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Abstract

Biopsy sampling is an effective technique to collect cetacean skin and blubber samples for various biological studies. However, determining the impact of this research practice is important, as impact may vary among sites, species and gear used. We examined the short-term behavioural reactions of four small (160-278 cm in length) delphinid species (*Stenella longirostris*, *Stenella attenuata*, *Tursiops aduncus* and *Peponocephala electra*) to remote biopsy sampling around the island of Mayotte (12^{o}50'S, 45^{o}10'E, SW Indian Ocean). Two scales of behavioural reactions were considered: 1- the behavioural reaction of the individual, and 2- the reaction of the focal group to which the targeted individual belonged. Three main categories of behavioural responses were defined on the basis of the character and duration of behavioural response: low, moderate and strong. This study underlines that biopsy sampling induces moderate reactions of individuals. No inter-specific variations of responses, at the scale of individuals or focal groups, were observed. In other words, smaller delphinids were not more reactive than larger ones. No effect of group size was observed on the strength of behavioural reactions. However, it was clear that biopsy success during sampling sessions was higher in species with large group size. Finally, in the spinner dolphin (*S. longirostris*), we investigated whether initial behavioural state affected the level of reaction. Resting and socialising groups showed a stronger response than milling and travelling groups. This study confirms the limited impact of remote biopsy sampling in small delphinids, especially in the spinner dolphin. However, as a precautionary approach, in situations where it is possible, biopsy sampling of milling and travelling dolphins may be preferred.

Keywords: animal welfare, delphinids, group reactions, individual reactions, Indo-Pacific bottlenose dolphin, melon-headed whale, pantropical spotted dolphin, spinner dolphin, remote biopsy sampling.

Introduction

In wildlife studies, some invasive techniques may be used to collect biological samples to answer a variety of questions which may be of particular relevance for management and conservation purposes. It is critical that the impact of such research practices is quantitatively assessed and managed, as the process of sample collection may negatively impact individuals and/or populations over a range of scales (e.g. injuries, individual stress, individual/group displacement, change of behaviour, etc.).

The use of skin and blubber biopsy samples from free-ranging cetaceans is a widespread and powerful technique to answer many questions, including population genetics (stock identity, social organization, population size, phylopatry, genetic connectivity, Amos & Hoelzel 1990; Bérubé *et al* 1998), feeding ecology and trophic relationships using stable isotope and fatty acid analyses (Herman *et al* 2005; de Stephanis *et al* 2008; Gross *et al* 2009), and pollutant analysis (Godard *et al* 2004). In order to collect samples, modified crossbows, rifles and hand held biopsy poles have been used, both for large and small cetaceans, including delphinids (Weinrich *et al* 1991; Barrett-Lennard *et al* 1996; Krützen *et al* 2002; Bilgmann *et al* 2007).

The behavioural effect of biopsy sampling has been investigated in large whales, such as right whales (*Eubalaena glacialis* and *E. australis*, Brown *et al* 1991; Best *et al* 2005), humpback whales (*Megaptera novaeangliae*, Weinrich *et al* 1991; Clapham & Mattila 1993), other large balaenopterid whales (Gauthier & Sears 1999), and delphinids such as short-beaked common (*Delphinus delphis*, Bearzi 2000), bottlenose dolphin (*Tursiops* spp., Krützen *et al* 2002; Bilgmann *et al* 2007; Gorgone *et al* 2008) and Indo-Pacific humpback dolphin (*Sousa chinensis*, Jefferson & Hung 2008).

The International Whaling Commission considers biopsy sampling to be acceptable, since no long-term effects (change of behaviour) have been shown on individuals and populations

(International Whaling Commission 1991). Levels of short-term reactions to biopsy sampling could potentially vary among species, populations and individuals. However, for both small and large cetaceans, the behavioural impact of biopsy sampling is generally considered to be low. Responses from the animals can be typified as reactions to a noxious stimulus of brief duration and low-to-moderate amplitude (Weinrich *et al* 1992; Best *et al* 2005; Bilgmann *et al* 2007; Jefferson & Hung 2008). In small cetaceans, a case of death has been reported in a short-beaked common dolphin, underlining that remote biopsy sampling is not without risk (Bearzi 2000). Consequently, the use of less invasive sampling techniques may be preferred. Other methods include skin swabbing and faecal sampling (Harlin *et al* 1999; Parsons *et al* 1999). However, these techniques provide a limited amount of material, and DNA may not be of sufficient quality to undertake multiple markers analyses and other analyses (such as pollutant analyses, for example). Biopsy sampling is generally preferred for molecular genetic studies (Parsons *et al* 2003) and other analyses such as those of stable isotopes (Gross *et al* 2009). In addition, the use of remote techniques, using a gun or a crossbow, is more effective than a pole system for studies of population structure and parentage because animals can be sampled even if they do not bowride. Remote sampling also allows the individual identification of targeted dolphins (Bilgmann *et al* 2007). Proper identification of bowriding animals is generally not possible (good photograph angle).

The objective of this study is to characterize short-term reactions of four small delphinid species to remote biopsy sampling: the spinner dolphin (*Stenella longirostris*, 160-208 cm), the pantropical spotted dolphin (*Stenella attenuata*, 160-260 cm), one of the smallest delphinids, the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*, 230-270 cm) and the melon-headed whale (*Peponocephala electra*, 240-278 cm), one of the least known delphinids. This study provides, to the best of our knowledge, the first information on the effect of biopsy sampling on these species.

In order to collect skin and blubber samples for stable isotope, genetic and histopathological investigations, remote biopsy sampling was conducted from December 2004 to October 2008. Levels of behavioural reactions were recorded at two different scales: 1- the individual reaction of the sampled dolphin and 2- the behavioural reaction of the focal group to which the targeted animal belonged. The latter component of the study has not been investigated in previous studies for any other cetacean, as far as we are aware, and allows understanding the impact of remote biopsy sampling at a broader scale, i.e. groups and not only targeted individuals.

Materials and methods

Study area

The island of Mayotte (45°10'E, 12°50'S), which is part of the Comoros archipelago, is located in the northern Mozambique Channel (western Indian Ocean) between Madagascar and Southeast Africa (Figure 1). Its surface area is 376 km^2. This territory is composed of two main islands: the main inhabited island, on the east and on the barrier reef, a smaller inhabited island. The other islands are small islets dispatched all over the lagoon. The island of Mayotte is characterized by the presence of high marine mammal diversity (22 species including 12 delphinids; Kiszka *et al* 2007). The most common species are the spinner dolphin, the pantropical spotted dolphin, the Indo-Pacific bottlenose dolphin and the melon-headed whale; these are resident year-round (Kiszka *et al* 2007).

Biopsy collection

From December 2004 to September 2008, small-boat-based cetacean surveys were conducted throughout the year in Mayotte waters in sea conditions not exceeding Beaufort 3. Observation effort concentrated mostly on the lagoon and over the insular slope in adjacent waters of the barrier reef. Biopsy attempts were made opportunistically, when groups and individuals were easily approachable and when conditions were optimal (Beaufort < 2, dolphins closely approaching the boat). Optimal weather conditions allowed stability of the research boat and better chances to sample the animals successfully and safely. Several types of boats were used: a 7-m catamaran equipped with two, four-stroke, 60-hp outboard engines; a 7-m mono hull boat equipped with two, two-stroke, 40-hp outboard engines; a 6.4-m cabin cruiser equipped with one, four-stroke, and 150-hp outboard engine; and a 10.8-m cabin cruiser equipped with two, four-stroke, 115-hp outboard engines. Biopsies were collected by using a crossbow (BARNETT Veloci-Speed® Class, 68-kg draw weight) with Finn Larsen (Ceta-Dart, Copenhagen, Denmark) bolts and tips (dart 25-mm long, 5-mm-diameter). A conical plastic stopper caused the bolt to rebound after the impact with the dolphin. The dolphins were hit below the dorsal fin when sufficiently close (3-10 m) to the research boat. Focal groups/individuals were approached under power at speeds of 1-4 knots. Blubber and skin biopsy samples were preserved individually in 90% ethanol before shipping and subsequent analysis. Biopsy sampling was conducted under French scientific permit #78/DAF/2004 (September 10, 2004) and permit #032/DAF/SEF/2008 (May 16, 2008) after examination of the project by Conseil National de Protection de la Nature.

Behavioural observations

During biopsy sampling sessions, an observer recorded behavioural reactions of dolphins at two different scales: the targeted individual and the focal group with which the targeted individual was associated. The focal group was defined as a group of dolphins engaged in the same activity and travelling in the same direction (Shane 1990). Three levels of behavioural reaction were defined for individuals and focal groups. These reactions followed Hooker *et al* (2000) and were adapted for the species investigated in this study:

1. No reaction: the individual and focal group continued to show the same behaviour as before the biopsy attempt;
2. Moderate reaction: the individual or the focal group modified its behaviour but gave no prolonged (>5 min) evidence of behavioural disturbance; reactions included e.g. acceleration, twitch and immediate dive and simple immediate dive. A dive was considered as a behavioural response to biopsy sampling when it lasted more than 5 minutes;
3. Strong reaction: the individual or the focal group modified its behaviour in a succession of percussive behaviours (strong and short-lived reactions), including escape from the research boat of the individual or/and focal group (leaping, breaches, tail slaps).

Data analysis

We investigated the occurrence (events and their proportions) of reactions described above and factors responsible for the variability of reactions (group size, species, activity), at the scale of hit/targeted individuals as well as focal groups. Group size was defined prior to biopsy sampling as the number of animals at the surface within five body lengths of each other (Smolker *et al* 1992). The estimates of group size were more stochastic for spinner dolphins, pantropical spotted dolphins and melon-headed whales, as group size for these species was important (mostly > 50 individuals). Determining absolute group size was not possible for large groups of delphinids. The predominant behaviour was recorded as the activity displayed by the majority of the animals of the group during the first 10 minutes. These data were collected during scan sampling of the group (Mann 1999) using six different behavioural categories: travelling, milling, resting, feeding/foraging, playing and socializing (Shane 1990).

Analysis of individual behavioural reactions were differentiated when the animal was missed (the bolt did not reach the animal) or hit. An individual is considered as hit when the bolt reached the body. There was no differentiation between biopsy hit providing or not providing a sample. We tested how group size may affect individual behavioural reactions, especially for the most frequently sampled species, the spinner dolphin. For this species, we also investigated the effect of initial behavioural state on the levels of reaction and the long term effect of biopsy sampling. In this later case, we hypothesised that avoidance behaviour would increase across the study period. Significance of this increase has been tested using a Pearson's correlation. For comparisons, Fisher exact tests, Kruskal Wallis tests and contingency table analyses were performed using Rv2.10.0 (R Development Core Team, 2009).

Results

Biopsy sampling was undertaken from December 2004 to September 2008 (n = 271 attempts, n = 193 samples). Four species (spinner dolphin, pantropical spotted dolphin, melon-headed whale and Indo-Pacific bottlenose dolphin) constituted 96% of the biopsies sampled (n = 259 attempts, n = 181 samples). Other species included the Fraser's dolphin (*Lagenodelphis hosei*, n = 7), the common bottlenose dolphin (*Tursiops truncatus*, n = 2), the Indo-Pacific humpback dolphin (n = 2) and the short-finned pilot whale (*Globicephala macrorhynchus*, n = 1), but this data was not been included in this study. We used three types of vessels to undertake biopsy sampling, but no significant differences of individual behavioural reactions were found between boat types (all species combined, $\chi^2 = 3.7$, $df = 6$; $P = 0.391$).

Among the four species, no significant inter-specific differences in reactions were recorded, both at the scales of individuals (Fisher exact test; $P = 0.9$) and groups ($P = 0.643$). Sampling success (a hit) varied between species from 65 to 78% (Table 1). On 34 occasions overall, the hit was successful but no sample was retained in the biopsy tip. Individual behavioural reactions to remote biopsy sampling were recorded on 252 occasions (180 hits, 72 misses), while focal group behavioural reactions were recorded on 271 occasions (193 hits, 78 misses). There were no statistical differences between individual behavioural reactions between biopsy hits and misses (all species combined, Fisher exact test, $P = 0.068$). Similarly, at the scale of focal groups, no significant differences between biopsy hits and misses were found (all species combined, KW test; $H = 0.702$; $df = 1$; $P = 0.402$).

At the individual scale, 94% of individual reactions were moderate, i.e. twitch and immediate dive, and simple immediate dive (Figure 2, Table 2). Strong reactions (tail slap, leaping, successive breaches and escape) only represented 2% of behavioural responses of individual dolphins. Escape and leaping was only observed in spinner and spotted dolphins. Increase of speed was observed once in a bottlenose dolphin group (Table 2).

Group behavioural reactions were frequent (54% of sampling sessions), with dive being the commonest moderate reaction (45%, Figure 3, Table 2). Strong reactions of focal groups were rare, representing only 4% of responses. These strong reactions consisted of increased swimming speed or escape (Table 2, Figures 2 and 3).

We did not find any correlation between group size and behavioural reactions (Fisher exact test; $P = 0.431$). However, there is a clear relationship between the mean specific group size and the mean number of biopsies collected per biopsy session (Table 1). The average number of biopsies collected during each session was the lowest for the Indo-Pacific bottlenose dolphin, which had the lowest mean group size (Table 1).

On six occasions, hit dolphins were observed bow-riding just after being sampled (fresh wound of the biopsy hit observed below the dorsal fin or in adjacent areas). These cases were observed in the pantropical spotted dolphin (n = 2 events), spinner dolphin (n = 2) and Indo-

Pacific bottlenose dolphin (n = 2). During sampling sessions, significant signs of avoidance of the research vessel by groups were observed on few occasions: in spinner dolphins (n = 2, after one and four biopsy attempts) and in melon-headed whales (n = 2, after one and six biopsy attempts).

We hypothesized that group reactions to biopsy sampling would differ according to activity (milling/travelling, resting, socializing, and playing). We tested this for spinner dolphins, as the dataset for that species was the largest. A statistical difference was found between group reactions and initial behavioural states in which spinner dolphin groups were engaged (Fisher exact test; $P = 0.041$). Spinner dolphins predominantly showed a stronger response to biopsy sampling when resting and socialising. When milling and travelling, reactions were moderate. We did not observe changes of dolphin reactions (increase of avoidance behaviour) to the research vessel prior to biopsy sampling over the study period (nearly four years; Pearson's correlation, $r = 0.324$, $P > 0.05$).

Discussion

In this study, we observed behavioural reactions of four delphinid species to remote biopsy sampling. The biopsy success reached 65-78%, which is consistent with previous studies. This was mostly due to the high accessibility of the targeted species. They generally came close to the research vessel, especially dolphins of the genus *Stenella*, often coming to ride waves created by the bow of the boat. No significant inter-species differences were found in reactions to remote biopsy sampling. Indeed, the smallest species (spinner and pantropical spotted dolphins) did not have a higher occurrence of moderate reactions than larger ones (Indo-Pacific bottlenose dolphin and melon-headed whale), as might have been expected. However, the strongest reactions, such as breaches and escape, occurred (but were very rare).

Such extreme reactions were only observed in the smaller species, especially spinner and pantropical spotted dolphins. However, due to the small sample size for Indo-Pacific bottlenose dolphins and melon-headed whales, we can not exclude that these species could also react strongly, like spinner and spotted dolphins do.

The mean number of biopsies per session was greater for species with a larger mean group size, i.e. melon-headed whales, spinner and pantropical spotted dolphins. In larger groups, animals are more accessible for biopsying, as there are more individuals to choose from. This is likely to be not just a function of the behaviour or the group reaction, but also because of the higher number of individuals.

Despite the fact that we used three different types of boats, no differences in reactions were found among boats. These differences have been documented in other studies, with generally stronger reactions when smaller boats were used (Bilgmann *et al* 2007). However, in this later study, sampling was done on bowriding dolphins, and the boat types and length differed to a much larger extent than in the study presented here.

Delphinids around Mayotte exhibited short-term behavioural reactions to biopsy attempts, characterized by acceleration, twitch and immediate dive and simple immediate dive. Strong reactions to biopsy sampling were previously recorded in common bottlenose dolphins (Parsons *et al* 2003). Conversely, reactions of common bottlenose dolphins appear to be minimal in other areas such as in eastern US (Gorgone *et al* 2008). Dolphins of all species sampled react in a similar fashion to biopsy hits and misses. This has been previously documented for other species such as the Indo-Pacific humpback dolphin (Jefferson & Kung 2008), meaning that the hit of the bolt on the water has a significant effect on the reactions of dolphins at the proximity of the impact. In the study presented here, focal groups were frequently impacted by biopsy sampling, meaning that remote biopsy sampling does have a broader effect on small cetaceans, i.e. on adjacent individuals belonging to the group. This effect was also greater on species constituting small groups, i.e. Indo-Pacific bottlenose dolphins, as the biopsy success decreased during sampling sessions for such species. The group behavioural reactions of spinner and pantropical spotted dolphins were relatively low, apparently because they formed larger aggregations. However, results underlined that there is a variability of reactions according to initial behavioural state. Indeed, for the spinner dolphin, we observed that the animals had stronger reactions to remote biopsy sampling when resting and socialising. When milling and travelling, reactions were more moderate. This suggests that remote biopsy sampling should be preferably conducted during travelling and milling activities.

Animal welfare implications

Overall, conducting remote biopsy sampling is effective on small delphinids and induces a limited short-term (less than 5 minutes) behavioural impact on hit and missed individuals, including in the smallest delphinid species (especially dolphins of the genus *Stenella*). However, we observed that biopsy sampling does not only impact hit individuals, but groups to which the targeted individual belongs. No long-term effect of biopsy sampling was observed, such as an increase of avoidance of the research vessel of the animals. This confirms that the method has no long term impact on the animals. However, as a precautionary approach, our findings suggest that biopsy sampling may preferably be conducted when the animals are milling or travelling. However, it is critical to reconsider practicing biopsy sampling to answer scientific questions.

Acknowledgements

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Table 1: Number of attempts, biopsy samples collected and sampling success in delphinids sampled around the island of Mayotte from December 2004 to September 2008.

Species	n attempts	n samples	% success	Number of biopsy sessions	Mean group size	Average n biopsies/ session
Stenella longirostris	137	96	70	30	70.5	3.2
Stenella attenuata	77	50	65	20	78.5	2.5
Peponocephala electra	23	18	78	5	310	3.6
Tursiops aduncus	22	17	77	15	6.3	1.1

Table 2: Individual and focal group behavioural reactions of *Stenella longirostris*, *Stenella attenuata*, *Peponocephala electra* and *Tursiops aduncus* to remote biopsy sampling (numbers represents events).

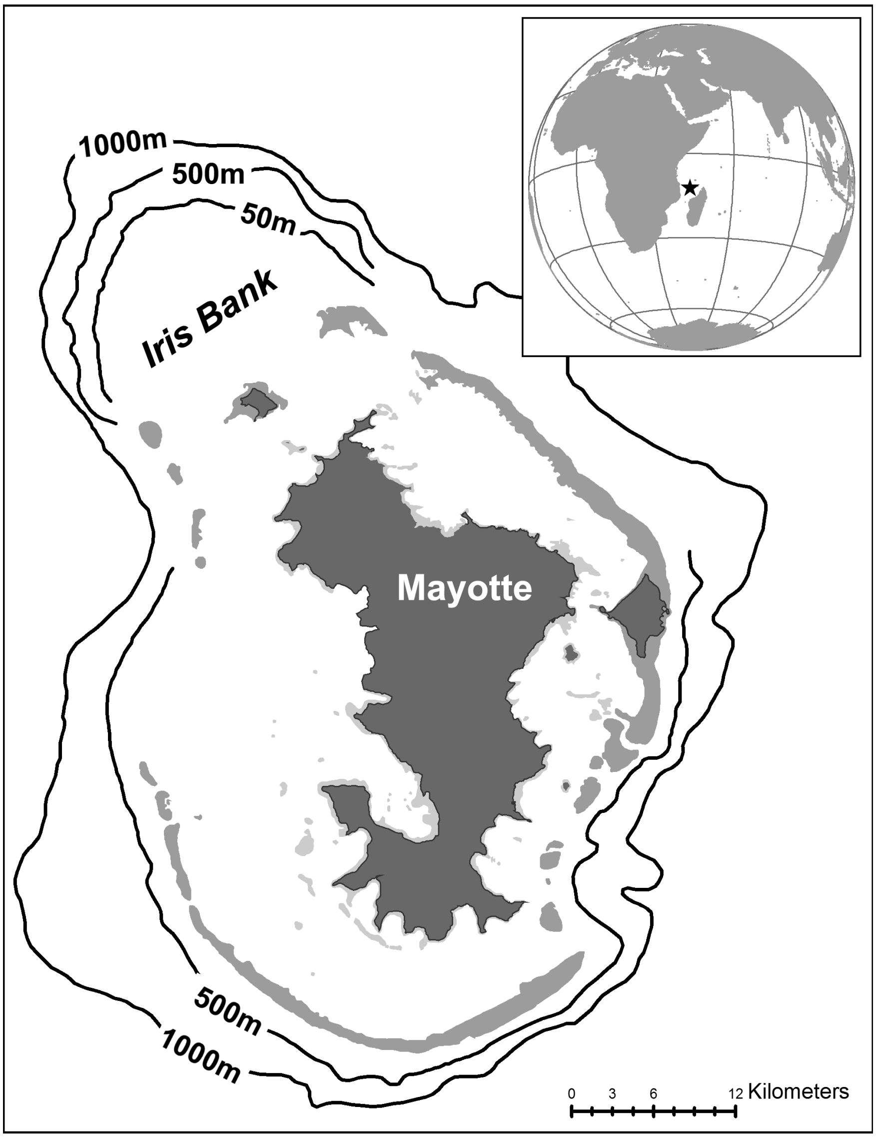
	Stenella longirostris	*Stenella attenuata*	*Peponocephala electra*	*Tursiops aduncus*
Individual reactions				
Twitch and dive	83	21	8	10
Successive breaches	1	1	0	0
Tail slap	2	0	0	0
Escape (leaping)	1	0	0	0
No reaction	1	0	0	0
Group behavioural reactions				
Dive	41	19	7	9
Increase swimming speed	2	0	0	0
Escape	1	1	0	1
No reaction	40	28	10	6

Figure captions

Figure 1: Location and map of the study area.

Figure 2: Individual behavioural reactions of delphinids (*Stenella longirostris*, *Stenella attenuata*, *Tursiops aduncus* and *Peponocephala electra*) to a biopsy hit or miss.

Figure 3: Focal group behavioural reactions of delphinids (*Stenella longirostris*, *Stenella attenuata*, *Tursiops aduncus* and *Peponocephala electra*) to a biopsy hit or miss.

432 

433 Figure 1

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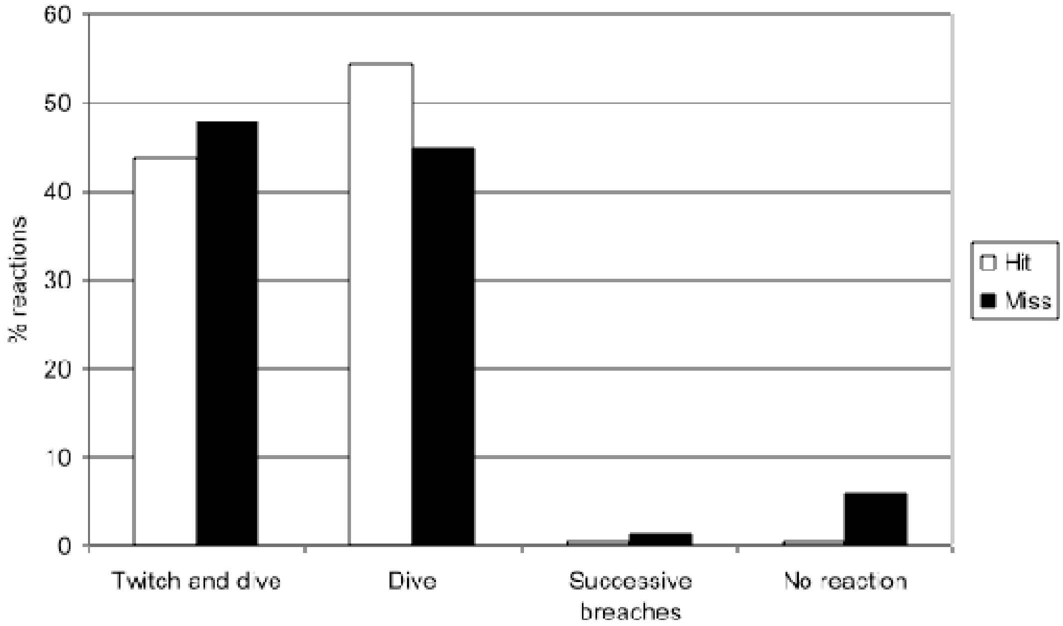

Figure 2

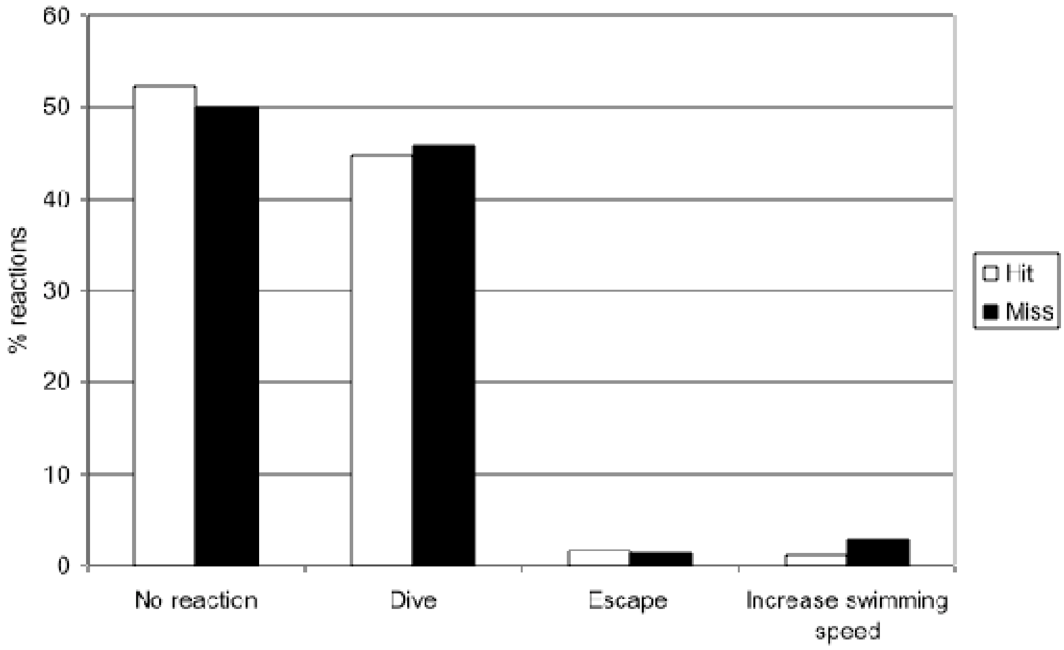

Figure 3