Journal of the Marine Biological Association of the United Kingdom, 2010, 90(8), 1701–1711.
doi:10.1017/S0025315409991561

Behavioural reactions of wintering humpback whales (*Megaptera novaeangliae*) to biopsy sampling in the western South Atlantic

MAURICIO CANTOR, TAIANA CACHUBA, LUENA FERNANDES AND MÁRCIA H. ENGEL
Instituto Baleia Jubarte, Rua Barão do Rio Branco, 26, CEP 45900-000, Caravelas, BA, Brazil

Molecular analysis has become a powerful tool in cetacean ecology since it supports efficient conservation policies. Remote biopsy sampling is the most efficient method to obtain epithelial material for analysis purposes; however, as an intrusive technique it presents inherent costs, evidenced by behavioural reactions. Clarifying which factors influence these responses is essential to assess its impact and prevent possible long-term effects. For eleven winters, samples from humpback whales were collected in the Abrolhos Bank, the main breeding ground of this species in the western South Atlantic. We analysed the influence of several characteristics of the shot, vessels, groups and behaviour on the frequency and intensity of the whales' response. The majority of biopsied whales did not show any detectable response. Among those that responded, a low-level category of reaction was most frequent. The use of larger boats resulted in less intense responses. Responses were influenced by group size and behavioural state: large groups, which were involved in aggressive mating behaviour, reacted less frequently than smaller groups. Females with calves showed less intense reactions than non-lactating females. The behaviour of the animals prior to and during the boat approach also affected their response: resting whales responded more intensely than whales involved in social or travel activities. Comparison with previous studies confirmed that reactions vary in intensity according to location: whales biopsied in feeding grounds responded with more intensity than those in breeding grounds, which in turn responded more intensely than whales in migration. This study reinforces existing evidence that biopsy sampling is unlikely to have long-term effects and can thus continue to be used as one of the main tools to access information which is vital for conservation.

Keywords: biopsy, behavioural responses, short-term effect, humpback whale, *Megaptera novaeangliae*, breeding ground

Submitted 11 February 2009; accepted 30 October 2009; first published online 12 February 2010

INTRODUCTION

Molecular analysis has been widely used as a tool in recent cetacean studies as it produces answers to some of the crucial issues concerning their biology and ecology. Although photo-identification and behavioural studies have provided us with a wealth of information, more complete and reliable results are produced when these are interpreted together with genetic (Mesnick *et al.*, 1999) and toxicological (Metcalfe *et al.*, 2004) data. Furthermore, many questions can only be answered through the use of new molecular technology, which will provide scientific support for the elaboration of efficient management and conservation policies (Hoelzel & Amos, 1988; Hoelzel, 1991; IWC, 1991).

Small fragments of epidermis provide sufficient genetic material to reveal specific information about individual identification (Paslbøll *et al.*, 1997), sex (Paslbøll *et al.*, 1992), genetic variability (Jackson *et al.*, 2008) and gene flow (Baker *et al.*, 1994). It allows the investigation of questions on social organization and population structure (Baker *et al.*, 1993; Cerchio *et al.*, 2005), mating systems (Clapham & Paslbøll, 1997), stock identification and effective population size (Rosenbaum *et al.*, 2000), trophic levels (Gendron *et al.*, 2001), migration patterns and habitat use (Baker *et al.*, 1990; Engel *et al.*, 2008). In addition, studies on toxicology and contaminants (Fossi *et al.*, 2000), inferences on feeding habits, nutritional condition (Borobia *et al.*, 1995) and pregnancy (Mansour *et al.*, 2002) can also be conducted through the molecular analysis of blubber.

Standard protocol for biopsy sample collection involves the use of a crossbow or rifle (Lambertsen, 1987). Although alternative non-invasive methods exist, such as sampling from stranded animals (Cunha & Sole-Cava, 2007), faecal matter (Green *et al.*, 2007) or sloughed skin (Clapham *et al.*, 1993), they can only be collected opportunistically and do not always provide enough high quality DNA (Parsons *et al.*, 2003). Remote biopsy sampling is therefore considered the most efficient, straightforward and ethical method of obtaining fresh high quality samples of specific individuals for any type of molecular analysis (IWC, 1991). However, it is essential to assess the impact of biopsy sampling, which is an intrusive method and may have some negative effects (IWC, 1991).

On-going studies involving biopsy collection have been carried out since 1997 to monitor the humpback whales (*Megaptera novaeangliae*; Borowski, 1781) that migrate

Corresponding author:
M. Cantor
Email: m.cantor@ymail.com

annually to the east and north-east coast of Brazil to reproduce during the austral winter. Remnant of a population extensively exploited until the past century, this stock has undergone a recent population growth (Zerbini *et al.*, 2004; Morete *et al.*, 2008) but remains vulnerable to human disturbances such as entanglement (Pizzorno *et al.*, 1998), noise from vessel traffic (Sousa-Lima & Clark, 2008) and direct targeting by whale-watching tourism (Simões *et al.*, 2005; Morete *et al.*, 2007). This work has been crucial to determine the genetic variability of this population, its gene flow with other southern hemisphere breeding stocks and has supported South Georgia and South Sandwich Islands as the main feeding area for this population instead of the Antarctic Peninsula (Engel *et al.*, 2008). Furthermore, information about the social structure and effective population size has been recently analysed (Cypriano-Souza, 2008). While studies in other populations showed biopsy sampling to cause minimal disturbance (Weinrich *et al.*, 1991, 1992; Clapham & Mattila, 1993; Brown *et al.*, 1994; Gauthier & Sears, 1999) risks may still exist (Bearzi, 2000), in particular because this is the main breeding and calving ground in the western South Atlantic (Engel, 1996; Martins *et al.*, 2001; Freitas *et al.*, 2004; Andriolo *et al.*, 2006).

This study represents the most detailed analysis of the short-term behavioural responses of humpback whales to biopsy collection, as it has considered all the potential factors related to this methodology which might affect the species' behaviour, such as shots fired, type of vessels, group size, behaviour of the targeted group and whether samples are collected on breeding, feeding grounds or during migration. Additionally, this is the first effort to clarify these impacts in the western South Atlantic breeding ground.

MATERIALS AND METHODS

Study area and sampling methods

Biopsy samples were collected from humpback whales during the austral winter (July to November), throughout the breeding seasons of 1997 to 2007. The area studied extends across the Abrolhos Bank (16°40′S to 19°30′S, 37°25′W to 39°45W), an extension of the continental shelf on the eastern coast of Brazil, but most samples were obtained near the Abrolhos Archipelago (Figure 1). Four to five-day cruises were conducted weekly along planned routes. Whenever a group of whales was sighted, it was approached to 20 m on a gradually converging course.

Whales were sampled using a system adapted from Lambertsen (1987): a regular Kantas crossbow (draw weight 150 lbs) with polycarbonate and aluminium darts 55.5 cm long and 0.8 cm in diameter. A custom-built stainless steel tube tip (0.8 cm diameter, 2.5 cm length) was screwed at the end of the dart, inside which three backward-pointing barbs were silver-soldered to hold the sample. The stopper, a flat plastic piece 2.6 cm in diameter at the base of the tip, was used to control penetration to a maximum depth of the tip length and cause the dart to recoil once the sample was taken. Before loading the dart into the crossbow, biopsy tips were sterilized in 92.8% ethanol and, after each shot, they were cleaned and boiled for ten minutes, to minimize wound infection and contamination of the sample. Skin samples for genetic analysis were kept in 70% ethanol or DMSO (Amos & Hoelzel, 1990) and stored at −20°C until processed; blubber for contaminant studies was stored in laminated paper at −16°C.

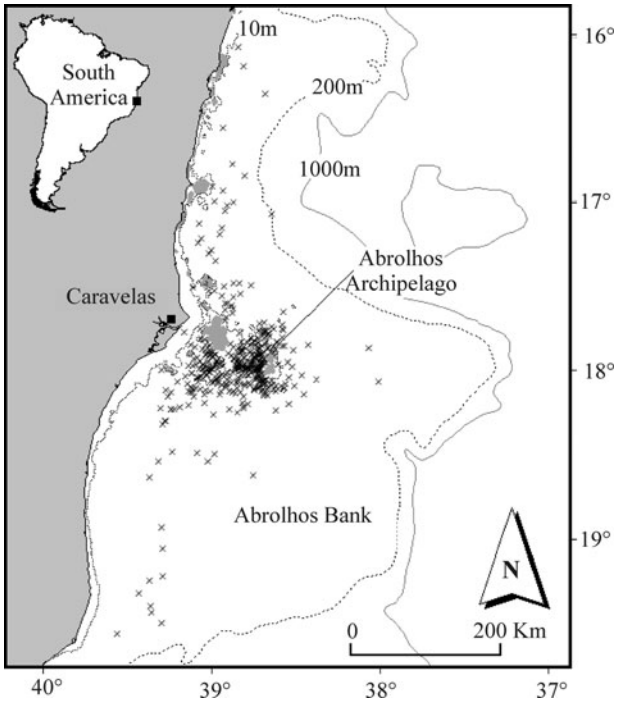

Fig. 1. Location of biopsy collection from humpback whales along the Abrolhos Bank, between 1997 and 2007. The crosses (X) represent successful shots (when a sample was obtained) and the grey marking represents the coral reefs.

Only adult-sized animals were sampled, chosen randomly. Multiple sampling of individuals was avoided through examination of identity using dorsal fins. Between 1997 and 2006, priority was given to the photo-identification of individuals, thus the biopsy attempts were only initiated after 30 minutes near the group and in many cases the groups were approached from behind, instead of alongside. The darts were fired from distances between 10 and 25 m, to hit the whale's dorsal surface, preferably perpendicularly, usually when the whale arched its body just before diving. We waited until the whole group reappeared on the surface before turning the vessel back into course. Sampling was aborted in the event of bad weather, poor light conditions or groups that were difficult to approach.

Data collected and definitions

The shot itself includes both hits (when sample was obtained) and unsuccessful shots (hit without sample and misses). Behaviour reactions were collected *ad libitum* (Altmann, 1974) by two observers on the boat with naked eye throughout the biopsy procedure. These data were categorized hierarchically as follows (see Brown *et al.*, 1994; Best *et al.*, 2005): 'No Response', when the whale continues its pre-biopsy behaviour with no change detectable; 'Startle', when some body part of the whale (other than the flukes) makes a sudden but subtle movement, such as a muscle contraction in the region of hit; 'Fluke Move', when a small, non-forceful movement of the flukes was observed—the flukes are moved either dorso-ventrally or laterally without striking the

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

surface of the water and without producing any spray or white water; 'Immediate Dive' (Weinrich *et al.*, 1992) and 'Submerge' (Gauthier & Sears, 1999) were placed in this category; 'Fluke Slap', when the whale flexes the caudal region forcefully and strikes the surface of the water with the ventral side of the fluke—spray or white water is produced.

In order to determine which factors may influence the frequency and intensity of immediate behavioural responses, characteristics of biopsy shots, vessels used, approach type, group behaviour and group characteristics were recorded. Influence of study location on the reactions and effects on individual re-sightings were also studied.

BIOPSY SHOT CHARACTERISTICS

The characteristics of biopsy shots recorded were region of hit (anterior, below or posterior of the dorsal fin), depth of sample (skin with blubber samples came from deeper dart penetration than only skin samples), and retrieval system used (with or without tether—a monofilament line connected to a reel and attached to the crossbow). Stronger reactions are expected to hits on the peduncle or fluke, to deeper penetration of dart and to retrieval systems, which can lead to entanglement problems.

VESSELS USED

The vessel characteristics examined were vessel length (small—4.0 to 7.5 m or large—13.0 to 16.0 m), engine horsepower (25 HP to 250 HP) and engine location (outboard or inboard).

APPROACH TYPE

Characteristics examined included speed of approach (0–4 and 5–9 knots), total contact period (the time the vessel remained approximately 100 m from the group to attempt to photograph and biopsy), and pursuit period (the time that the group was pursued by the vessel until the proper distance had been achieved to trigger the dart).

GROUP BEHAVIOUR

The group behaviour was recorded prior to boat approach and during the total contact period by one-zero sampling (Altmann, 1974) on standardized data sheets. These data were placed into three behavioural states: social, travel and resting (as Brown *et al.*, 1994). Social behaviour included points where more than one animal in close proximity breached, pectoral slapped, peduncle slapped, fluke slapped, rolled or breathed forcefully (Gauthier & Sears, 1999), tail breached (Clapham, 2000), side fluked, head slapped, fluke swished, pectoral waved, fluke waved and/or were oriented in different directions relative to each other (cf. Bryden & Corkeron cited in Brown *et al.*, 1994). The aggressive behaviour (bubble streams, tail thrashes and tail slashes; Tyack & Whitehead, 1983) and 'tail up' behaviour (Morete *et al.*, 2003) were also included in this category. Travel behaviour was registered when one or more group members were moving and not exhibiting any of the behaviours listed above for social behaviour. For groups containing more than one individual, all members must have been oriented in the same direction relative to each other (Brown *et al.*, 1994). Resting behaviour occurred, when one or more members were stationary and not exhibiting any of the behaviours listed for social behaviour or travel. For groups of more than one individual, all members must have been oriented in the same direction relative to each other (Brown *et al.*, 1994).

GROUP CHARACTERISTICS

Groups were defined as either a lone whale or affiliations of whales within 100 m of each other, moving in the same general direction in a coordinated manner (Whitehead, 1983). Group characteristics recorded included group size (one, two, three, four, and more than four whales; Brown *et al.*, 1994); group type (non-competitive and competitive groups—the groups containing three or more adults, with a definite structure of nuclear animal and escorts and exhibiting aggressive behaviour and fast movement; Tyack & Whitehead, 1983); behavioural role of target whale in the group (mothers, member of a pair, member of a non-competitive trio, escort to a mother–calf pair and member of a competitive group); and sex of target animal. In most cases, sex was determined by molecular techniques, which was carried out by PCR amplification followed by *Taq*I digestion of the ZFX/ZFY region of the sex chromosomes following the protocol of Palsbøll *et al.* (1992) modified by Bérubé & Palsbøll (1996) (Cypriano-Souza, 2008). Because samples collected in 2006 and 2007 (N = 118) are still being analysed, we determined the sex by the whale's behavioural role in the group: individuals in close contact with calves are invariably females; in competitive groups, nuclear animals are generally females and the other participants are invariably males (Tyack & Whitehead, 1983; Clapham *et al.*, 1992); and a whale in close proximity to a mother–calf pair ('escort' is generally male; Medrano *et al.*, 1994).

LOCATION

In order to analyse the influence of study location on the reactions to biopsy sampling, we compared our results with those of studies conducted in different breeding grounds and areas used for other purposes, such as feeding and migration.

As some of them classified the responses differently (Weinrich *et al.*, 1991, 1992; Clapham & Mattila, 1993, Gauthier & Sears, 1999), we associated the categories according to Brown *et al.* (1994): 'No Response' = 'No Reaction', 'Startle' and 'Fluke Move' = 'Low-level Reaction', 'Fluke Slap' = 'Moderate Reaction', and 'Strong Reaction' (defined by those authors as a reaction with multiple forceful activities and/or high-energy behaviours) was not encountered.

EFFECT ON INDIVIDUAL RE-SIGHTINGS

Possible long-term effects were inferred from the Humpback Whale Institute's photo-identification catalogue by comparing the frequencies of at least once re-sighted animals for biopsied and unbiopsied whales.

Data analysis

Maximum likelihood Chi-square of log-linear analysis of frequency tables were used for Biopsy Shots Characteristics, Group Behaviour and Group Characteristics. Only the models of interest, i.e. those that had the variable 'Reaction', were selected to be tested and the unsuccessful shots were excluded from this analysis. The models were excluded one by one and when a model did not fit the data, we concluded that the variables were interrelated. The Fleiss' Kappa was calculated to determine the degree of similarity between paired frequencies of behaviour prior to boat approach and during

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

contact period. In addition, McNemar's test was used for analysis of behaviour change, comparing the most frequent behaviour state with the remaining, in absence and presence of a boat. Contingency tables and Pearson's Chi-square were conducted to compare our results with the literature including vessels used comparisons and effect on individual re-sightings. Partition Chi-square tests were used to evaluate what levels contributed more to the obtained discrepancies. Approach type characteristics were analysed using ANOVA (the pursuit period) and Kruskal–Wallis test (the total contact period). Percentages were compared by Tukey-type multiple comparisons for proportions and significant results are represented by giving a $q_{0.05,\infty,3}$ value >3.314 and $q_{0.005,\infty,2} > 2.772$ (Zar, 1999). For all tests, a probability of <0.05 was accepted as significant.

RESULTS

A total of 542 shots were fired and 444 samples were obtained between 1997 and 2007. Behavioural reactions were registered for 484 shots: 396 from hits and 88 from unsuccessful shots. The most frequent category for both was 'No Response' (53.8%, $\chi^2 = 200.91$, df = 3, $P < 0.001$ and 52.8%, $\chi^2 = 48.27$, df = 3, $P < 0.001$, respectively). Among the whales which did respond, the most frequent category was 'Fluke Move' (47.5%, $\chi^2 = 42.00$, df = 2, $P < 0.00$ and 64.3%, $\chi^2 = 21.00$, df = 2, $P < 0.001$, respectively). There was no difference in responses to hits or unsuccessful shots ($\chi^2 = 4.24$, df = 3, $P = 0.237$).

Influence of biopsy shot characteristics

The reaction to biopsy sampling was not influenced by any characteristic of the biopsy shot considered, such as the region of hit (N = 95; 60% of them reached below the dorsal fin, 24.2% posterior and 15.8% the anterior area), the depth of sample (77.5% of N = 222 contained blubber) and the use of tethered darts (N = 149). The exclusion of any one model in log-linear analysis (N = 74) did not result in significant difference (each tested model is in Table 1).

Influence of vessels used

The use of vessels of different lengths, power and location of engine resulted in different categories of responses to collection of biopsy ($\chi^2 = 71.14$, df = 8, $P < 0.001$) (Table 2). Combining responses registered from large vessels with inboard engines and small vessels with outboard engines, different whale responses were noted ($\chi^2 = 25.64$, df = 2, $P < 0.001$). Darting from small and less powerful boats resulted in higher frequency of more intense responses and lower frequency of 'No Response' than from the larger ones (Moderate Reaction = 31.9 and 18.8%, $q_{0.05,\infty,2} = 12.14$, No Response = 45.1 and 53.6%, $q_{0.05,\infty,2} = 6.83$, respectively). In addition, 'Strong Reaction' was not registered in any attempts with larger boats.

Influence of approach type

Vessel speed when approaching (0–4 kn: N = 13, 5–9 kn: N = 13) did not influence the response to biopsy darting (due to the small sample size all the reactions were combined in one category: $\chi^2 = 0.15$, df = 1, $P = 0.695$). Contact period had no influence either ($\mu = 39.9 \pm 19.9$ minutes, range = 5 to 160, N = 396, $H_{3,396} = 4.10$, $P = 0.250$). However, in the period during active pursuit to reach a distance of 20 m ($\mu = 18.9 \pm 10.4$ minutes, range = 5 to 40, N = 54), the category 'No Response' was more frequent (53.7%, SS = 1072.88, df = 3, MS = 357.63, F = 3.81, $P = 0.016$).

Influence of group behaviour

The presence of the vessel altered the frequencies of behaviour observed prior to approach (Travel: from 42.91 to 65.20%, Social: from 34.46 to 29.05%, Resting: from 13.51 to 7.43%), thus the degree of similarity between behavioural states observed before and after vessel approach was low ($\kappa = 0.364 \pm 0.051$, cf. Landis & Koch, 1977). Only travelling behaviour increased in frequency in the presence of vessels (N = 127 prior to approach to N = 185 during the total contact period). Groups exhibiting behaviour other than travel decreased behaviour state frequency in the presence of the vessel (Social: N = 102 to N = 86; Resting: N = 40 to N = 22), reverting mainly to the Travel state (Social to Travel: $P = 0.010$, Resting to Travel: $P = 0.001$, McNemar's test).

The combined effect of the group behaviour prior to boat approach and during the contact period influenced the frequency of reaction to biopsy darting, as indicated by the model in which these variables were excluded (N = 326; Table 1). Whales biopsied while travelling and socializing showed 'No Response' most often (partition Chi-square: $\chi^2 = 88.92$, df = 3, $P < 0.001$ and $\chi^2 = 78.55$, df = 3, $P < 0.001$, respectively), while resting whales exhibited the 'Fluke Move' and 'No Response' with equal frequency ($\chi^2 = 8.86$, df = 3, $P = 0.031$).

Influence of group characteristics

Log-linear analysis was applied to the 357 cases in which reaction, group size ($\mu = 3.0 \pm$ SD = 1.31, range = 1 to 9, N = 396), group type (109 competitive groups) and sex of target animal (165 males and 167 females) were recorded on the same hit. As shown by the model in which the variables group size and group type were excluded, the combined effect of the number of individuals in the target group and its competitive character influenced the reaction to biopsy darting (Table 1). It also indicated that the sex of target animals did not interfere with intensity of reaction.

Furthermore, individuals with different behavioural roles responded differently to darting ($\chi^2 = 46.83$, df = 15, $P < 0.001$—100 mothers, 123 members of a pair, 25 members of a non-competitive trio, 50 escorts to a mother–calf pair, 30 singletons and 126 members of a competitive group). Reaction to biopsy differed between mothers with calves and non-lactating females ($\chi^2 = 9.37$, df = 3, $P = 0.025$). Females with their calves showed no reaction on 59.6% of the shots (N = 109), while non-lactating females reacted more frequently to biopsy (43.1% of 'No Response', N = 51). Among those females that did react, the same intensity of reaction occurred in the presence and absence of calves ($\chi^2 = 5.05$, df = 2, $P = 0.08$).

Influence of location

Response frequencies obtained in this study differed from those obtained in similar studies ($\chi^2 = 158.76$, df = 10,

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

Table 1. Results of log-linear analysis for 'Biopsy Shot Characteristics', 'Group Behaviour' and 'Group Characteristics'. 1 = 'Reaction' (four categories), 2 = 'Region of the Hit' (three categories), 3 = 'Depth of Sample' (two categories), 4 = 'Retrieval System Used' (two categories), 5 = 'Group Behaviour Prior to Boat Approach' (three categories), 6 = 'Group Behaviour During the Contact Period' (three categories), 7 = 'Group Size' (five categories), 8 = 'Group Type' (two categories), 9 = 'Sex of Target Animal' (two categories). Excluded models related to significant results (*) indicate that variables compounding it are interrelated.

	Model tested	Maximum likelihood Chi-square	df	*P*
Biopsy shot characteristics	1,2,3,4,21,31,41,321,421,431	4.67	6	0.587
	1,2,3,4,21,31,41,321,421	7.49	9	0.586
	1,2,3,4,21,31,41,321	13.30	15	0.579
	1,2,3,4,21,31,41	21.64	21	0.421
	1,2,3,4,21,31	23.11	23	0.454
	1,2,3,4,21	23.60	25	0.543
	1,2,3,4	26.52	29	0.598
Group behaviour	1,5,6,51,61,651	0.00	0	1.000
	1,5,6,51,61	105.33	16	< 0.001*
	1,5,6,51,651*	0.00	0	1.000
	1,5,6,651*	0.00	0	1.000
Group characteristics	1,7,8,9,71,81,91,871,971,981,9871	0.00	0	1.000
	1,7,8,9,71,81,91,871,971,981	4.78	16	0.997
	1,7,8,9,71,81,91,871,971	11.70	20	0.926
	1,7,8,9,71,81,91,871	36.58	36	0.442
	1,7,8,9,71,81,91	292.12	52	< 0.001*
	1,7,8,9,71,81,871*	39.63	39	0.442
	1,7,8,9,71,871*	39.62	39	0.442
	1,7,8,9,871*	39.63	39	0.442

Table 2. Different vessel characteristics (location and power of engine and length of the vessel) and the reactions of humpback whales to the biopsy shots.

Engine (HP)		Length (m)	Successful shots	No response (%)	Low level (%)	Moderate level (%)	Source
Inboard	250	15.5	263	143 (54.4)	82 (31.2)	38 (14.5)	This study 1
Inboard	250	14.2	61	27 (44.3)	14 (23.0)	20 (32.8)	This study 2
Inboard	90–120	13.0–16.0	38	24 (63.2)	4 (10.5)	10 (26.3)	This study 3
Outboard	70–90	5.0–7.5	206	71 (34.5)	38 (18.5)	87 (42.2)	Gauthier & Sears, 1999
Outboard	50	4.5	203	119 (58.6)	48 (23.7)	36 (17.7)	Brown *et al.*, 1994
Outboard	25–30	4.0–5.5	565	249 (44.7)	127 (22.5)	188 (33.3)	Clapham & Matilla, 1993

$P < 0.001$; Figure 2). Whales biopsied in the western South Atlantic reacted differently from those observed in a western North Atlantic breeding ground (Clapham & Mattila, 1993: $\chi^2 = 23.34$, df = 2, $P < 0.001$) and in all feeding grounds studied (Weinrich *et al.*, 1991: $\chi^2 = 79.25$, df = 2, $P < 0.001$; Weinrich *et al.*, 1992: $\chi^2 = 71.28$, df = 2, $P < 0.001$; Gauthier & Sears, 1999: $\chi^2 = 41.78$, df = 2, $P < 0.001$). However, our results were similar to those observed in a migration area (Brown *et al.*, 1994: $\chi^2 = 1.301$, df = 2, $P = 0.520$). The intensity of response to sampling darts was different in areas used for different purposes, i.e. between breeding, feeding and migration areas ($\chi^2 = 111.75$, df = 4, $P < 0.001$). Whales biopsied in feeding grounds responded more intensely than those in breeding grounds and the latter responded more intensely than whales in migration (Figure 2).

Effect on individual re-sightings

The Humpback Whale Institute photo-identified 2210 whales between 1989 and 2005, 429 of which (19.4%) have been re-sighted at least once. During our study, we sampled 83 whales that matched this catalogue, 10 of which (12.1%) were re-sightings. Difference in the proportion of biopsied and unbiopsied re-sighted whales has not been detected ($\chi^2 = 2.15$, df = 1, $P = 0.143$).

DISCUSSION

Cetaceans are exposed to numerous human disturbances and generally react to them by avoiding the source of stimulus. Vessel traffic, industrial activities (Richardson *et al.*, 1985), whale watching (Bejder *et al.*, 2006) and noise (Sousa-Lima & Clark, 2008) result in greater behaviour change than that reported for collection of biopsies. In fact, the impact of remote biopsy sampling has been compared to a mildly noxious stimulus (Weinrich *et al.*, 1992). We documented one more case study where no major disturbance could be detected. The range of behavioural responses observed in our study area is comparable with those reported for several other species (Whitehead *et al.*, 1990; Brown *et al.*, 1991; IWC, 1991; Barrett-Lennard *et al.*, 1996; Jahoda *et al.*, 1996; Weller *et al.*, 1997; Gauthier & Sears, 1999; Hooker *et al.*, 2001; Best *et al.*, 2005; Gorgone *et al.*, 2008; Jefferson & Hung, 2008). Although stronger reactions have been reported under unusual circumstances (Brown *et al.*, 1991; Weinrich *et al.*, 1991), such as stuck darts or entangled retrieval lines, we did not detect intense behaviours, even under similar situations. In addition, all responses were part of the natural repertoire of the species and may occur in other contexts (Weinrich *et al.*, 1992; Gauthier & Sears, 1999). Reactions can be provoked by missed shots too, suggesting that an

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

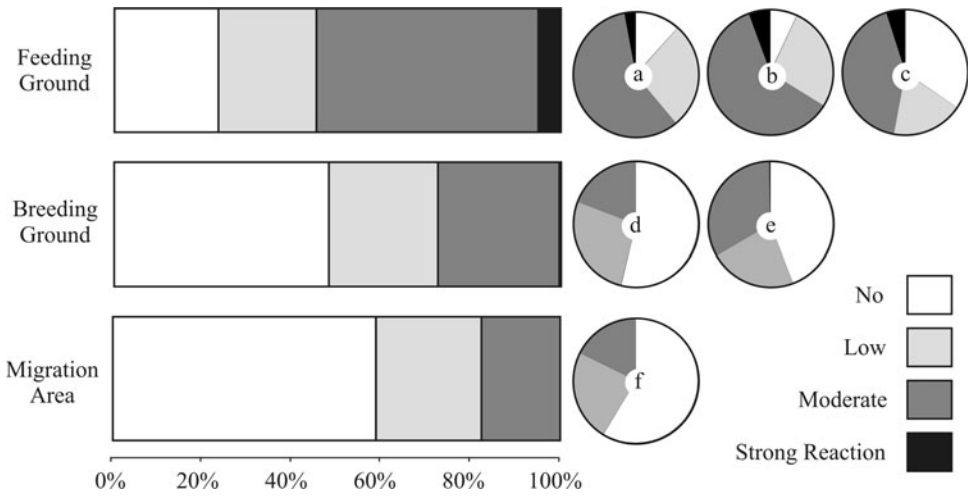

Fig. 2. Frequencies of humpback whales' reactions to biopsy sampling conducted in areas used for different purposes. (a) Weinrich *et al.*, 1991, N = 103; (b) Weinrich *et al.*, 1992, N = 71; (c) Gauthier & Sears, 1999, N= 206; (d) this study, N = 396; (e) Clapham & Mattila, 1993, N = 565; (f) Brown *et al.*, 1994, N = 203.

unexpected disturbance or a stimulus other than the contact with the dart itself, such as the sound of the dart hitting the water or the approach of a boat, can also incite the response (Weinrich *et al.*, 1991; Krützen *et al.*, 2002; Gorgone *et al.*, 2008).

Similarly, lesions caused by the dart are minor compared with wounds from intraspecific interactions (cf. Pack *et al.*, 1998) or other anthropogenic factors (cf. Pizzorno *et al.*, 1998). Studies carried out on dolphins found no evidence of infection or difficult healing (Krützen *et al.*, 2002; Jefferson & Hung, 2008), even with more intrusive procedures than biopsy sampling (Weller *et al.*, 1997). Although non-intrusive techniques, such as skin swabbing (Harlin *et al.*, 1999) and use of a biopsy pole (Bilgmann *et al.*, 2007), pose lower risk of injury and frequency of responses, they do not obtain blubber samples or, occasionally, sufficient amount of skin. Success rates using remote biopsy sampling are also generally higher (Weinrich *et al.*, 1991, 1992; Brown *et al.*, 1994; Gauthier & Sears, 1999), further attesting to the effectiveness of this technique.

With regards to the characteristics of the shot measured in this study, namely region of hit, depth of sample and retrieval system, there was no variation in the behavioural responses observed. Although the surface just below the dorsal fin is the recommended target region for biopsy collection, in some cases distance and weather conditions did not allow this area to be precisely reached. However, we found that hits anterior and posterior to the dorsal fin did not cause a specific category of response either; similarly, reactions to radio tag implantation also occur independently of the body region hit (Watkins, 1981). A tail flick reaction (Weinrich *et al.*, 1992), equivalent to our 'Fluke Slap', could be expected as a reflexive response to a dart hitting the peduncle or fluke. Nevertheless, this behaviour is also exhibited as a result of close approach by a vessel (Watkins, 1981; Weinrich *et al.*, 1992; personal observation), suggesting that this reaction may contain both intuitive and intentional components (Weinrich *et al.*, 1992).

Another characteristic of the shot expected to influence reaction was the depth of the sample, based on studies with small cetaceans whose longer tissue samples were associated to stronger reactions in bottlenose dolphins (Krützen *et al.*, 2002). However, in our study, deeper samples did not trigger stronger reactions, consistent with the low level reactions caused by the much deeper intra-muscular radio tag implantation in other balaenopterids (Watkins, 1981). Similarly, although Gauthier & Sears (1999) suggested the high frequency of response by minke whales (*Balaenoptera acutorostrata*) to be partly related to the relatively longer samples in their study that compared length of blubber sample to responses in four species of balaenopterids, no significant relationship between frequency of reaction and sample size was found.

In recent studies (Gauthier & Sears, 1999; Best *et al.*, 2005), retrieval line systems have been avoided out of concern for the risk of entanglement and strong responses (Weinrich *et al.*, 1991). However, we found that tethered darts did not increase the response of our study animals. In fact, the use of a tether was beneficial to expedite dart recovery, reducing the number of boat manoeuvers needed and the time spent in the vicinity of the group. Even in cases of contact (N = 2) and entanglement (N = 2) with the line, the responses were low and moderate level (Startle and Fluke Slap), respectively.

Although avoidance reactions to vessel approach are evident with or without biopsy attempts, the presence of a vessel could be associated with darting, increasing avoidance behaviours (c.f. Clapham & Mattila, 1993; Gorgone *et al.*, 2008). Whales can evade vessels either vertically, reducing surface time, i.e. dive time and breath intervals increase and speed of travel decreases, or horizontally (the opposite) (Jahoda *et al.*, 2003). Weinrich *et al.* (1992) further suggested that boat presence may influence surface interval/dive time rates. As such, another parameter expected to influence reaction to biopsy was the type of vessel and engine used. Studies carried out from smaller and less powerful boats with outboard engines (Clapham & Mattila, 1993; Brown *et al.*, 1994; Gauthier & Sears, 1999), registered higher frequency and intensity of responses to biopsy attempts than our observations from larger boats with more powerful inboard engines. Likewise, although the species and techniques used were different, Bilgmann *et al.* (2007) compared small boats (5.6 to 6 m, 90 to 115 HP, outboard engine) to boats bigger than

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

ours (16 to 25 m, 115 to twin 600 HP, inboard engine) when conducting an experiment with biopsy pole on small cetaceans (*Tursiops* sp. and *Delphinus delphis*) and found that smaller boats resulted in stronger reactions.

Although all types of vessels produce underwater noise, which may induce avoidance, studies with humpbacks also reported only minor behaviour changes to larger whale-watching boats when compared to smaller, but noisier ones (Au & Green, 2000). Ross (1976) explained that most of the noise of boats is caused by propeller cavitations and that outboard engines of small boats cause greater cavitations than inboard engines of large boats, thus producing more sound since they need more revolutions per minute (RPM) to get a similar thrust (Au & Green, 2000).

The types of approach and permanence period with a group of whales are also factors which might influence the probability of reaction to biopsy darting. Especially for lactating females, a slow and patient approach produces less evasion, which increases the sampling success and reduces harassment (Clapham & Mattila, 1993). Although the approach speed of up to 9 knots appears not to influence the response to darting, more data are necessary to address this question further.

Behavioural or photo-identification studies prior to biopsy sampling increase the period of permanence of the boat with the whales and thus the potential of disturbance. Brown *et al.* (1994) suggested that the threshold of response to darting may be lower when the whales are previously subjected to other forms of human annoyance. However, in our study, the intensity of response to biopsy procedures was not affected by the period of time our research boat remained near the whales. Moreover, during the active pursuit to get within firing range whales were less prone to react, probably because in this case the stimulus can be anticipated, as was the case with radio tag implants (Watkins, 1981). Repetitive biopsy attempts can also alter the whale's state of alertness, indeed several species showed a less intense or similar response to successive hits (Brown *et al.*, 1994; Gauthier & Sears, 1999).

The presence of a vessel can also alter the whales' essential behaviour states, such as resting and reproduction. In the presence of boats, other studies in the same area verified a reduction of resting in groups of females with calves (Morete *et al.*, 2007) and interruption of the song or evasion by singer males (Sousa-Lima & Clark, 2008). Throughout our study, when the boat approached, groups involved in social and resting activities usually changed these states to travel, as a means of avoiding the source of disturbance. Weinrich *et al.* (1992), monitored focal groups prior to and after biopsy and registered a slight increase in frequency of agonistic behaviours (hard tail flicks and trumpet blows). Thus the majority of whales might have been sampled in a disturbed state. Since it is not possible to collect samples without a boat, this behaviour change appears to be inevitable.

The tolerance threshold of an individual to a stimulus seems to be also related to the activity in progress at the time of sampling. When feeding or socializing, the animals generally ignore the disturbing stimulus, differently from inactive animals (Watkins, 1986; cf. IWC, 1991). In our study area, travelling humpbacks responded less frequently than resting animals, as observed in animals feeding in the Gulf of Maine (Weinrich *et al.*, 1992), and strong reactions, although rare, were shown by a resting individual in another breeding ground (Clapham & Mattila, 1993). Similarly, Barrett-Lennard *et al.* (1996) noted that resting killer whales show more intense reactions to biopsy sampling than others. However, during migration their reaction to sampling is not affected by the behaviour state (Brown *et al.*, 1994).

This study, as others (Clapham & Mattila, 1993; Cerchio, 2003; Best *et al.*, 2005), found that in the breeding grounds, the behavioural role of an individual, determined by group size, seems to influence the reaction to biopsy darting, while sex is not a relevant factor. Population dynamics and group characteristics reflect the competitive character of the breeding grounds (Chittleborough, 1965), where large groups are usually involved in violent and fast-moving behaviours (Tyack & Whitehead, 1983; Clapham *et al.*, 1992) with intense contact and agonistic intra-sexual interactions that can have fatal consequences (Pack *et al.*, 1998). These group characteristics and aggressive behaviour influence the reactions to skin sampling. Lone animals and small groups tend to respond more intensely, while in competitive groups, where the animals are in high excitation and expect physical contact, the brief stimulus of a dart may be less noticed (Clapham & Mattila, 1993).

The behavioural role of individuals in competitive groups affected the intensity of response to biopsies as well. Nuclear animals, presumably females, respond more than males which dispute for them. Also, the hierarchy among males may be critical in response intensity: secondary escorts respond more than challengers, and these in turn respond more than principal escorts (Clapham & Mattila, 1993). Principal escorts are the focus of the aggressive contact (cf. Herman *et al.*, 2008) thus they are probably subjected to greater physical contact than that delivered by the hit of a dart. A challenger, which is attempting to displace the principal escort, is prepared for physical contact but probably not as much as his opponent. Finally, the secondary escort appears to be more sensitive to an unexpected stimulus, since it is not actively challenging the principal escort (Cerchio, 2003).

Mother and calf pairs, in contrast to non-lactating females, also show low response to the dart stimulation during the reproductive season. This observation is typical of breeding areas where constant contact with their calves makes mothers less prone to reaction because they are less surprised by a further tactile stimulus (Clapham & Mattila, 1993; cf. Cerchio, 2003). On one occasion during this study, a calf was witnessed breaching repeatedly on its mother's back, who did not present any response when biopsied between these breaching events. Calves, however, which seem to be naive and probably more sensitive, present the stronger reactions (Cerchio, 2003). It should be noted though that a study with right whales (*Eubalaena australis*) in a breeding ground (Best *et al.*, 2005) found non-lactating females responded less frequently than mothers. This variation may be due to differences in body morphology between the species. Gauthier & Sears (1999) proposed that morphological diversity (body size and thickness of the epithelial tissues) was the main cause of the differences in behavioural reactions to biopsy darting in four species of balaenopterids (*B. musculus, B. physalus, B. acutorostrata* and *M. novaeangliae*).

Location can be a main contributor to variation in biopsy sampling responses, resulting in different frequencies and intensities of reaction to darting: whales biopsied in feeding grounds respond with more intensity than in breeding grounds (Clapham & Matilla, 1993), which in turn respond more intensely than whales in migration (Brown *et al.*, 1994).

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

The humpback whale's migratory behaviour includes more than just travelling and some reproductive behaviours are not uncommon. Throughout the northward and southward migration periods, small groups and male–female pairs are most common (Brown & Corkeron, 1995) and mating attempts and mate guarding can occur (Clapham, 2000). Brown *et al.* (1994) found that gender is the most important factor governing an individual's response to biopsy sampling in a migration area, while group size did not interfere. Since competitive groups and mother–calf pairs are rare in this period, the response threshold of females is therefore more likely to be crossed than that of males.

In the feeding grounds, group compositions are different, dependent mainly on the unpredictable distribution of prey. Solitary whales and small groups are the most common (Weinrich & Kuhlberg, 1991), while some brief cooperation in foraging groups can be observed (Hain *et al.*, 1982) but agonistic encounters are rare (Clapham, 2000) and, consequently, tactile stimulus among the individuals is infrequent. Therefore, Weinrich *et al.* (1991) and Gauthier & Sears (1999) observed that neither group size nor gender interfered in the reaction to biopsy sampling. Likewise, mothers and calves biopsied in feeding grounds react as frequently as other groups (Weinrich *et al.*, 1991; Clapham & Mattila, 1993; unpublished data). This is probably due to the rapid maturation process of juveniles (Chittleborough, 1965), who receive gradually less attention from their mothers and become independent early (Baraff & Weinrich, 1993). As such the calves, who are a few months older in the feeding grounds than in the breeding grounds, receive less intense care from their mothers and therefore the physical contact between them is inferior. The less significant physical stimulus on the feeding grounds may explain why whales are more surprised by the sampling dart there than in any other location.

Although immediate reactions can be observed in every study involving biopsy collection, there is no significant evidence that this procedure leads to any long-term negative impacts on individuals or their populations. Even though low, the rate of photo-identification recaptures of biopsied and unbiopsied animals are equivalent (Weinrich *et al.*, 1991; Clapham & Mattila, 1993; Weller *et al.*, 1997; Best *et al.*, 2005; this study), suggesting that darting does not alter the movements or distribution of individuals. Based on long-term studies of the effects on the reproduction of right whales (Best *et al.*, 2005), we may also expect no adverse impacts on their reproductive cycles or calf survival.

This study provides new data supporting low level responses by whales to remote biopsy sampling procedures. This method results in only limited annoyance and the behavioural responses observed are instantaneous and comparable to other human harassments. The intensity of reactions to the artificial darting stimulus was influenced by some individual characteristics as well as variables at the time of sampling. The extent of excitation and physical contact between individuals is determined by group size and behaviour, which in turn depends on location and seasonal variations and influences levels of reaction: breeding humpbacks adopt different roles in a group, and thus react with different intensity than migrating or feeding whales. Also, because biopsy sampling is an abrupt stimulus, if the whales are more active prior to the hit then the animals can be expected to react less intensely.

We conclude that the continuation of biopsy sampling studies in the western South Atlantic breeding ground is unlikely to be harmful for this population. From a wildlife conservation perspective, human disturbances are only of concern if they affect survival or fertility, consequently decreasing population size (Gill *et al.*, 2001). Hence, in the absence of these long-term impacts, and bearing in mind all the vital conclusions that can be obtained from biopsy material, the short-term level of disturbances incurred may be considered acceptable.

ACKNOWLEDGEMENTS

Biopsy samples were collected under permission of CMA/IBAMA (03-06 Proc. 02034.000097/01-25). PARNAM allowed the collection within the National Marine Park of Abrolhos (05/2006 Proc. PNA005-06). We thank the staff of Instituto Baleia Jubarte for executive management and the crew, researchers and interns for assistance in the field. We are grateful to Norsul Cia de Navegação for research boat maintenance; Paulo Enrique Cardoso Peixoto for help in data analysis; Jennifer Lewis; Leonardo Wedekin and the anonymous referees for important comments and suggestions; Ana Lúcia Cypriano-Souza for molecular genetic analysis; Leonardo Wedekin for preparing the map; Fernando Guilherme-Silveira for ideas on first drafts; Michelle Borsz and Maria Cecília Magnani for proofreading English; and David Janiger for making several papers available. The Instituto Baleia Jubarte is sponsored by Petróleo Brasileiro S.A. (PETROBRAS).

REFERENCES

Altmann J. (1974) Observational study of behavior: sampling methods. *Behaviour* 49, 227–265.

Andriolo A., Martins C.C.A., Engel M.H., Pizzorno J.L., Mas-Rosa S., Freitas A., Morete M.E. and Kinas P.G. (2006) The first aerial survey to estimate abundance of humpback whale (*Megaptera novaeangliae*) in the breeding ground off Brazil. *Journal of Cetacean Research and Management* 8, 307–311.

Amos B. and Hoelzel A.R. (1990) DNA fingerprinting cetacean biopsy samples for individual identification. *Report of the International Whaling Commission* Special Issue 12, 79–85.

Au W.W.L. and Green M. (2000) Acoustic interactions between humpback whales and whale-watching boats. *Marine Environmental Research* 49, 469–481.

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

Baker C.S., Perry A., Bannister J.L., Weinrich M.T., Abernethy R.B., Calambokidis J., Lien J., Lambertsen R.H., Ramirez J.U., Vasquez O., Clapham P.J., Alling A., O'brien S.J. and Palumbi S.R. (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proceedings of the National Academy of Sciences of the United States of America* 90, 8239–8243.

Baker C.S., Slade R.W., Bannister J.L., Abernethy R.B., Weinrich M.T., Lien J., Urban- R.J., Corkeron P., Calambokidis J., Vasquez O. and Palumbi S.R. (1994) Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. *Molecular Ecology* 3, 313–327.

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

Baraff L. and Weinrich M.T. (1993) Separation of humpback whale mothers and calves on a feeding ground in early autumn (*Megaptera novaeangliae*). *Marine Mammal Science* 9, 431–434.

Barrett-Lennard L.G., Smith T.G. and Ellis G.M. (1996) A cetacean biopsy system using lightweight pneumatic darts and its effect on the behavior of killer whales. *Marine Mammal Science* 12, 14–27.

Bearzi G. (2000) First report of a common dolphin (*Delphinus delphis*) death following penetration of a biopsy dart. *Journal of Cetacean Research and Management* 2, 217–221.

Bejder L., Saumel A., Whitehead H., Gales N., Mann J., Connor R., Heithaus M., Watson-Capps J., Flaherty C. and Krützen M. (2006) Decline in relative abundance of bottlenose dolphins exposed to long-term disturbance. *Conservation Biology* 20, 1791–1798.

Bérubé M. and Palsbøll P. (1996) Identification of sex in cetaceans by multiplexing using ZFX and ZFY specific primers. *Molecular Ecology* 5, 283–287.

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

Bilgmann K., Griffiths O.J., Allen S.J. and Möller L.M. (2007) A biopsy pole system for bow-riding dolphins: sampling success, behavioral responses and test for sampling bias. *Marine Mammal Science* 23, 218–225.

Borobia M., Gearing P.J., Simard Y., Gearing J.N. and Beland P. (1995) Blubber fatty acids of finback and humpback whales from the Gulf of St. Lawrence. (*Balaenoptera physalus, Megaptera novaeangliae*). *Marine Biology (Berlin)* 122, 341–353.

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

Brown M.R. and Corkeron P.J. (1995) Pod characteristics of migrating humpback whales (*Megaptera novaeangliae*) off the east Australian coast. *Behavior* 132, 163–179.

Brown M.R., Corkeron P.J., Hale P.T., Schultz K.W. and Bryden M.M. (1994) Behavioral responses of east Australian humpback whales (*Megaptera novaeangliae*) to biopsy sampling. *Marine Mammal Science* 10, 391–400.

Cerchio S. (2003) *Paternity, polygyny and alternative mating tactics in humpback whales* (Megaptera novaeangliae). PhD thesis. University of Michigan, Ann Arbor, USA.

Cerchio S., Jacobsen J.K., Cholewiak D.M., Falcone E.A. and Merriwether D.A. (2005) Paternity in humpback whales, *Megaptera novaeangliae*: assessing polygyny and skew in male reproductive success. *Animal Behavior* 70, 267–277.

Chittleborough R.G. (1965) Dynamics of two populations of the humpback whale, *Megaptera novaeangliae* (Borowski). *Australian Journal of Marine and Freshwater Research* 16, 33–128.

Clapham P.J. (2000) The humpback whale: seasonal feeding and breeding in baleen whale. In Mann J., Connor R.C., Tyack P.L. and Whitehead H. (organizers) *Cetacean societies—field studies of dolphins and whales*. Chicago: The University of Chicago Press, pp. 173–196.

Clapham P.J. and Mattila D.K. (1993) Reactions of humpback whales to skin biopsy sampling on a West Indies breeding ground (*Megaptera novaeangliae*). *Marine Mammal Science* 9, 382–391.

Clapham P.J. and Palsbøll P.J. (1997) Molecular analysis of paternity shows promiscuous mating in female humpback whales (*Megaptera novaeangliae*, Borowski). *Proceedings of the Royal Society of London, Series B Biological Sciences* 264, 95–98.

Clapham P.J., Palsbøll P.J., Mattila D.K. and Vasquez O. (1992) Composition and dynamics of humpback whale competitive groups in the West Indies (*Megaptera novaeangliae*). *Behavior* 122, 182–194.

Clapham P.J., Palsbøll P.J. and Mattila D.K. (1993) High energy behaviors in humpback whales as a source of sloughed skin for molecular analysis. *Marine Mammal Science* 9, 214–220.

Cunha H.A. and Sole-Cava A.M. (2007) Molecular sexing of tucuxi dolphins (*Sotalia guianensis* and *Sotalia fluviatilis*) using samples from biopsy darting and decomposed carcasses. *Genetics and Molecular Biology* 30, 1186–1188.

Cypriano-Souza A.L. (2008) *Caracterização genética da população de baleias jubarte,* Megaptera novaeangliae, *da Área de Reprodução do Oceano Atlântico Sul Ocidental baseada em microssatélites nucleares.* MSc thesis. Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil.

Engel M. (1996) Comportamento reprodutivo da baleia jubarte (*Megaptera novaeangliae*) em Abrolhos. *Etologia* 14, 275–284.

Engel M.H., Fagundes N.J.R., Rosenbaum H.C., Leslie M.S., Ott P.H., Schmitt R., Secchi E., Dalla Rosa L. and Bonattelo S.L. (2008) Mitochondrial DNA diversity of the Southwestern Atlantic humpback whale (*Megaptera novaeangliae*) breeding area off Brazil, and the potential connections to Antarctic feeding areas. *Conservation Genetics* 9, 1253–1262.

Fossi M.C., Marsili L., Neri G., Casini S., Bearzi G., Politi E., Zanardelli M. and Panigada S. (2000) Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants. *Marine Environmental Research* 50, 517–521.

Freitas A.C., Kinas P.G., Martins C.C.A. and Engel M.H. (2004) Abundance of humpback whales on the Abrolhos Bank wintering ground, Brazil. *Journal of Cetacean Research and Management* 3, 225–230.

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

Gendron D., Aguiniga S. and Carriquiry J.D. (2001) 8^15n And 8^13c in skin biopsy samples: a note on their applicability for examining the relative trophic level in three rorqual species. *Journal of Cetacean Research and Management* 3, 41–44.

Gill J.A., Norris K. and Sutherland W.J. (2001) Why behavioral responses may not reflect the population consequences of human disturbance. *Biological Conservation* 97, 265–268.

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

Green M.L., Herzing D.L. and Baldwin J.D. (2007) Noninvasive methodology for the sampling and extraction of DNA from free-ranging Atlantic spotted dolphins (*Stenella frontalis*). *Molecular Ecology Notes* 7, 1287–1292.

Hain J.H.W., Karter G.R., Kraus S.D., Mayo C.A. and Winn H.E. (1982) Feeding behavior of humpback whale (*Megaptera novaeangliae*) in the western North Atlantic. *Fishery Bulletin* 80, 259–268.

Harlin A.D., Würsig B., Baker C.S. and Markowitz T.M. (1999) Skin swabbing for genetic analysis: application to dusky dolphins (*Lagenorhynchus obscurus*). *Marine Mammal Science* 15, 409–425.

Herman E.Y.K., Herman L.M., Pack A.A., Marshall G., Shepard C.M. and Bakhtiari M. (2008) When whales collide: Crittercam offers insight into the competitive behavior of humpback whales on their Hawaiian wintering grounds. *Marine Technology Society Journal* 41, 35–43.

Hoelzel A.R. (1991) Report of the workshop on the genetic analysis of cetacean populations. *Report of the International Whaling*

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

Commission (Genetic Ecology of Whales and Dolphins) Special Issue 13, 3–22.

Hoelzel A.R. and Amos W. (1988) DNA fingerprinting and 'scientific' whaling. *Nature (London)* 333, 305.

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

International Whaling Commission (IWC) (1991) Report of the *ad-hoc* working group on the effect of biopsy sampling on individual cetaceans. *Report of International Whaling Commission* Special Issue 13, 23–27.

Jackson J.A., Patenaude N.J., Carroll E.L. and Baker C.S. (2008) How few whales were there after whaling? Inference from contemporary mtDNA diversity. *Molecular Ecology* 17, 236–251.

Jahoda M., Airoldi S., Azzellino A., Biassoni N., Borsani J.F., Cianfanelli L., Lauriano G., Sciara G.N.D., Panigada S., Vallini C. and Zanardelli M. (1996) Behavioral reactions to biopsy-darting on Mediterranean fin whales. *European Research on Cetaceans* 10, 43–47.

Jahoda M., Lafortuna C.L., Biassoni N., Almirante C., Azzellino A., Panigada S., Zanardell I.M. and di Sciara G.N. (2003) Mediterranean fin whale's (*Balaenoptera physalus*) response to small vessels and biopsy sampling assessed through passive tracking and timing of respiration. *Marine Mammal Science* 19, 96–110.

Jefferson T.A. and Hung S.K. (2008) Effects of biopsy sampling on Indo-Pacific humpback dolphins (*Souza chinensis*) in a polluted coastal environment. *Aquatic Mammals* 34, 310–316.

Krützen M., Barré L.M., Möller L.M., Heithaus M.R., Simms C. and Sherwin W.B. (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 large whales and its use for cytogenetics. *Journal of Mammalogy* 68, 443–445.

Landis J.R. and Koch G.G. (1977) The measurement of observer agreement for categorical data. *Biometrics* 33, 159–174.

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

Martins C.C.A., Morete M.E., Engel M.H., Freitas A., Secchi E.R. and Kinas P.G. (2001) Aspects of habitat use patterns of humpback whales in the Abrolhos Bank, Brazil, breeding ground. *Memoirs of the Queensland Museum* 47, 563–570.

Medrano L., Salinas M., Salas I., Ladron De Guevara P., Aguayo A., Jacobsen J. and Baker C.S. (1994) Sex identification of humpback whales, *Megaptera novaeangliae*, on the wintering grounds of the Mexican Pacific Ocean. *Canadian Journal of Zoology* 72, 1771–1774.

Mesnick S.L., Clapham P. and Dizon A.E. (1999) A note on the collection of associated behavioral data with biopsy samples during cetacean assessment cruises. *Journal of Cetacean Research and Management* 1, 205–211.

Metcalfe C., Koenig B., Metcalfe T., Paterson G. and Sears R. (2004) Intra- and inter-species differences in persistent organic contaminants in the blubber of blue whales and humpback whales from the Gulf of St Lawrence, Canada. *Marine Environmental Research* 57, 245–260.

Morete M.E., Freitas A., Engel M.H., Pace R.M. III and Clapham P.J. (2003) A novel behavior observed in humpback whales on wintering grounds at Abrolhos Bank (Brazil). *Marine Mammal Science* 19, 694–707.

Morete M.E., Bisi T.L. and Rosso S. (2007) Mother and calf humpback whale responses to vessels around the Abrolhos Archipelago, Bahia, Brazil. *Journal of Cetacean Research and Management* 9, 241–248.

Morete M.E., Bisi T.L., Pace R.M. III and Rosso S. (2008) Fluctuating abundance of humpback whales (*Megaptera novaeangliae*) in a calving ground off coastal Brazil. *Journal of the Marine Biological Association of the United Kingdom* 88, 1229–1235.

Pack A.A., Salden D.R., Ferrari M.J., Glockner-Ferrari D.A., Herman L.M., Stubbs H.A. and Straley J.M. (1998) Male humpback whale dies in competitive group (*Megaptera novaeangliae*). *Marine Mammal Science* 14, 861–873.

Palsbøll P.J., Vader A., Bakke I. and El-Gewely M.R. (1992) Determination of gender in cetaceans by polymerase chain reaction. *Canadian Journal of Zoology* 70, 2166–2170.

Palsbøll P.J., Allen J., Bérubé M., Clapham P.J., Feddeersen T.P., Hammond P.S., Hudson R.R., Jogensen H., Katona S., Larsen A.H., Larsen F., Lien J., Mattila D.K., Sigurjonsson J., Sears R., Smith T., Sponer R., Stevick P. and Oien A.N. (1997) Genetic tagging of humpback whales (*Megaptera novaeangliae*). *Nature (London)* 388, 767–769.

Parsons K.M., Durban J.W. and Claridge D.E. (2003) Comparing two alternative methods for genetic sampling of small cetaceans. *Marine Mammal Science* 19, 224–231.

Pizzorno J.L.A., Brito L.J., Dorneles P.R., Azevedo A.F. and Do N. Gurgel I.M.G. (1998) Review of strandings and additional information on humpback whales, *Megaptera novaeangliae*, in Rio de Janeiro, southeastern Brazilian coast (1981–1997). *Report of International Whaling Commission* 48, 443–446.

Richardson W.J., Fraker M.A., Würsig B. and Wells R.S. (1985) Behavior of bowhead whales *Balaena mysticetus* summering in the Beaufort Sea: reactions to industrial activities. *Biological Conservation* 32, 195–230.

Rosenbaum H.C., Best P.B., Findlay K.P., Engel M.H., Pomilla C., Razafindrakoto Y., Morete M.E., Vely M., Freitas A.C., Baker C.S., Jenner C., Jenner M-N and Bannister J. (2000) *Mitochondrial DNA variation among humpback whales from the wintering grounds in the South Atlantic and Southwestern Indian Oceans*. Report of Scientific Committee of International Whaling Commission, SC/52/IA11 (unpublished).

Ross D. (1976) *Mechanics of underwater noise*. New York: Pergamon Press.

Simões D.G., Macedo R.H.F. and Engel M.H. (2005) Turismo de observação de cetáceos como ferramenta no estudo do comportamento de baleias jubarte (*Megaptera novaeangliae*). *Revista de Etologia* 1, 3–14.

Sousa-Lima R.S. and Clark C.W. (2008) Modeling the effect of boat traffic on the fluctuation of humpback whale singing activity in the Abrolhos National Marine Park, Brazil. *Canadian Acoustics* 36, 174–181.

Tyack P. and Whitehead H. (1983) Male competition in large groups of wintering humpback whales. *Behavior* 83, 132–154.

Watkins W.A. (1981) Reactions of three species of whales *Balaenoptera physalus, Megaptera novaeangliae*, and *Balaenoptera edeni*, to implanted radio tags. *Deep-Sea Research* 28A, 589–599.

Watkins W.A. (1986) Whale reactions to human activities in Cape Cod waters. *Marine Mammal Science* 2, 251–262.

Weinrich M.T. and Kuhlberg A.E. (1991) Short-term association patterns of humpback whale (*Megaptera novaeangliae*) groups on their feeding grounds in the southern Gulf of Maine. *Canadian Journal of Zoology* 69, 3005–3011.

Weinrich M.T., Lambertsen R.H., Baker C.S., Schilling M.R. and Belt C.R. (1991) Behavioral responses of humpback whales (*Megaptera*

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press

novaeangliae) in the Southern Gulf of Maine to biopsy sampling. *Report of the International Whaling Commission (Genetic Ecology of Whales and Dolphins)* Special Issue 13, 91–97.

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

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

Whitehead H. (1983) Structure and stability of humpback whale groups off Newfoundland. *Canadian Journal of Zoology* 61, 1391–1397.

Whitehead H., Gordon J., Mathews E.A. and Richard K.R. (1990) Obtaining skin samples from living sperm whales. *Marine Mammal Science* 6, 316–326.

Zar J.H. (1999) *Biostatistical analysis*, 4th edition. Upper Saddle River, NJ: Prentice-Hall.

and

Zerbini A.N., Andriolo A., da Rocha J.M., Simões-Lopes P.C., Siciliano S., Pizzorno J.L.A., Waite J.M., DeMaster D.P. and Van Blaricom G.R. (2004) Winter distribution and abundance of humpback whales, *Megaptera novaeangliae*, off Northeastern Brazil. *Journal of Cetacean Research and Management* 6, 101–107.

Correspondence should be addressed to:
M. Cantor
Instituto Baleia Jubarte, Rua Barão do Rio Branco 26, CEP 45900-000, Caravelas, BA, Brazil
email: m.cantor@ymail.com

https://doi.org/10.1017/S0025315409991561 Published online by Cambridge University Press