globicephala macrorhynchus*, to biopsy sampling

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Remote biopsy sampling has been used for almost 40 yr (Winn *et al.* 1973) to obtain tissue samples for various analyses, and is particularly important because most cetaceans are difficult or impossible to capture safely at sea (Noren and Mocklin 2011). It is generally assumed that there are few, if any, detrimental effects on the fitness of sampled animals. In support of this assumption, there has only been one documented case of death of a cetacean from a biopsy dart (Bearzi 2000), although it is possible deaths have occurred that have not been described in the published literature. Failure of the dart stopper and the poor body condition of the common dolphin (*Delphinus delphis*) were believed responsible for the death of this animal, as the dart penetrated through its thin blubber layer.

Many studies have reported qualitative and semiquantitative descriptions of the reactions of individual whales and dolphins to biopsy attempts. In most cases, descriptions of the response to biopsy sampling used qualitative and sometimes subjective observations, all limited to the behavior of sampled animals at the surface. Furthermore, the criteria used to assess response varied across studies, hindering comparisons across species and sites. Recently Noren and Mocklin (2011) performed an extensive and very useful review of the behavioral and physical reactions of mysticetes and odontocetes to biopsy attempts and standardized categories of behavioral response. They concluded that most odontocetes exhibit a low level of response (*e.g.*, short-term startle response, immediate dive, increase of speed), while low and moderate levels of responses were equally prevalent for mysticetes. Their review also revealed that wounds from biopsy darts typically heal quickly, with no signs of infection.

It is apparent from Noren and Mocklin's (2011) review that there has been little quantitative analysis of the behavioral reactions of cetaceans to biopsy sampling due

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to the difficulties of observing animals underwater after a biopsy attempt has been made. Most prior evaluations of the reaction of cetaceans to biopsy sampling have relied on observations made from onboard the sampling vessel immediately following impact of the biopsy dart and seldom provide information regarding the subsequent subsurface behavior of the animal. Digital acoustic recording tags (Johnson and Tyack 2003), or DTAGs, provide a novel opportunity to collect information on the subsurface behavior of cetaceans following biopsy sampling. We used DTAGs to examine the behavioral responses of short-finned pilot whales, *Globicephala macrorhynchus*, to biopsy attempts by quantifying behavior of animals prior to and following biopsy attempts. We examined five categories of behavior: foraging behavior, surface time, fine-scale body orientation, fluking rate and amplitude, and group vocalization rate.

We tagged whales during the summers of 2010 and 2011 off Cape Hatteras, North Carolina. The DTAG records sounds produced by the tagged whale and other sounds within the audio range of the tag's two hydrophones at a sampling rate of 192 kHz. The tag also records pitch, roll, heading, and depth using 3-axis accelerometers, magnetometers, and a pressure sensor, sampled at a frequency of 50 Hz. These measures can be used to reconstruct body orientation and movement patterns. The tags are attached noninvasively using four suction cups and are programmed to release after a predetermined time interval. A VHF antenna on the tag allowed us to locate and track these animals when they were at the surface. Once the tag was released and retrieved, data were downloaded *via* an infrared port for calibration and analysis.

We used two rigid-hulled inflatable boats (RHIB): the *Exocetus* (a 6 m RHIB with a single four-stroke 90 hp engine) and the *Balaena* (an 8 m RHIB with two four-stroke 90 hp engines) to collect samples. The use of two different engine configurations could be a confounding variable that influences behavioral responses to biopsy sampling (Gorgone *et al.* 2008), but we did not observe any qualitative differences in the responses of pilot whales to the two boats. We collected biopsy samples using 25 × M8, 25 mm stainless-steel sampling tips, 25 × ACC 3-71 shaft bolts, fired from a crossbow with 150 lb pull strength (Weller *et al.* 1997). We sampled eight whales in a total of 12 biopsy attempts (defined as any instance of contact between a biopsy dart and the body of the whale regardless of whether a tissue sample was collected) after DTAGs were attached. Records of two of the whales could not be used due to problems of data configuration on the DTAGs, giving a final sample of six pilot whales and eight total biopsy attempts. In 2010 we attempted to biopsy whale 186b three times before obtaining a tissue sample.

To identify the precise time a biopsy dart made contact with a whale, we listened to the audio record on the DTAG for the sound of the dart using Matlab. This sound was audible in all seven of the 2010 biopsy attempts, and one of two in 2011. This enabled us to define precise pre- and postbiopsy periods for subsequent analyses.

Biopsy attempts were typically made either just after the DTAG was attached or just before it was programmed to release from the whale. The time elapsed between attachment of the DTAG and biopsy attempts varied between 00:08:19 and 3:45:26. We analyzed 30 min periods before and after each biopsy attempt to assess changes in the foraging behavior of the animal (mean ± SD: 27.3 ± 3.2 min prebiopsy, and 32.4 ± 3.5 min postbiopsy). We examined the rate of foraging dives (dives/min), the depths of these dives, and the rate of prey capture attempts (as indicated by echolocation buzzes in the audio record; buzzes/min). We defined a foraging dive as any submergence deeper than 20 m during which echolocation buzzes occurred (Aguilar de Soto *et al.* 2008) and determined whether whales stopped foraging after a biopsy attempt. If a dive was underway once the 30 min analysis period was over, we included the entire dive in our analyses. For whale 186b, we could not analyze the effect of the first two biopsy attempts on foraging behavior because the two attempts were less than 30 min apart.

We measured surface time (defined as the amount of time spent in the upper 3 m of the water column) for 30, 15, and 5 min before and after the biopsy attempt for each whale. If a surface period started before the 30, 15, or 5 min cutoff and/or ended after the cutoff, it was included in the analysis, extending some analyses slightly beyond the desired time period (mean $\pm$ SD: 27.3 $\pm$ 3.2 min prebiopsy, and 32.4 $\pm$ 3.5 min postbiopsy) We did not include surface periods containing a biopsy attempt in analyses.

We used TrackPlot (Version 2.2), a custom visualization program (Ware *et al.* 2006), to extract body orientation and acceleration data for each whale to examine the immediate response of whales to biopsy attempts. We calculated absolute change in body orientation over a series of 0.8 s time steps, before and after each biopsy attempt (Agostinelli 2009, Champley 2009, R Development Core Team 2011). We combined heading and pitch into one measurement, "pointing angle" as in Miller *et al.* (2004), but examined roll separately. Pointing angle was calculated using greater circle distance (Agostinelli 2009), a visual depiction of which can be found in figure 1 of Miller *et al.* (2004). First, we compared the average roll and pointing angle 4.8 s before the biopsy attempt to 4.8 s after the biopsy attempt (using a paired *t*-test) for the six whales, to determine the short-term effect of the biopsy attempt. We chose this time interval because analysis over a longer time period could disguise an ephemeral response. To compare the change in body orientation during the biopsy sampling period as compared to surface periods at least 30 s before the biopsy attempt, we randomly selected 100 surface periods, each 12 time steps (9.6 s) long, for all six animals. Using these 100 randomly selected control sections as the experimental mean and the 12 time steps centered around the biopsy attempt as the null, we executed a one-sample *t*-test for each biopsy attempt.

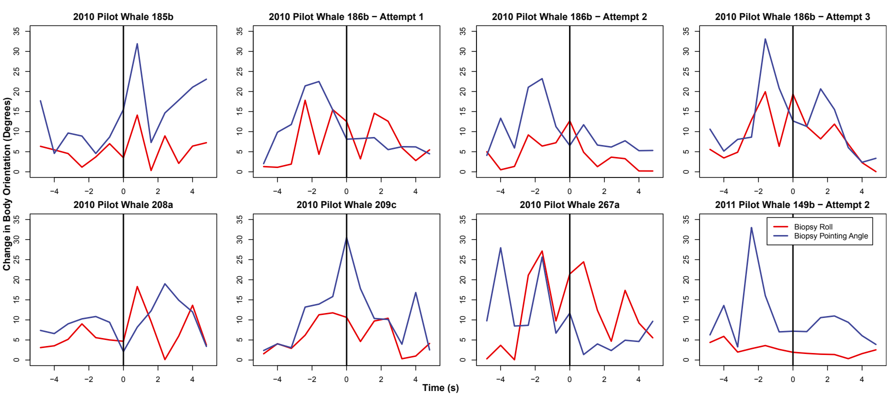

Figure 1. Absolute change in body orientation. The blue line is the pointing angle during the biopsy attempt and red is the roll angle during the biopsy attempt. The vertical black line is the time the biopsy dart struck the animal.

An increase in fluke rate and/or fluke amplitude may contribute to a startle response. We calculated fluke rate in TrackPlot by taking the second derivative of the pitch angle for the entire track, after which a Fourier analysis (FFT) of the 25.6 s time sequence (or 32 time steps) is carried out over the desired time window using a Hamming filter. The centroid of this metric gives the fluking wavelength and the integral provides the relative energy, providing a fluking rate where the frequency is equal to 1/wavelength and fluke amplitude is a relative measure of the magnitude of fluke motion. We used a TrackPlot function specifically designed to calculate fluke amplitude and rate over 32 time steps (25.6 s) before and after the biopsy attempt. Some whales dove immediately after a biopsy attempt (we defined a dive as any submergence deeper than 3 m). Fluke amplitude for whales that submerged deeper than 3 m below the surface decreased greatly, so we did not analyze fluke amplitude for these whales.

We used Matlab to examine the audio record 30 s before and after each biopsy attempt and analyzed these records using Adobe Audition (Version 2.0) in 3 s segments. We assumed that individuals in the group of the tagged whale produced all of the vocalizations in the audio record. We summed occurrences of all whistle types and social buzzes, but did not include echolocation clicks because they are used primarily in foraging. After determining normality of the data, we employed a paired t-test to determine significance of any differences observed in the group vocalization rate before and after each biopsy attempt.

Only one whale (185b) did not dive in the 30 min before or after a biopsy attempt. During the third biopsy attempt, whale 186b did not make any foraging dives, but dove to 21 m before the biopsy attempt and to 22 m and 34 m in the 30 min afterwards. Whale 149b executed a nonforaging dive to 23 m before the biopsy attempt and afterwards dove to 501 m, producing 15 foraging buzzes. Three whales (208a, 209c, and 267a) exhibited foraging behavior before and after biopsy attempts (Table 1).

There was no significant difference between the cumulative time spent within 3 m of the surface 30 min before and 30 min after biopsy attempts. On average, whales spent 535 $\pm$ 164 s near the surface in the 30 min before a biopsy attempt and 501 $\pm$ 137 s near the surface after the attempt (t = 0.55, df = 5, P = 0.61). We repeated these analyses for 15 and 5 min pre- and postbiopsy with no difference in the results.

Pilot whales did not demonstrate a significant difference in body orientation 4.8 s after the biopsy attempt as compared to before. However, five of the six whales analyzed showed a significant increase in both pointing angle and roll during the 4.8 s before and after the biopsy attempt when compared to the 100 randomly selected control periods of 9.6 s (Fig. 1). Only one whale (267a) did not exhibit a significant increase in both pointing angle and roll during this time period. This whale significantly increased its roll, but significantly decreased its pointing angle.

There was a general trend of increased fluke rate after a biopsy attempt, but fluke amplitude dropped quickly during a dive because whales fluke strongly before gliding during descent. In the three instances during which the whales did not dive immediately after a biopsy attempt we observed an increase in fluke amplitude, but our sample size is too small for proper analysis of this trend.

The first biopsy attempts for whales 185b, 186b, 208a, and 267a, and second biopsy attempt of whale 149b elicited an increase in group vocalization rates (t = –5.0, df = 4, P = 0.007; Table 2). There were two exceptions to this trend. Group vocalizations increased after the first biopsy attempt of whale 186b, but the latter

Table 1. Foraging behavior of short-finned pilot whales 30 min before and after each biopsy attempt. Foraging dives are defined as submergences deeper than 20 m during which the whale produced echolocation clicks and buzzes. Whales 185b and 186b did not exhibit any foraging dives.

Whale data		30 min before biopsy attempt				30 min after biopsy attempt			
ID	Year tagged	Rate of foraging dives	Echolocation clicks present?	Rate of buzzes (buzzes/min)	Mean ± SD depth (m)	Rate of foraging dives	Echolocation clicks present?	Rate of buzzes (buzzes/min)	Mean ± SD depth (m)
208a	2010	0.10/min	Yes	0.25	307 ± 8	0.03	Yes	0.03	300
209c	2010	0.4/min	Yes	0.18	202	0.03	Yes	0.20	276
267a	2010	0.04/min	Yes	1.04	281	0.05	Yes	1.51	370 ± 54
149b (2)	2011	0	No	0	NA	0.03	Yes	0.42	501

Table 2. Group vocalization rate for 30 s before and after each biopsy attempt, rates are provided in vocalizations per minute. We attempted to biopsy whale 186b three times before we were successful, with the previous two attempts contacting the whale without retrieving a tissue sample. Group size was determined to be all individuals within 30 m (or 10 body lengths) of the focal, or tagged, pilot whale.

Whale ID	Vocalization rate prebiopsy attempt	Vocalization rate postbiopsy attempt	Group size
185b	0	18	Unavailable
186b (1)	16	42	12–14
186b (2)	24	30	12–14
186b (3)	38	34	12–14
208a	24	42	Unavailable
209c	34	0	4
267a	2	16	5
149b (2)	6	12	16

two attempts did not elicit significant increases, although the vocalization rate remained high. The group containing whale 209c was the only group to show a significant decrease in group vocalization rate following the biopsy attempt.

Our study, albeit based on a small sample size, provides the first quantitative behavioral responses of short-finned pilot whales to biopsy sampling. Our most important finding is that we found no evidence that biopsy sampling disrupted foraging behavior, as the three animals that were foraging before the biopsy attempt continued to do so afterwards. The most striking change in behavior we observed was for whale 149b, which began to forage immediately after the biopsy attempt. This metric is tied to the health of the sampled whales and suggests that biopsy sampling does not impart a short term detriment to this important aspect of their behavior.

Sampled whales showed an increase in absolute change in body orientation and submergence was a common immediate reaction to biopsy attempts, although there was no overall change in time spent within 3 m of the surface. There was typically (although not always) an increase in group vocalization rates in response to initial biopsy attempts (see Table 3 for a synthesis). Our results support Noren and Mocklin's (2011) findings of an ephemeral and generally low intensity response to biopsy sampling in terms of behavioral state, body orientation, surface time, and fluke amplitude and rate. The most striking response was an increase in group vocalization rate 30 s after an initial biopsy attempt was made for most, but not all, whales.

Taken together, our fine-scale measurements constitute a quantitative description of a startle response (Noren and Mocklin 2011). No whales showed a difference in their absolute change in body orientation before the biopsy attempt as compared to after the biopsy attempt. However, five of the six whales showed a temporary increase in absolute change in body orientation during the biopsy attempt, when compared to nonbiopsy control periods. We conclude that these whales exhibited a startle response in the form of a "flinch," an ephemeral change in body orientation in response to the biopsy attempt process that lasts several seconds. This flinch is likely in response to not only the penetration of the biopsy dart, but also to our vessel, which approached within 5–10 m from the focal whale during the biopsy attempt. However, some whales did not react until the moment the dart was heard on the DTAG audio record. This may indicate that some whales respond more to the biopsy dart, while others may respond more to the vessel approach (Fig. 1).

Table 3. A synthesis table for all parameters measured for each incidence of attempted biopsy sampling. First column is whale ID with the biopsy attempt number in parentheses. The second column is time lapsed between DTAG attachment and biopsy attempt. Third column is results of the effect of the biopsy attempt on foraging behavior, followed by the effect on surface interval to dive time ratio, then whether the entire biopsy sampling process elicited an absolute significant change in body orientation. The sixth column provides which whales submerged deeper than 3 m after a biopsy attempt, followed by the description of increase or decrease of fluke amplitude, fluke rate, and group vocalization rate, respectively.

Whale ID	Time lapsed	Foraging behavior	Body flinch	Submergence after biopsy	Fluke amplitude	Fluke rate	Group vocalization rate
185b	0:54:56	NA	Yes	Yes	Decrease	Decrease	Increase
186b (1)	0:45:07	NA	Yes	Yes	Decrease	Increase	Increase
186b (2)	0:52:28	NA	Yes	Yes	Decrease	Increase	Increase
186b (3)	1:22:38	NA	Yes	Yes	Decrease	Decrease	Decrease
208a	0:34:54	Unaffected	Yes	Yes	Decrease	Decrease	Increase
209c	2:35:47	Unaffected	Yes	No	Increase	Increase	Decrease
267a	0:08:19	Unaffected	Yes	No	Increase	Increase	Increase
149b (2)	3:45:26	Unaffected	Yes	No	Increase	Increase	Increase

In summary, analysis of data from the DTAG provided us with continuous observations of foraging behavior, time spent near the surface, three-dimensional body orientation, fluking rate and fluking amplitude, and group vocalization rate for the tagged whales. We acknowledge that our sample size is small and reactions to biopsy sampling vary from individual to individual as noted in Noren and Mocklin (2011). Nevertheless, we conclude that short-finned pilot whales demonstrate a low level of response to biopsy attempts, similar to that described qualitatively for other species of odontocetes. Our quantification of the behavioral reactions of short-finned pilot whales to remote biopsy sampling has, in large part, agreed with the findings of Noren and Mocklin (2011). Our study has provided quantitative measures that demonstrate the response of short-finned pilot whales to biopsy attempts is transitory, unlikely to cause any long-lasting behavioral changes, or exert any effect on the fitness of the sampled individual. It would be interesting to employ the approach used here with other odontocete species (particularly those with different patterns of social organization) and with mysticetes to replicate Noren and Mocklin's (2011) meta-analysis with a more quantitative set of response measures.

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