A QUANTITATIVE ANALYSIS OF THE RESPONSE OF SHORT-FINNED PILOT WHALES, *GLOBICEPHALA MACRORHYNCHUS*, TO BIOPSY ATTEMPTS

by

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Abstract. Remote biopsy sampling is a common method used to obtain tissue samples from wild cetaceans. Using this technique, researchers typically obtain a small sample of skin and blubber using a biopsy tip fired from a crossbow or modified air rifle. Analysis of these tissues can provide important information on specific identity, sex, pollutant levels, diet, and reproductive status, which are critical to studies of free-ranging cetaceans. Biopsy sampling is generally considered to be a relatively benign procedure, but all prior attempts to evaluate its impact have been subjective assessments of the behavioral response of individuals at the surface. The goal of the present study is to provide a quantitative assessment of the immediate effects of biopsy attempts on the behavior of short-finned pilot whales (*Globicephala macrorhynchus*) equipped with digital acoustic recording tags (DTags) off Cape Hatteras, North Carolina. A biopsy attempt was defined as any instance of contact between a biopsy dart with an animal. A series of five metrics was examined to determine if behavior of whales was affected by a biopsy attempt, including: foraging behavior (number of dives, depth, and number of prey capture attempts); time spent within 3 m of the surface; fine-scale body orientation; fluke rate and amplitude; and group vocalization rate. The short-term reactions to biopsy attempts appear to be ephemeral and should not compromise the fitness of the animal, although the effects of increasing the group vocalization rate after a biopsy attempt should be examined further. The results of this analysis provide the first subsurface, quantitative assessment of the short-term effects of biopsy sampling on cetaceans.

Key Words: biopsy, behavioral responses, pilot whales, vocalization rate, DTag

INTRODUCTION

Samples of tissue obtained from wild cetaceans provide researchers with important information on the specific identity (*e.g.* Gales *et al.* 2002; Willis *et al.* 2004), sex (Winn *et al.* 1973), pollutant levels (Brown *et al.* 1991; Focardi *et al.* 1991; Woodley *et al.* 1991), dietary history (Hooker *et al.* 2001b; Herman *et al.* 2005; Krahn *et al.* 2007a), age (Herman *et al.* 2008), reproductive status (Monsour *et al.* 2002; Kellar *et al.*2009), which are critical to studies of free-ranging cetacean populations (Baker *et al*. 1990; Baker *et al.* 1993; Lambertsen 1987). Remote biopsy sampling has been used for almost 40 years to obtain tissue samples from these species because many dolphins and whales are difficult or impossible to capture safely at sea. However, despite the importance of this sampling technique, there has been relatively little quantitative assessment of the effects of biopsy attempts on the behavior of free-ranging cetaceans and a general assumption that this method has few detrimental effects on the fitness of these animals. There has only been one documented case of death of a cetacean from a biopsy dart (Bearzi 2000). The stopper of the dart and the poor body condition of the common dolphin **(***Delphinus delphis*) were believed to responsible for the death of this animal, as the stopper failed to halt the dart from penetrating too deeply through its the thin blubber layer.

Many studies have reported qualitative and semi-quantitative descriptions of the reactions of individual whales and dolphins to biopsy attempts (e.g. Aguilar & Nadal 1984; Weller *et al.* 1997; Weinrich *et al.* 1992; Hooker *et al.* 2001a; Krutzen *et al.* 2002). But in many cases the description of the response of these animals to biopsy attempts has been subjective and the criteria used to assess response vary across studies, hindering comparisons across species and sites. Recently Noren & Mocklin (2011) performed an extensive review of the behavioral and physical reactions of mysticetes and odontocetes to biopsy attempts and standardized the categories of these behavioral responses. These authors concluded that the most predominant response for odontocetes is low (*e.g* short-term startle response, immediate dive, increase of speed), while low and moderate responses are equally prevalent for mysticetes. Wounds from biopsy darts appear to heal relatively quickly, with no signs of infection.

Despite the useful recent review by Noren and Mocklin (2011), there has been very little quantitative description of the short- or long-term behavioral reactions of cetaceans to biopsy sampling due to the difficulties of observing animals under water after an attempt has been made. The objective of this paper, therefore, is to quantify the behavioral responses of short-finned pilot whales, *Globicephala macrorhynchus*, to biopsy attempts using data from digital acoustic tags or DTags (Johnson & Tyack 2003) attached to the animals. We describe the behavior of these animals prior to and following the biopsy attempt in five categories: (1) foraging behavior; (2) surface time; (3) body orientation; (4) fluke amplitude and rate; and (5) group vocalization rate.

METHODS

Field methods. We tagged short-finned pilot whales with DTags (Johnson & Tyack 2003) during the summers of 2010 and 2011 off Cape Hatteras, North Carolina (Figure 1). These archival tags record: (1) acoustic behavior of the tagged whale, as well as any sounds within the audio range of the two hydrophones; (2) body orientation (pitch, heading, roll) using 3-axis magnetometers and accelerometers; and (3) depth and time, with all sampling occurring at 50 Hz. These tags are attached non-invasively using four suction cups, which can be programmed to release at a pre-determined time. The VHF antenna on the tag allows for tracking of these animals when they are at the surface but out of sight, as well as facilitating recovery of the tag once it has detached from

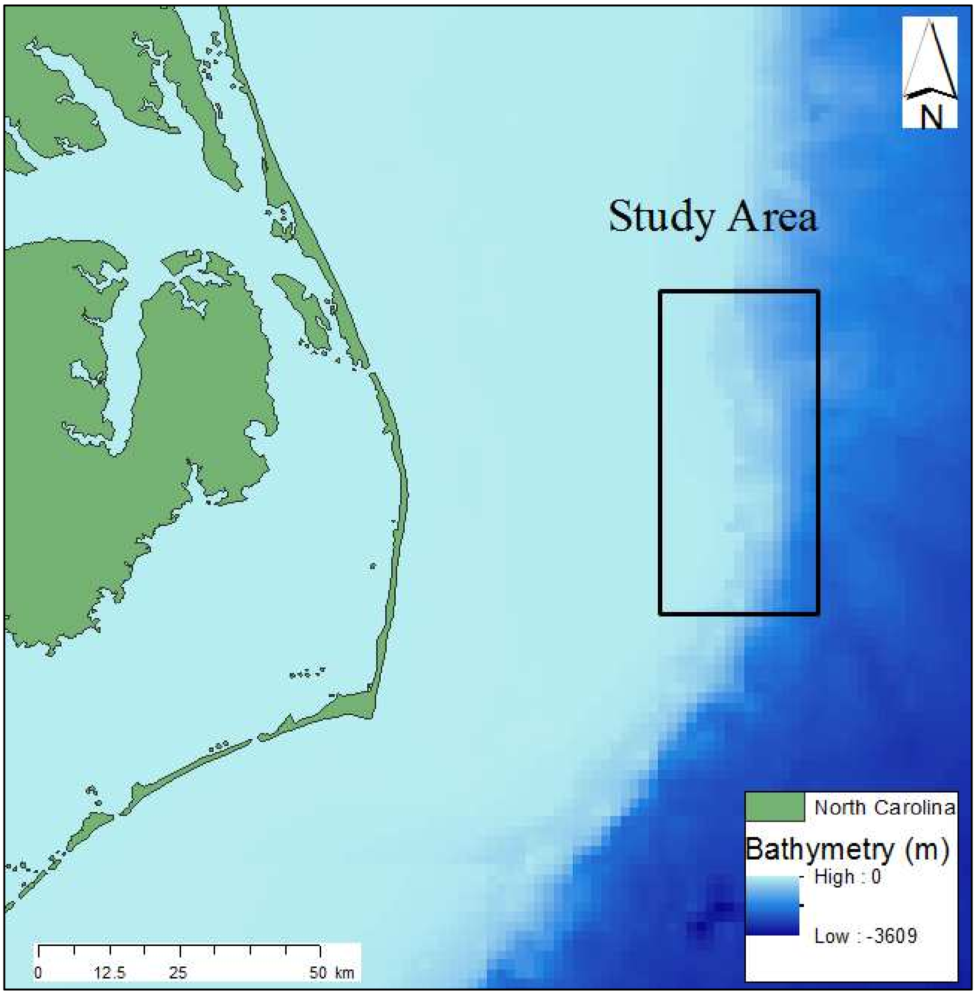

Figure 1. Location of tagged short-finned pilot whales, off Cape Hatteras, North Carolina.

the whale. Once the tag is retrieved, data are downloaded via an infrared port for calibration and analysis.

We obtained biopsy samples from eight pilot whales while DTags were attached, from a total of 12 biopsy attempts. A biopsy attempt was defined as any instance of contact between a biopsy dart comes and the body of the whale, whether a tissue sample was gathered successfully or not. We collected biopsy samples using a 25 x M8 25 mm stainless-steel sampling tipped dart fired from a crossbow with 150-lb pull strength (Weller *et al.* 1997). Of the eight whales biopsied, two could not be used for analyses due to problems of data configuration on the DTags, giving a final sample of six pilot whales and eight total biopsy attempts (Table 1). We attempted to biopsy whale 186b three times, with the first two biopsy attempts contacting the whale, but not producing a tissue sample. We also attempted to biopsy whale 149b twice, but only the first, unsuccessful hit of the biopsy was audible on the DTag record, so the second attempt could not be used in the analysis. While the focus of this paper was to quantify behavioral reactions to biopsy attempts of these short-finned pilot whales, there are likely some behaviors they exhibit due to the proximity of the biopsying vessel as well (Williams *et al.* 2002; Noren *et al.* 2009).

Precise biopsy times. To identify the precise time a biopsy dart made contact with a whale, we listened to the audio record for the 'thump' of the dart, which was audible in all seven of the

Table 1. Summary of short-finned pilot whales biopsy sampled while a DTag was attached.

Whale ID	Year Tagged	Tag on time (H:M:S)	Tag off time (H:M:S)	Duration
185b	2010	14:30:46	20:20:00	5:49:14
186b	2010	14:32:47	20:03:00	5:30:13
208a	2010	14:50:21	23:47:00	8:56:39
209c	2010	13:19:38	20:09:00	6:49:22
267a	2010	15:19:00	33:19:00	18:00:00
149b	2011	10:33:11	14:24:10	3:50:59

Table 2. Precise time of biopsy attempts for each pilot whale in terms of seconds from tag on. We made multiple attempts of whale 186b.

Whale ID	Year Tagged	Time of Biopsy Attempt (sec from tag on)
185b	2010	3297.0
186b	2010	2708.0
186b	2010	3149.0
186b	2010	4957.7
208a	2010	2218.7
209c	2010	3539.6
267a	2010	10957.8
149b	2011	13140.0

2010 biopsy attempts, and one of two in 2011 (Table 2). This enabled us to define pre- and post-biopsy periods for subsequent analyses.

Foraging behavior. Biopsy attempts were typically made either just after the DTag was attached or just before it was programmed to release from the whale. Due to this constraint, we analyzed relatively short periods (30 minutes) before and after each biopsy attempt to assess changes in the foraging behavior of the animal. We examined the number of foraging dives, the depths of these dives, and the number of prey capture attempts, as indicated by echolocation buzzes in the audio record. We considered any submergence deeper than 20 m during which buzzes occurred, to be a foraging dive (Aguilar de Soto *et al.* 2008). For whale 186b which we attempted to biopsy multiple times, we could not analyze the effect of the first two biopsy attempts on foraging behavior because the two attempts were not longer than 30 minutes apart.

Surface time. We defined surface time as the time each whale spent in the upper 3 meters of the water column for 30 minutes before and after the biopsy attempt for each whale, using a custom software application, the TrackPlot visualization program, to determine depth.

Body orientation. We used TrackPlot (Ware *et al.* 2006) to extract body orientation and acceleration data for each whale. We calculated absolute change in body orientation over 0.8 second time steps, the default for the TrackPlot program, for 5 seconds before and after each biopsy attempt (Agostinelli 2009; Champley 2009; R Development Core Team 2011). Heading and pitch were combined into one measurement, 'pointing angle' as in Miller *et al.* (2004), but we examined roll separately. As a control we isolated 10 second segments of absolute change in body orientation not associated with biopsy attempts for each animal. We took the pointing angle and roll in response to the biopsy attempt and compared this to the mean pointing angle and roll of the control periods for each pilot whale using a paired T-test.

Fluke amplitude and rate. We calculated fluke amplitude and rate using TrackPlot (Ware *et al.* 2006) over 32 time steps, the default for the TrackPlot program, before and after the biopsy attempt for a total of 25.6 seconds (Figure 2). As some whales dove immediately after a biopsy attempt, we defined a dive as a submergence deeper than 3 m.

Group vocalization rate. We used Matlab to examine the audio record 30 seconds before and after the biopsy attempt. We analyzed this audio record within Adobe Audition (Version 2.0) in three-second segments, which we determined to be the ideal time in which we were able to enumerate the number of social calls in the audio range of the DTag. We summed all whistle types and social buzzes during these times, but did not count echolocation clicks because they are used primarily during foraging (Figure 3). A paired T-test was used to determine significance of any differences observed in the group vocalization rate 30 seconds before and after the biopsy attempt, once we determined normality of the data.

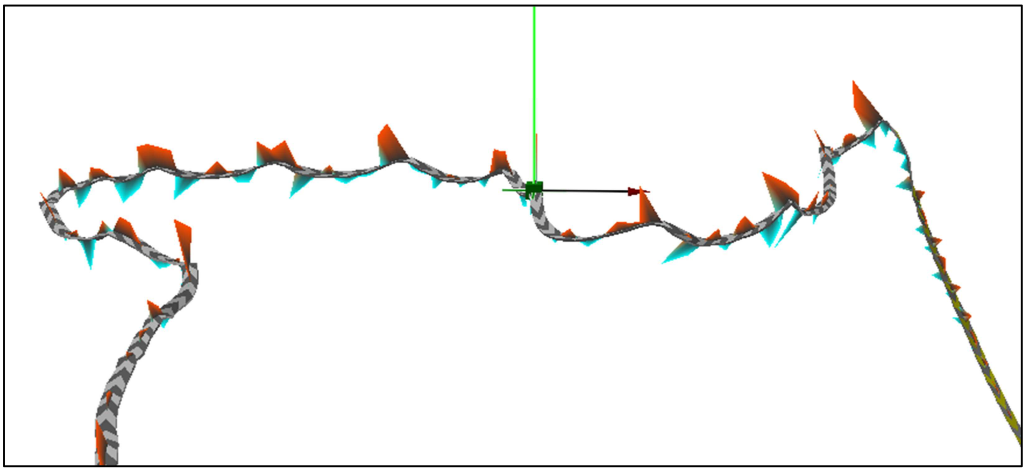

Figure 2. Visualization of fluke rate and amplitude from TrackPlot (Ware *et al.* 2006). Red indicates an upward fluke stroke, blue indicates a downward fluke stroke, and the green box indicates the location of the whale, while the arrows on the ribbon track indicates the direction of movement of the whale.

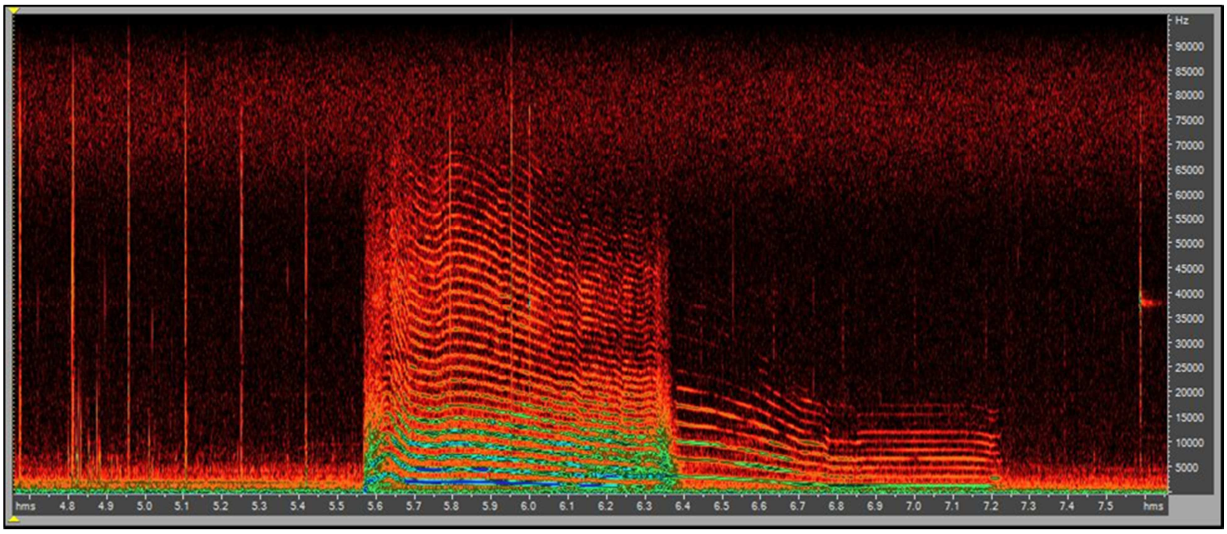

Figure 3. An example of a spectrogram from whale 267a. The tall vertical lines are echolocation clicks, while the strong calls with multiple harmonics are frequency modulated whistles, the first presumably by the tagged whale, the second by a nearby group member.

Table 3. Foraging behavioral change of pilot whales 30 minutes before and after the biopsy attempt.

Whale Data		30 minutes before biopsy attempt			30 minutes after biopsy attempt		
ID	Year Tagged	# of Dives	# of Buzzes	Depth (m)	# of Dives	# of Buzzes	Depth (m)
185b	2010	0	NA	NA	0	NA	NA
186b	2010	1	0	20.6	2	0	22.3, 33.5
208a	2010	2	2, 3	315, 298.8	1	1	300.3
209c	2010	1	5	201.6	1	6	276.0
267a	2010	1	29	280.8	2	21, 38	316.0, 424.0
149b	2011	1	0	23.2	1	15	500.7

RESULTS

Foraging behavior. Whale 185b was the only whale that did not forage in the 30 minutes before or after a biopsy attempt. On the third biopsy attempt, whale 186b did not perform any foraging dives, but did dive to 20.6 m and in the 30 minutes afterwards completed two non-foraging dives to 22.3 m and 33.5 meters. Whales 208a, 209c, 267a, and 149b did not vary their behavior with respect to foraging prior to and following biopsy attempts (Table 3).

Table 4. Surface time in seconds for 30 minutes before and after the biopsy attempt.

Whale ID	Pre-Biopsy Attempt (s)	Post-Biopsy Attempt (s)
185b	594	673
186b	282	419
208a	446	427
209c	777	560
267a	544	312
149b	570	614

Surface time. There was no significant difference between the cumulative time spent within 3 m of the surface before and after biopsy attempts (Table 4). On average, the whales spent 535 ± 164 seconds within 3 m of the surface in the 30 minutes before the biopsy attempt, and 501 ± 137 seconds within 3 m of the surface after the biopsy attempt (t = 0.55, df = 5, *P* = 0.61).

Body orientation. Four of the six whales analyzed showed a significant increase in the pointing angle and roll during the five seconds before and after the biopsy attempt as compared to the control periods (Figure 4, Table 5 for *P*-values). The two whales which did not show a significant increase in both pointing angle and roll during this time period was whale 208a, which only showed a significant increase in pointing angle, and whale 267a, which showed a significant increase in roll, but a significant decrease in pointing angle.

Fluke amplitude and rate. In four biopsy attempts, the whales dove immediately one whale dove after seven seconds, and three whales did not dive within 15 seconds afterwards. Fluke amplitude dropped quickly after each dive because whales typically fluke several times strongly at the beginning of a submergence (Table 6). Fluke amplitude was not analyzed statistically, but a two-tailed T-test on fluke rates did not reveal any effect of the biopsy attempt (t = -0.96, df = 5, *P* = 0.38).

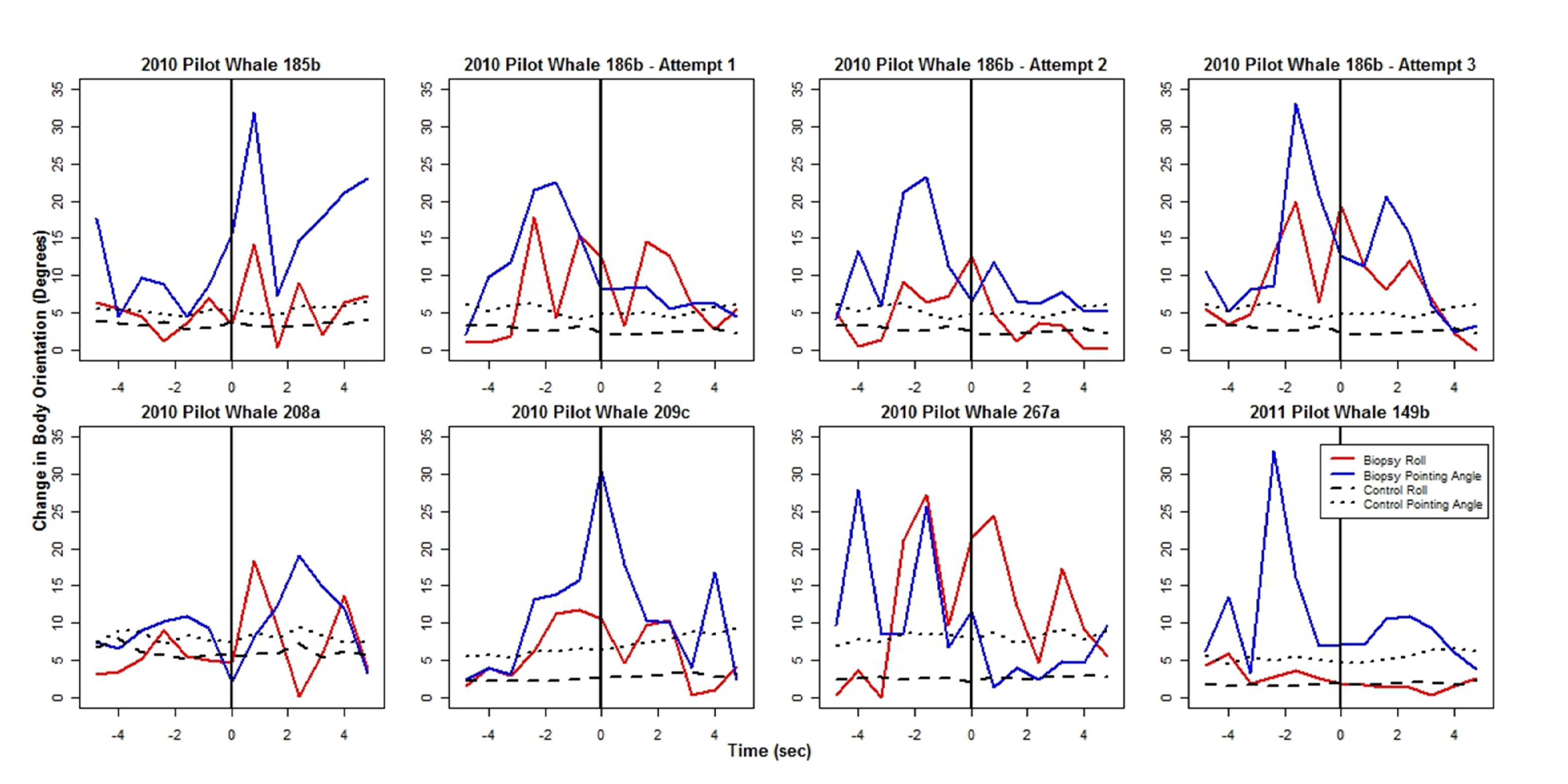

Figure 4. Absolute change in body orientation. The blue line is pointing angle during the biopsy attempt and red is the roll angle during the biopsy attempt. The dashed lines are controls for both pointing angle and roll during non-biopsy control sampling periods. The vertical black line is the time the biopsy dart struck the animal.

Group vocalization rate. All the first biopsy attempts for whales 185b, 186b, 208a, 267a, and 149b elicited an increase in group vocalization rates 30 seconds after the biopsy attempt (t = -5.0, df = 4, *P* = 0.007, Table 7). There were two exceptions to this trend: whales 186b and 209c. Group vocalizations increased after the first biopsy attempt of whale 186b, but the second two attempts did not elicit significant increases. Whale 209c was the only individual to show a significant decrease in group vocalization rate.

Table 5. P-values for t-tests of body orientation 5 seconds before and after the biopsy attempt as compared to a control non-biopsy attempt section of surface data.

		PA		Roll	
Whale ID	**df**	**t**	**P-value**	**t**	**P-value**
185b	77	-38.3	2.20E-16	-38.8	2.20E-16
186b	34	-17.1	2.20E-16	-46.6	2.20E-16
186b	34	-18.3	2.20E-16	-48.7	2.20E-16
186b	34	-14.7	2.80E-16	-42.3	2.20E-16
208a	74	-0.2	0.85	-3.8	2.80E-04
209c	89	-2.83	5.70E-03	-47.1	2.20E-16
267a	55	4.6	2.50E-05	-27.7	2.20E-16
149b	46	-7.7	8.30E-10	-49.9	2.20E-16

Table 6. Fluke amplitude and rate for 25.6 seconds before and after the biopsy attempt. Four short-finned pilot whales dove immediately after the biopsy attempt, *one dove seven seconds after the biopsy attempt, and three did not dive within the 30 seconds after the biopsy attempt.

	Rate (fluking/0.8 sec)		Relative Amplitude		
Whale ID	**Pre-Biopsy Attempt**	**Post-Biopsy Attempt**	**Pre-Biopsy Attempt**	**Post-Biopsy Attempt**	**Dive after biopsy attempt?**
185b	0.285	0.203	0.115	0.012	Yes
186b	0.168	0.304	0.139	0.056	Yes
186b	0.205	0.325	0.155	0.031	Yes
186b	0.224	0.163	0.128	0	Yes
208a	0.22	0.217	0.057	0.004	Yes*
209c	0.244	0.318	0.075	0.194	No
267a	0.29	0.303	0.061	0.123	No
149b	0.306	0.341	0.109	0.11	No

Table 7. Group vocalization rate for 30 seconds before and after the biopsy attempt. Whale 186b we attempted to biopsy three times before we were successful, with the previous two attempts contacting the whale, but without retrieving a tissue sample.

Whale ID	Pre-Biopsy Attempt	Post-Biopsy Attempt
10_185b	0	9
10_186b	8	21
10_186b	12	15
10_186b	19	17
10_208a	12	21
10_209c	17	0
10_267a	1	8
11_149b	3	6

DISCUSSION

Our results supported Noren and Mocklin's findings of an ephemeral and generally low intensity response to remote 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 seconds after a biopsy attempt was made.

Foraging behavior. Biopsy attempts did not result in a cessation of foraging behavior. There was a great amount of individual variation in the behavioral state of the whales, but the overall pattern of foraging whales was to sustain their foraging efforts after a biopsy attempt. The greatest change in behavior we observed was for whale 149b in 2011 which began to forage immediately after the biopsy attempt.

Surface time. We saw no significant change in time spent within 3 m of the surface before and after a biopsy attempt. This was in contrast to the findings of Weinrich *et al.* (1992) who showed an overall decrease in surface to dive time ratio for a sample of 9 of 16 biopsied humpback whales (*Megaptera novaeangliae*) in the southern Gulf of Maine. This was also in contrast to the findings of Janik *et al*. (1996), where 24 of 34 bottlenose dolphins (*Tursiops truncatus*) showed a decreased number of surfacings within the first minute of a boat passing the group of dolphins.

Body orientation. This fine-scale measurement can provide a quantitative description of exactly what constitutes a startle response (Noren & Mocklin 2011). Four of the six whales showed an increase in absolute change in body orientation which quickly dissipated and the remaining two whales only exhibited an increase in pointing angle or roll. We conclude that these whales exhibited a startle response in the form of a 'flinch' to the biopsy attempt process, which not only includes the penetration of the biopsy dart, but also the change in body orientation due to the proximity of the vessel the researchers are in.

Fluke amplitude and rate. It was difficult to ascertain the reactions of whales in terms of their fluke amplitude and rate because three of the whales dove immediately after a biopsy attempt. However, there was no significant change in fluke rate, which indicates no difference in speed after the biopsy attempt.

Group vocalization rate. An increased rate in vocalizations after a biopsy attempt may be an indicator of disturbance (Esch *et al.* 2009). Indeed, the possibility that the entire group was disturbed by the biopsy attempt is supported by previous studied as non-target animals in the group have been shown to react biopsy sampling in (Krutzen *et al.* 2002; Gorgone *et al.* 2008). However, there were some interesting exceptions to this response. We attempted to biopsy whale 186b three times. After the first attempt, the group vocalization rate increased significantly, as expected. However, after the second and third attempts group vocalization rate did not decrease to the initial pre-biopsy attempt rate. Unfortunately, we were unable to estimate group sizes for whale 186b during the biopsy attempts. The group of whale 209c was the only case in which the vocalization rate decreased after a biopsy attempt. This was also the only group where we could determine conclusively that the sampled whale was itself calling regularly to another whale in its group, using the amplitude of the calls. After the biopsy attempt, the entire group became silent.

In conclusion, 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 & Mocklin (2011). The short-term reactions to biopsy attempts appear to be ephemeral and should not compromise the fitness of the animal, although the effects of increasing the group vocalization rate after a biopsy attempt should be examined further. 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.

Literature Cited

Agostinelli, C. 2009.CircStats: Circular Statistics, from "Topics in circular Statistics" (2001). R package version 0.2-4. http://CRAN.R-project.org/package=CircStats

Aguilar de Soto, N., M.P. Johnson, P.T. Madsen, F. Díaz, I. Domínguez, A. Brito and P. Tyack. 2008. Cheetahs of the deep sea: deep foraging sprints in short-finned pilot whales off Tenerife (Canary Islands). Journal of Animal Ecology 77: 936–947.

Baker, C. S., S. R. Palumbi, R. H. Lambertsen, M. T. Weinrich, J. Calambokidis and S. J. O'Brien. 1990. Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. Nature 344: 238–240.

Baker, C.S., A. Perry, J.L. Bannister, *et al*. 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. Proceedings of the National Academy of Sciences 90: 8239–8243.

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: 217–222.

Brown, M.W., S. D. Kraus and D. E. Gaskin. 1991. Reaction of North Atlantic right whales (*Eubalaena glacialis*) to skin biopsy sampling for genetic and pollutant analysis. Report of the International Whaling Commission (Special Issue 13): 81–89.

Champely, S. 2009. pwr: Basic functions for power analysis. R package version 1.1.1. http://CRAN.R-project.org/package=pwr

Esch, H. C., L. S. Sayigh, J. E. Blum and R. S. Wells. 2009. Whistles as potential indicators of stress in bottlenose dolphins (*Tursiops truncatus*). Journal of Mammalogy 90: 638–650.

Focardi, S., G. Notarbartolo Di Sciara, C. Venturino, M. Zanardelli and L. Marsili. 1991. Subcutaneous organochlorine levels in finback whales (*Balaenoptera physalus*) from the Ligurian Sea. European Research on Cetaceans 5: 93–96.

Gales, N. J., M. L. Dalebout and J. L. Bannister. 2002. Genetic identification and biological observation of two free-swimming beaked whales: Hector's beaked whale (*Mesoplodon hectori*, Gray, 1871), and Gray's beaked whale (*Mesoplodon grayi*, Von Haast, 1876). Marine Mammal Science 18: 544–551.

Gorgone, A. M., P. A. Haase, E. S. Griffith and A. A. Hohn. 2008. Modeling response of target and nontarget dolphins to biopsy darting. Journal of Wildlife Management 72: 926–932.

Herman, D. P., D. G. Burrows, P. R. Wade, *et al*. 2005. Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. Marine Ecology Progress Series 302:275–291.

Herman, D. P., C. O. Matkin, G. M. Ylitalo, *et al*. 2008. Assessing age distributions of killer whale *Orcinus orca* populations from the composition of endogenous fatty acids in their outer-blubber layers. Marine Ecology Progress Series 372: 289–302.

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

Hooker, S. K., S. J. Iverson, P. Ostrom and S. C. Smith. 2001*b*. Diet of northern bottlenose whales inferred from fatty-acid and stable-isotope analyses of biopsy samples. Canadian Journal of Zoology 79:1442–1454.

Janik, V.M., and P.M. Thompson. 1996. Changes in surfacing patterns of bottlenose dolphins in response to boat traffic. Marine Mammal Science 12: 597–602.

Johnson, M.P., and P.L. Tyack. 2003. A digital acoustic recording tag for measuring the response of wild marine mammals to sound. IEEE Journal of Oceanic Engineering 28: 3–12.

Kellar, N. M., M. L. Trego, C. I. Marks, S. J. Chivers, K. Danil and F. I. Archer. 2009. Blubber testosterone: A potential marker of male reproductive status in short-beaked common dolphins. Marine Mammal Science 25: 507–522.

Kiszka, J., B. Simon-Bouhet, F. Charlier, C. Pusineri and V. Ridoux. 2010. Individual and group behavioural reactions of small delphinids to remote biopsy sampling. Animal Welfare 19: 411–417.

Krahn, M. M., D. P. Herman, C. O. Matkin, *et al*. 2007*a*. Use of chemical tracers in assessing the diet and foraging regions of eastern North Pacific killer whales. Marine Environmental Research 63: 91–114.

Krützen, M., L. M. Barré, L. M. Möller, M. R. Heithaus, C. Simms and W. B. Sherwin. 2002. A biopsy system for small cetaceans: Darting success and wound healing in *Tursiops* spp. Marine Mammal Science 18: 863–878.

Lambertsen, R. H. 1987. A biopsy system for whales and its use for cytogenetics. Journal of Mammalogy 68: 443–445.

Mansour, A. A. H., D. W. McKay, J. Lien, J. C. Orr, J. H. Banoub, N. Øien and G. Stenson. 2002. Determination of pregnancy status from blubber samples in minke whales (*Balaenoptera acutorostrata*). Marine Mammal Science 18: 112–120.

Miller, P.J.O., M.P Johnson and P.L. Tyack. 2004. Sperm whale behaviour indicates the use of echolocation click buzzes “creaks” in prey capture. Proceedings of the Royal Society of London. Series B: Biological Sciences 271: 2239–2247.

Noren, D. P., A. H. Johnson, D. Rehder and A. Larson. 2009. Close approaches by vessels elicit surface active behaviors by Southern Resident killer whales. Endangered Species Research 8:179–192.

Noren, D.P., and J.A. Mocklin. 2012. Review of cetacean biopsy techniques: Factors contributing to successful sample collection and physiological and behavioral impacts. Marine Mammal Science 28: 154–199.

R_Development_Core_Team (2011) R: A language and environment for statistical computing; Computing RFfS, editor. Vienna, Austria. http://www.R-project.org/

Ware, C., R. Arsenault, M. Plumlee and D. Wiley. 2006. Visualizing the underwater behavior of humpback whales. Computer Graphics and Applications, IEEE 26: 14–18.

Weinrich, M.T., R.H. Lambertson, C.R. Belt, M.R. Schilling, H.J. Iken and S.E. Syrjala. 1992. Behavioral reactions of humpback whales Megaptera novaeangliae to biopsy procedures. Fishery Bulletin 90: 588–598.

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

Williams, R., D. E. Bain, J. K. B. Ford and A.W. Trites. 2002. Behavioural responses of male killer whales to a ‘leapfrogging’ vessel. Journal of Cetacean Research and Management

Willis, P. M., B. J. Crespi, L. M. Dill, R. W. Baird and M. B. Hanson. 2004. Natural hybridization between Dall’s porpoises (*Phocoenoides dalli*) and harbour porpoises (*Phocoena phocoena*). Canadian Journal of Zoology 82: 828–834.

Winn, H., W. Bischoff and A. Taruski. 1973. Cytological sexing of Cetacea. Marine Biology 23: 343–346.

Woodley, T. H., M. W. Brown, S. D. Kraus and D. E. Gaskin. 1991. Organochlorine levels in North Atlantic right whale (*Eubalaena glacialis*) blubber. Archives of Environmental Contamination and Toxicology 21: 141–145.