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Behavioural responses of Argentine coastal dusky dolphins (Lagenorhynchus obscurus) to a biopsy pole system

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Abstract

*In recent years, analysis of tissue samples has become a powerful tool in cetacean ecology since it provides information for effective conservation and management policies. Biopsy samples taken for such studies have been obtained by various means including crossbows, rifles, and pole systems. We investigated sampling success and the reaction of dusky dolphins (*Lagenorhynchus obscurus*) off the coast of Argentina to a biopsy pole system from 2008 to 2009. Using* ad libitum *behavioural sampling, we documented individual behavioural reactions to biopsy sampling immediately after a biopsy was taken. Log-linear models were used to determine whether there were significant differences in behavioural response for different group size, composition or behavioural context. In total, 109 sampling attempts were made and 58 biopsy samples were obtained (53.21% success rate). The intensity of individual response was influenced by group size and composition. Small groups (typically mother/calf) reacted more frequently than larger groups (generally mixed-age adults and juveniles). The behaviour of the animals prior to our approach for biopsy sampling also affected their response. Milling dolphins appeared to respond more intensively than dolphins socialising or travelling. In conclusion, biopsy sampling with the biopsy pole system presented here show generally mild and short-term reactions, though behaviour, group size and group composition affected the response. This information can be used to minimise the impact of biopsy sampling, and facilitate the collection of data critical to effective conservation strategies.*

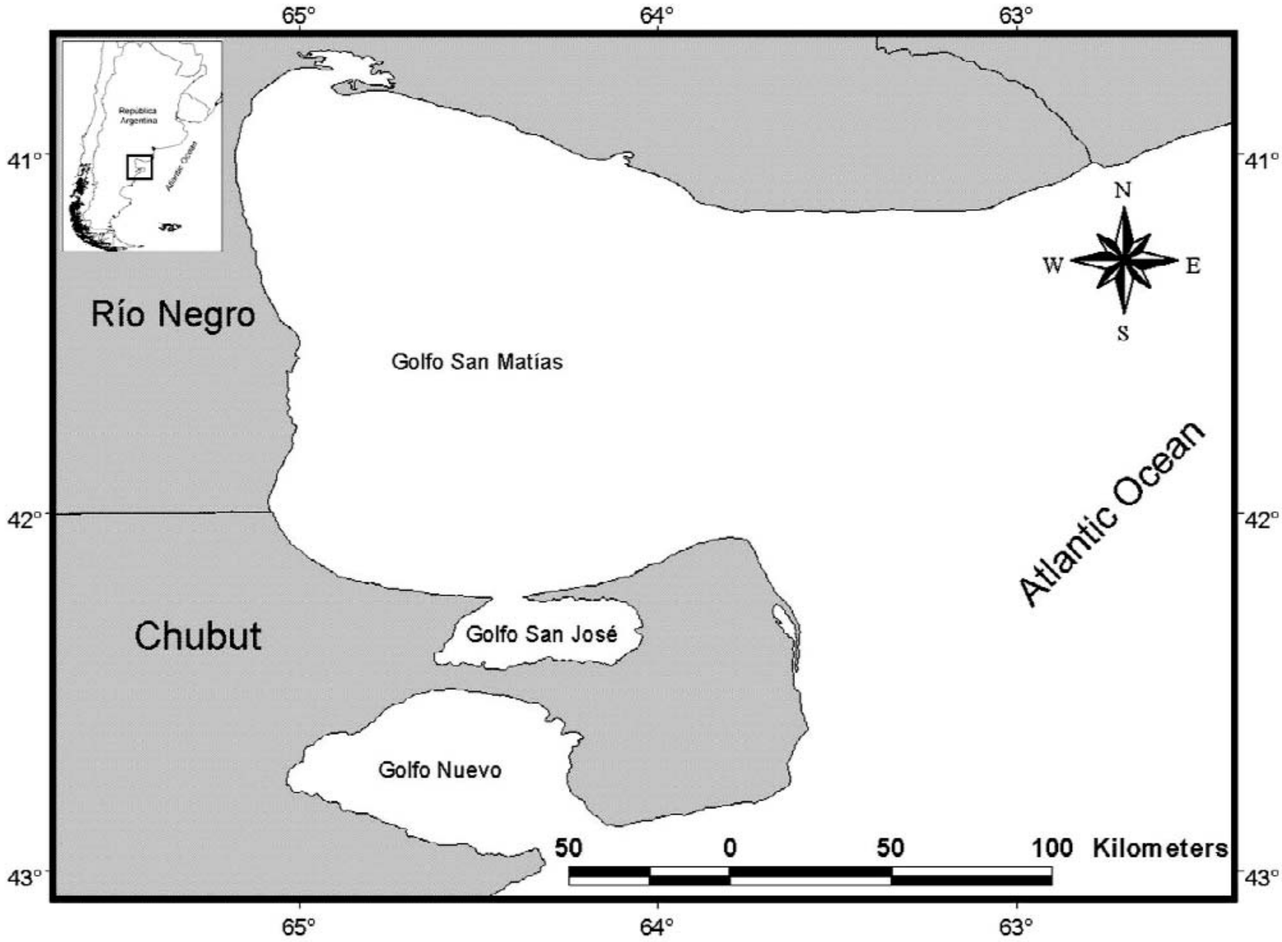
Keywords: *animal welfare, Argentina, behavioural response, biopsy pole system, dusky dolphins,* Lagenorhynchus obscurus

Introduction

Biopsy sampling from free-ranging cetaceans has been demonstrated to be useful for addressing questions regarding population genetics (Baker *et al* 1990; LeDuc *et al* 1999; Kiszka *et al* 2010), stable carbon and nitrogen isotope analyses (eg Walker *et al* 1999; Valenzuela *et al* 2009; Kiszka *et al* 2011) and contaminant levels (eg Fossi *et al* 2000; Fair *et al* 2010). Biopsy samples taken for genetic studies have been obtained using crossbows (Lambertsen 1987; Palsbøl *et al* 1991; Weinrich *et al* 1991, 1992; Gauthier & Sears 1999; Bearzi 2000; Hooker *et al* 2001; Gorgone *et al* 2008; Kiszka *et al* 2010; Quérouil *et al* 2010), rifles (Barret-Lennard *et al* 1996; Krützen *et al* 2002; Parsons *et al* 2003), pole systems (Bilgmann *et al* 2007), skin swabs (Hoelzel 1989; Milinkovitch 1994; Harlin *et al* 1999) or faecal sampling (Parsons *et al* 1999, 2003; Green *et al* 2007). Each of these procedures involves a degree of intrusion or disturbance, and so efforts have been made to minimise the impact (Weinrich *et al* 1991; Clapham & Mattila 1993; Brown *et al* 1994; Patenaude & White 1995; Gauthier & Sears 1999). Many of these studies have involved large baleen whales and remote biopsy systems (eg crossbows, rifles, etc). For small cetaceans, there are ample data on remote biopsy sampling systems (eg Weller *et al* 1997; Krützen *et al* 2002; Gorgone *et al* 2008; Jefferson & Hung 2008; Kiszka *et al* 2010; Quérouil *et al* 2010), but only one published study examined the behavioural response to a biopsy pole system (Bilgmann *et al* 2007).

Biopsy sampling, using either remote or pole system, is the most efficient method to obtain epithelial material for DNA analysis (Amos & Hoelzel 1990; Baker *et al* 1990, 1993; Hoelzel & Dover 1991; Hoelzel *et al* 1998; Gorgone *et al* 2008; Kiszka *et al* 2010). However, as an intrusive technique, it presents inherent costs. For instance, Bearzi (2000) reported the death of a common dolphin (*Delphinus delphis*) following biopsy sampling using a remote biopsy device (variable-power CO_2 dart projector); the dart stuck in the dorsal muscle mass and less than 2 min after the hit, the dolphin began catatonic head-up sinking and the animal died 16 min later. This case highlights the

Figure 1



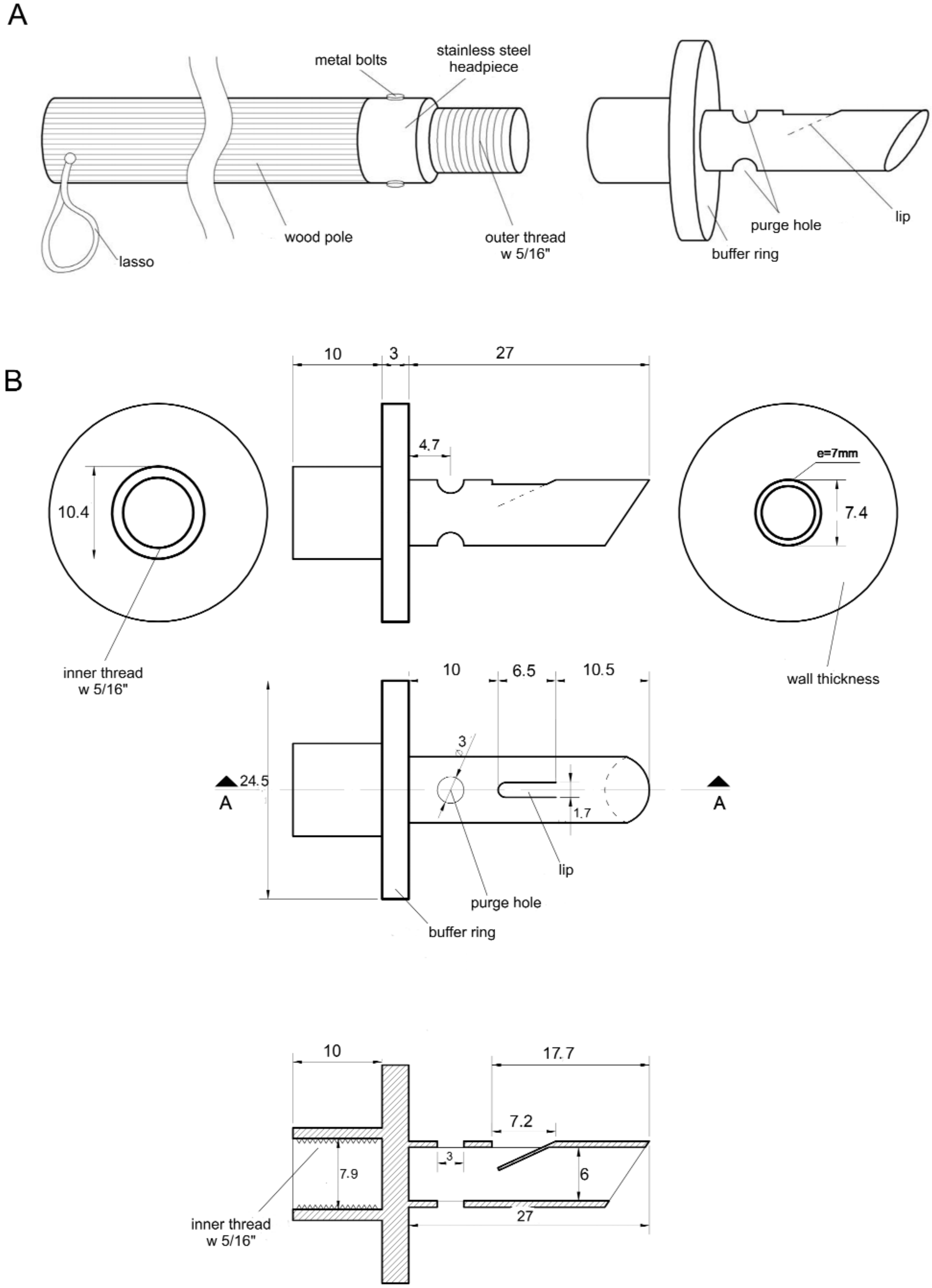
Study area, consisting of three gulfs, Golfo San Matías (GSM), Golfo San José (GSJ) and Golfo Nuevo (GN).

need for research into the potential impact of biopsy procedures. Such research, along with effective planning, can minimise the adverse effects of biopsy sampling.

When selecting a sampling technique to obtain biopsy samples, the conservation status of the species and target population, as well as the potential behavioural response of the target animals, should be considered (Bilgmann *et al* 2007). One of the problems with less-invasive sampling, such as faeces or skin-swabbing sampling, is that DNA may not be sufficient in quality and quantity to support the required molecular analyses (Harlin *et al* 1999; Parsons *et al* 1999). Biopsy samples provide sufficient DNA for repeated, multimarker genetic analyses, as well as isotopic or contaminants analysis (Amos & Hoelzel 1990; Borrel *et al* 2001; Natoli *et al* 2005, 2008; Valenzuela *et al* 2009; Cypriano-Souza *et al* 2010).

We investigated sampling success and behavioural response to the biopsy pole system for bow-riding dolphins in the context of different group sizes, group composition and behaviour. Biopsy samples were taken from dusky dolphins (*Lagenorhynchus obscurus*) off the Argentine coast during 2008–2009. The species is subject to incidental mortality in purse seine and trawling fisheries, and it was suggested that by-catch during the 1990s was probably close to the maximum sustainable level (Corcuera *et al* 1994; Crespo *et al* 1997; Dans *et al* 2003). In the last decade, substantial biological information for this species was obtained including abundance estimations (Schiavini *et al* 1999), reproductive biology (Dans *et al* 1997), diet composition (Koen Alonso *et al* 1998), parasite fauna (Dans *et al* 1999; Berón Vera *et al* 2008), behavioural ecology (Degrati *et al* 2008); habitat use (Garaffo *et al* 2007; 2010, 2011) and tourism interactions (Coscarella *et al* 2003; Dans *et al* 2008). However, aspects of the life history and stock identity still must be elucidated in order to model the dynamics of the population and the partitioning of genetic diversity among populations. This includes natural mortality factors and the identification of potential structure which may be affected differentially by fishery or tourism activities. Therefore, biopsy sampling is an important component of the broader study in support of effective conservation in the face of ongoing impact from fisheries and tourism. The primary objective of this study, therefore, is the development of a biopsy strategy that will entail minimal negative impact.

Figure 2



Showing (A) schematic of the biopsy pole system and detail of distal part, including biopsy tip and (B) detailed schematic of the biopsy tip.

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Materials and methods

Study area

We collected data during 2008 and 2009 in northern Patagonia, Argentina (see Figure 1); in the area that includes three gulfs, Golfo San Matías (GSM; 40º45' S-42º14' S and 65º05'-63º48'W), Golfo San José (GSJ; 42º25' S-42º 13' S and 64º37' W-64º02' W) and Golfo Nuevo (GN; 42º29' S-42º56' S and 65º03' W-64º0' W).

Biopsy sampling

Collection of samples was approved by Secretaría de Turismo and Dirección de Fauna y Flora Silvestre de la Provincia del Chubut (Research permit No 23/07) and Government Agency Provincia de Río Negro. Skin samples were exported under CITES permit No 032423, 31374/75/76, extended by Ministerio de Salud y Ambiente Secretaria de Ambiente y Desarrollo Sustentable, Buenos Aires, Argentina.

Biopsy samples were collected from a 6-m rigid-hull inflatable boat with a 60-horsepower (hp) and 4-stroke engine. The biopsy pole was 1.5-m long and made of wood (modified from Bilgmann *et al* 2007). The distal part of the pole contained a stainless steel headpiece with a 10-mm long outer thread (W 5/16"). The biopsy tips were stainless steel with a 10-mm posterior inner thread (W 5/16") that screwed into the headpiece. A 24.5-mm diameter, 3-mm thick buffer ring prevented deeper penetration. The biopsy tip was 27 mm in length and 7 mm in diameter, with a sharpened tip, a porthole at the base, and an internal 3-mm barb to hold the sample in place (Figure 2).

Once a sample was taken, wearing latex gloves, we extracted it from the biopsy tip by unscrewing the tip from the pole and, using sterilised forceps, pushed the sample into centrifuged tubes with a saturated NaCl/20% dimethyl sulphoxide solution (Amos & Hoelzel 1991). Once in the laboratory, the samples were stored at –20ºC until further analysis. Prior to each sampling day, biopsy tips were scrubbed using a toothbrush and then boiled for 20 min. Finally, we flamed the biopsy tip after dipping it into 100% ethanol and applied a broad-spectrum antibiotic (Iodo Povidona, Pervinox, Laboratorio Pheonix, Buenos Aires, Argentina). The biopsy tips were stored until use in a clean plastic container.

In all locations, we attempted to sample dusky dolphins that were travelling parallel and close to the vessel (bow-riding dolphins). Depending on the distance of the dolphin, the biopsy pole was either dropped or thrown lightly towards the animal, aiming at the body areas lateral to the base of the dorsal fin. The pole was held at an angle of between 60 and 90º to the water surface. Biopsy sampling took place only in a sea state less than Beaufort 3 and during daylight hours, because these conditions permitted the dolphins' movements to be observed.

When a group of dolphins was sighted, location (recorded using GPS), group size and composition, and the predominant group activity were recorded prior to biopsy sampling and after if any parameters changed. A group was defined as any collection of individuals located in close proximity (< 10 m) from one another (Smolker *et al* 1992). According to their composition, groups were classified as: adults and juveniles (consisting of animals of different lengths, all of which were larger than calves), mother/calf (when more than 80% of the individuals were mothers with their calves), and mixed (a combination of adults, juveniles, and calves, all of which formed a single unit). Age was assessed based on the body length of an individual. Dusky dolphins reach sexual maturity at the age of 6–7 years (Dans *et al* 1997), although three-year-old animals have almost the same length as adults (approximately 1.70 m; Dans 1999). Smaller animals were presumed to be juveniles; individuals that were less than two-thirds of adult length (and that were consistently accompanied by an adult) were considered calves (up to one year of age; Dans 1999). For mother/calf groups we left the group if no samples were taken after 10 min in order to avoid further harassment. Group size was assigned to one of the following categories: < 10, 11–20, 21–50, 51–70 and more than 70 animals (Degrati *et al* 2008).

Predominant group activity was defined as the activity in which most animals were engaged (Mann 1999) and assigned by instantaneous scan sampling (Altmann 1974). Activities were classified into one of the five categories: feeding (F); travelling (T); socialising (S); resting (R); and milling (M) (after Degrati *et al* 2008). During feeding, group members swam in circles or in a zigzag pattern, apparently to enclose a school of fish. Travelling consisted of persistent movement, with all group members swimming in the same direction. Socialising was characterised by frequent interactions between individuals, usually in the form of body contact and aerial displays. Resting involved individuals remaining stationary; apparently floating motionless on the surface. Milling consisted of low-speed movements with frequent changes in direction, resulting in little apparent overall directional movement by the group (for a review, see Degrati *et al* 2008). We conducted biopsy sampling during all behavioural categories, except for resting dolphins which were not sampled because they usually avoid the vessel.

Using *ad libitum* methods (Altmann 1974), we documented the response to the sampling procedure by recording the behaviour of each sampled dolphin after a biopsy was taken. We classified its reaction into one of the five categories following a protocol from Bilgmann *et al* (2007) and Krützen *et al* (2002): 0 for no noticeable reaction and the individual continues to bow-ride; 1 for a flinch, but the individual continues to bow-ride; 2 for an individual that accelerates underwater and leaves the bow; 3 for an individual that accelerates and leaves the bow followed by a single leap/porpoise ('porpoising' was, as described by Norris & Dohl [1980], a high-speed surface piercing motion in which long jumps are alternated with periods of swimming close to the surface); and 4 for an individual that accelerates and leaves the bow followed by multiple leaps and/or porpoises. The behavioural response of the individual was recorded whether the sampling attempt was successful (having collected a biopsy sample) or unsuccessful (no biopsy sample collected or pole thrown into the water but dolphin

Table 1 Behavioural responses of dusky dolphins (*Lagenorhynchus obscurus*)to biopsy pole system.

	Hit/miss			
Response	**SM**	**GSJ**	**GN**	**Total**
Category 0: no noticeable reaction and the individual continues to bow-ride	14/0	5/15	11/35	30/50
Category 1: for a flinch, but the individual continues to bow-ride	0/0	0/0	2/0	2/0
Category 2: for an individual that accelerates underwater and leaves the bow	2/0	12/0	12/0	26/0
Category 3: for an individual that accelerates and leaves the bow followed by a single leap/porpoise	0/0	0/0	0/1	0/1
Category 4: for an individual that accelerates and leaves the bow followed by multiple leaps and/or porpoises	0/0	0/0	0/0	0/0
Total	16/0	17/15	25/36	58/51

SM = Golfo San Matías; GSJ = Golfo San José; GN = Golfo Nuevo.

not hit). Categories 0–2 were considered mild and 3 and 4 as strong reactions to biopsy sampling. During a typical sampling event, the person taking the biopsy pole stood at the front of the vessel, with another observer close behind recording the biopsy sampling response and taking notes on the sampled individual (eg colour pattern, scars on the dorsal fin, and sex if possible). The second person, wearing latex gloves during the entire sampling event, was responsible also for changing the biopsy tips and preserving the sample.

Statistical analysis

Location, group size, behavioural state of the group and success or failure of the attempt were assessed as possible factors determining the individual's response to biopsy sampling. Frequencies were arranged in two-way contingency tables (hit × miss) for all data combined and a Yates-corrected Chi-squared test (χ^2) was applied when necessary. Also, the frequency data were arranged in three-way contingency tables (group size × sample × reaction; group composition × sample × reaction and behavioural state × sample × reaction). These tables were analysed by means of log-linear models to determine whether there was a significant behavioural response in the context of variation in group size, composition or behaviour. Each hypothesis of interest was tested incorporating the corresponding interaction to the null model (Caswell 2001). A four-way contingency table was not possible because of the large number of cells with a value of 0 (Caswell 2001). In all cases, a critical significance level of $\alpha = 0.05$ was used. In order to compensate for the small sample sizes in the tests, behavioural responses to biopsy sampling (1–4) were combined as reaction and (0) as no reaction to the biopsy sampling. Sampling events from different locations were pooled after confirming no significant differences due to location.

Sex ratio

To detect if there was a sex bias in the collection of samples, animals were genetically sexed using sex-chromosome specific primers ZFX and ZFY (Bérubé & Palsbøll 1996). The observed sex-ratio was tested for deviation from the expected 1:1 ratio using a Chi-squared test.

For all data analyses, SPSS version 15 statistical software was used (Pardo & Ruiz 2002).

Results

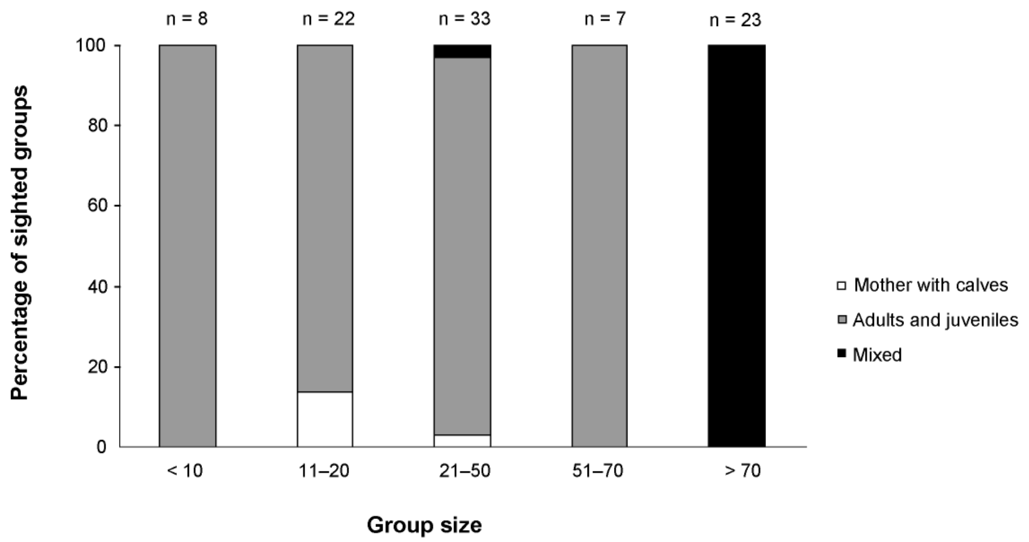
Biopsy sampling

In total, 109 sampling attempts were made between January 2008 and January 2009, and 58 biopsy samples were obtained (53.2% success rate). No significant differences were found between locations in the dolphin's response to biopsy sampling ($\chi^2 = 3.70$, $P = 0.146$, df = 2). No significant difference in response to hit versus miss was detected for all data combined ($\chi^2 = 0.11$, $P = 0.7358$, df = 1, Yates corrected χ^2). The most frequent behavioural response observed to biopsy sampling was accelerating and leaving the bow (category 2; Table 1), while the second most frequent response was no noticeable reaction and the individual continues to bow-ride (category 0; Table 1). When the biopsy attempt hit the water, biopsy sampling failed, no noticeable reaction was recorded and the individual continued bow-riding (Category 0; Table 1). On just one occasion the individual accelerated and left the research vessel leaping and porpoising (Category 3; Table 1). No individuals exhibited a category 4 reaction (Table 1). Most dolphins returned to the research vessel after being sampled and continued bow-riding. It was easy to observe the round, white biopsy wound and to avoid these animals for further sampling.

A total of 93 groups of dolphins were encountered during this study. Groups of 11–20, 21–50 and > 70 animals were the most commonly encountered; groups of < 10 and 51–70 dolphins occurred least frequently and at similar frequencies. With regard to age structure, groups of adults and juveniles were most frequently sighted, whereas mother and calf groups and mixed groups were sighted less frequently. Group size was significantly associated with group age composition ($\chi^2 = 93.06$, df = 8, $P < 0.001$; Figure 3). The larger groups were typically mixed, whereas the smaller groups were composed primarily of adults and juveniles without calves, taking into account that mother/calf groups were avoided most of the time during the sampling to minimise harassment and stress. All but one of the mother and calf groups encountered contained < 20 individuals (Figure 3).

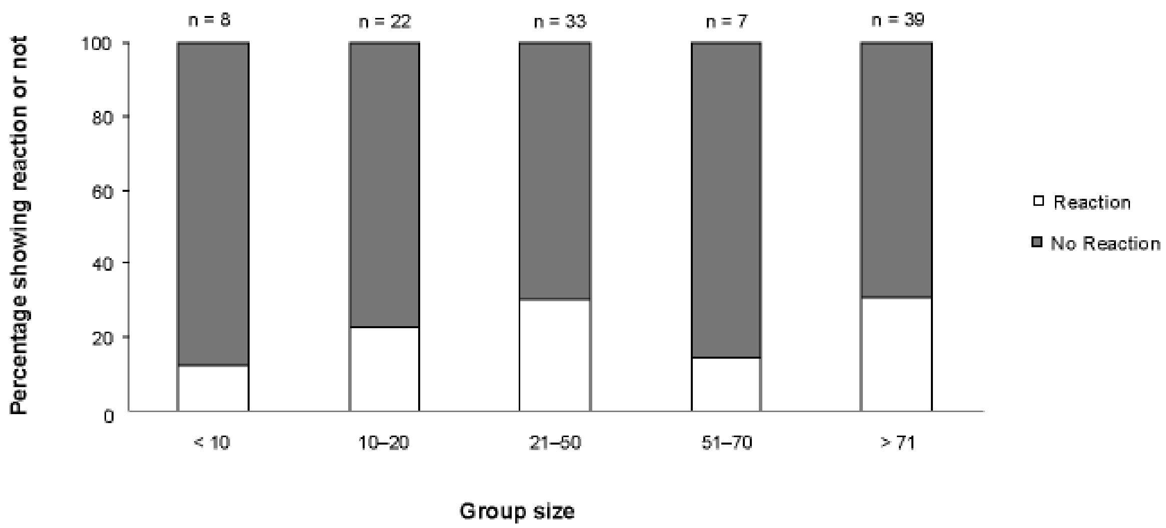
Dolphins in larger groups reacted to the biopsy pole sampling less frequently than dolphins in smaller groups

Figure 3



Group sizes and composition for dusky dolphins (*Lagenorhynchus obscurus*; n = 93) in the study area.

Figure 4



Group sizes of dusky dolphins (*Lagenorhynchus obscurus*; n = 109) and percentage of animals with reaction or no reaction to biopsy sampling.

(Figure 4). The behavioural response to successful compared to unsuccessful sampling attempts was significantly related to group size (ΔG = 30.47, df = 10, P < 0.001). Group composition also related to whether or not dolphins reacted to the biopsy sampling, with mother/calf groups reacting more frequently to biopsy sampling than adults and juveniles or mixed groups (ΔG = 28.41, df = 3, P < 0.001; Figure 5).

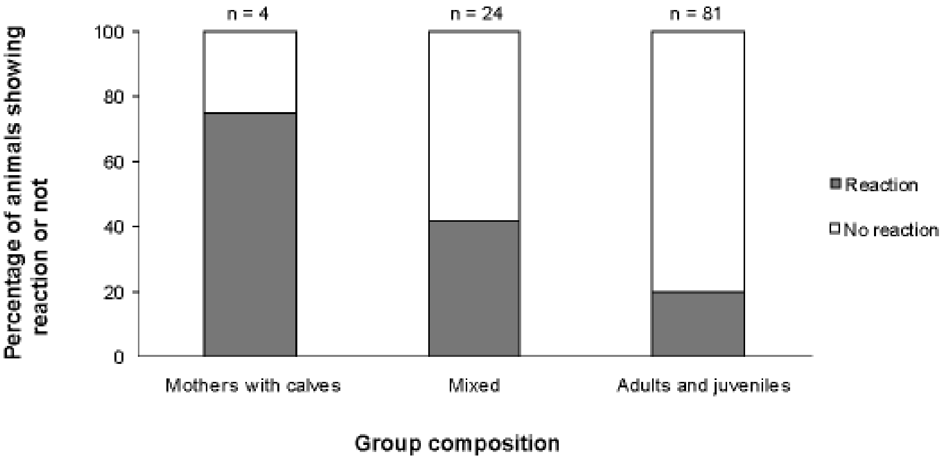
The behaviour that dolphins exhibited prior to biopsy sampling was related to the occurrence of a reaction of bow-riding dolphins to the pole-sampling procedure (ΔG = 33.24, df = 10, P < 0.001; Figure 6), although no group changed its behaviour during biopsy sampling. Milling dolphins responded more intensively than dolphins involved in social or travel activities (Figure 6).

No significant difference from a 1:1 sex ratio was detected for sampled individuals at any of the three locations (P-values varied from 0.5344 to 1.0).

Discussion

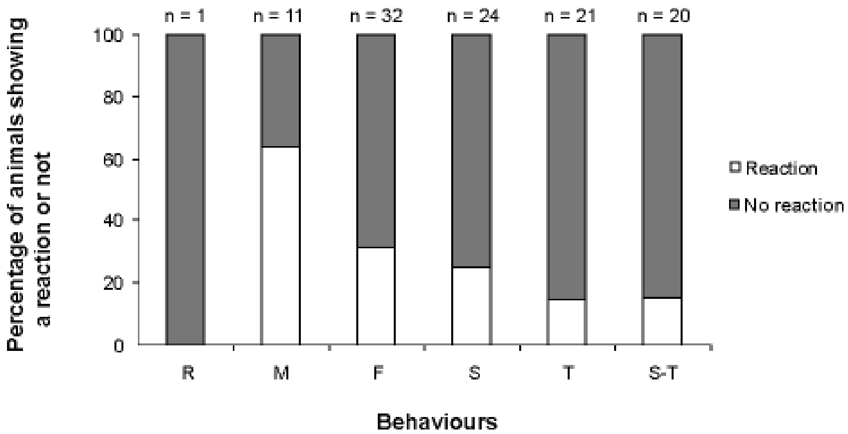
The biopsy pole system used in this study is a useful method for obtaining skin and blubber samples from coastal dusky dolphins off the coast of Argentina. This is the second study recording the behavioural response to biopsy sampling using a biopsy pole system, although that study involved a different species of dolphins in another region (Bilgman *et al*

Figure 5



Group composition of dusky dolphins (*Lagenorhynchus obscurus*; n = 109) and percentage of animals with reaction or no reaction to biopsy sampling.

Figure 6



Behaviours of dusky dolphin groups (*Lagenorhynchus obscurus*; n = 109) and percentage of animals with reaction or no reaction to biopsy sampling. R = resting, M = milling, F = feeding, S = socialising, T = travelling, S-T = socialising-travelling.

2007). Here, our goal was to identify the features that can alter the behaviours of biopsied dolphins so that researchers can reduce stress and keep negative impacts low.

The overall biopsy success rate in this study was 53%. When divided into the three locations, 100% of the sampling attempts on dolphins in Golfo San Matías were successful, 53% in Golfo San José and 41% in Golfo Nuevo. Bilgmann *et al* (2007), using the biopsy pole, reported that 87.5% of the sampling attempts on bottlenose dolphins (*Tursiops* spp) in New South Wales (NSW), 62.5% on bottlenose dolphins in South Australia (SA), 77.8% on common dolphins in NSW, and 72.5% on common dolphins in SA were successful. However, the majority of biopsy-sampling studies used remote biopsy systems, such as crossbow or rifles. The success rate reported using remote biopsy methods was 65% for killer whales (*Orcinus orca*) (Barret-Lennard *et al* 1996), 68% (Krützen *et al* 2002), 67% (Gorgone *et al* 2008), and 11.5% (Parson *et al* 2003) for bottlenose dolphins; 65% for pantropical spotted dolphin (*Stenella attenuata*), 70% for spinner dolphins (*Stenella longirostris*), 77% for Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) and 78% for the melon-headed whale (*Peponocephala electra*) (Kiszka *et al* 2010). Thus, the reported success rates were mostly quite similar using both sampling methods. Both methods are practical to implement, although should perhaps be used in different situations (bow-riding versus not bow-riding) and in some cases for answering different questions. For example, when an animal needs to be identified to assess social structure, the remote system may be less disruptive, while the pole system might be more useful when addressing questions of population structure in general. Using both the pole and remote systems in combination can reduce the overall time spent biopsy sampling and, consequently, the time spent with or near the animals. Therefore, the combination of pole and remote biopsy systems is recommended to facilitate research objectives and help reduce harassment and animal stress.

The animals showed mild reactions to the biopsy sampling procedure regardless of being hit or missed; this might indicate that the reaction was mainly caused by the unexpected disturbance rather than the biopsy sampling. In addition, when a vessel comes close to the dolphins during biopsy sampling, it may cause unintentional and unseen behavioural responses by dolphins that are not recorded by researchers. Responses may be caused by the closeness of the approach or to the type of vessel (large, noisy vessel versus small, quiet vessel). Coscarella *et al* (2003) and Dans *et al* (2008) found that dusky dolphins changed their behaviour in the presence of commercial vessels, especially with respect to feeding and travel behaviours. In this study, we used a similar type of vessel throughout to control for that variable, and during dolphin feeding behaviours we kept at a distance of > 100 m to help avoid interference with this behaviour.

During this study we recorded a strong reaction only once. On that occasion the dolphin left the research vessel and then began leaping and porpoising. It is worth noting that our perception of response is limited to activities at the surface, and significant underwater responses are possible. Bilgmann *et al* (2007), using a biopsy pole, found the same behavioural responses to successful and unsuccessful biopsy sampling for common and bottlenose dolphins. The results from this study on dusky dolphins and Bilgmann *et al* (2007) suggest that the biopsy pole technique generally produces moderate behavioural responses in small coastal cetacean species, whether individuals are hit or missed, and that the different species react similarly to the sampling procedure.

Therefore, the biopsy method used in this study mainly produced mild, short-term reactions, which is consistent with previous studies using pole and remote sampling methods (eg Weinrich *et al* 1992; Clapham & Mattila 1993; Brown *et al* 1994; Barrett-Lennard *et al* 1996; Weller *et al* 1997; Krützen *et al* 2002; Bilgmann *et al* 2007; Gorgone *et al* 2008; Kiszka *et al* 2010). It appears that the targeted dusky dolphins also have not altered their long-term behaviour since they are currently still easily approachable for systematic surveys.

The behavioural response to biopsy sampling using the pole method was significantly associated with group size, with dolphins that belong to the largest groups reacting less than those from smaller groups. Also, Kiszka *et al* (2010) found the same pattern for spinner and pantropical spotted dolphins. At the same time, dusky dolphin group size is significantly associated with group age composition, such that the largest groups are typically mixed groups, whereas the smallest groups are composed primarily of mothers and calves (Degrati *et al* 2008). Mother and calf groups reacted more frequently to biopsy sampling than adults and juveniles and mixed groups. Therefore, it could not be determined if group size or composition (or possibly both) was the causative factor.

During biopsy sampling we found that dolphins that were engaged in active behaviours, such as socialising, travelling or feeding, reacted less frequently to biopsy sampling than dolphins engaged in milling. Kiszka *et al* (2010) found that resting and socialising spinner dolphin groups showed a stronger response than milling and travelling groups. Degrati *et al* (2008) found that the most common diurnal behaviour of dusky dolphins was travelling, followed by milling and feeding, and they also found that mother and calf pairs spent more time milling and resting, whereas larger groups of adults and juveniles as well as mixed-age-class groups spent more time travelling and feeding. Hence, as a preventive measure, wherever possible, we recommend undertaking biopsy sampling from travelling dusky dolphins in large groups.

Dusky dolphin sex ratio was not biased in favour of males or females, based on samples collected in the three different Argentine gulfs. Several other studies also showed no significant bias (Krützen *et al* 2002, 2004; Möller & Beheregaray 2004; Bilgmann *et al* 2007). Skewed sex ratio was reported in samples of New Zealand's dusky dolphins, obtained by skin swabbing (Harlin *et al* 2003). In that study, the sex ratio varied between locations and collecting periods and was assumed to reflect seasonal variations in space occupation. Altogether, these results indicate that segregation by age and sex might occur in dusky dolphins and depends upon season and location. Further work is required along the Argentine coast to determine the dusky dolphins' spatio-temporal pattern and environmental determinants of age and sex segregation.

Animal welfare implications and conclusion

The biopsy pole system designed here is cost effective, easy to apply, and easy to set up multiple times during a boat trip. As a consequence, the time between biopsy attempts is minimal compared to the time involved in recovering the sample using long-distance devices, such as the recovery of bolts or darts when crossbow, pistol or rifles are used. Therefore, the pole system used in this study reduces the biopsy sampling time and helps to avoid harassment and animal stress. This implies that dolphin welfare can be improved if this method is used, where it is practical to do so. We also recommend that dolphins should be sampled only while travelling, in order to further minimise negative effects of sampling. Other advantages include a greatly reduced risk of the biopsy tip remaining attached in the animal, and increased accuracy, minimising hits to sensitive parts of the animal's body. For the remote systems, tether lines could become entangled, and the higher pressure applied to permit recoil of an untethered dart has, on at least one occasion, resulted in deep penetration and the death of a common dolphin (Bearzi 2000). In conclusion, biopsy sampling with the pole-system device presented here, is secure and easy, providing genetic samples with sufficient DNA for a multiple genetic marker approach, and blubber for isotopic or contaminant analysis.

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