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Efficiency and Effect Evaluation of Remote Biopsy Sampling on Indo-Pacific Humpback Dolphins (*Sousa chinensis*) in the Northern South China Sea

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Abstract

Remote biopsy sampling (RBS) is an important method to obtain epidermal samples from free-ranging cetaceans. The efficiency and effect of different RBS tools on the targeted animals remain a concern for scientists. The Paxarms Mk24c Projector (PMP) biopsy system, designed especially for small cetaceans, has proven to be practical for several small cetacean species. In the present study, we used a PMP to collect epidermal samples from free-ranging Indo-Pacific humpback dolphins (*Sousa chinensis*) distributed in coastal waters of the northern South China Sea to evaluate the efficiency and effectiveness of the PMP system and to assess any potential adverse impacts on Indo-Pacific humpback dolphins. Thirty-five tissue samples were obtained from 193 biopsy attempts, registering a sampling success rate of 18% in the present study. None of the dolphins that were targeted for biopsies (n = 193) using the PMP biopsy system showed any obvious adverse short-term reaction. Also, biopsy attempts, regardless of sampling success, did not significantly influence the targeted individual's reaction grade ($p > 0.05$). Similar to previous studies, sampling distances and Beaufort scales were key factors influencing sampling success. Based on the results of this study, we recommend that RBS on the Indo-Pacific humpback dolphin should be conducted within a distance of 25 m under sea state of Beaufort scale 3. A significantly higher sampling success rate was obtained when the animals were foraging and resting rather than travelling and socializing, regardless of different group sizes. The present study indicated a successful application of the PMP biopsy system for sampling from Indo-Pacific humpback dolphins in Chinese waters, and the PMP will contribute to studies relevant to Indo-Pacific humpback dolphin conservation for this vulnerable species throughout the world.

Key Words: Paxarms biopsy system, sampling success rate, small cetaceans, Sanniang Bay, Leizhou Bay

Introduction

Biological sampling is always a challenge when conducting research on free-ranging cetaceans because of their aquatic habitat and limited access to them above water. A common technique to collect tissue samples from cetaceans is the remote biopsy sampling (RBS) method (Noren & Mocklin, 2012). The first RBS system was developed with a harpoon in 1973, which was used to obtain research samples from humpback whales (*Megaptera novaeangliae*; Winn et al., 1973; Noren & Mocklin, 2012). Subsequent studies proved that RBS could provide high-quality epidermal samples efficiently without permanently injuring targeted animals (Krützen et al., 2002; Herman et al., 2005; Krahn et al., 2007; Noren & Mocklin, 2012). With thousands of epidermal samples from more than 40 species collected, RBS has become one of the most popular sampling methods for free-ranging cetaceans in research areas such as genetics (Winn et al., 1973; Krützen et al., 2002), toxicology (Aguilar & Borrell, 1994; Krahn et al., 2007), endocrinology (Kellar et al., 2006), and cytobiology (Mathews et al., 1988). The International Whaling Commission (1991) approved RBS as a non-invasive sampling method in 1991.

Indo-Pacific humpback dolphins (*Sousa chinensis*) are a coastal inshore cetacean species distributed in tropical to warm temperate areas (Jefferson & Karczmarski, 2001). They are found from central China (near the Yangtze River estuary) in the east through the Indo-Malay Archipelago, and westward around the coastal rim of the Indian Ocean to as far west as the Orissa coast of India (Jefferson et al., 2015). In 2016, a status of "Vulnerable" (VU) was proposed for the Indo-Pacific humpback dolphin (International Union for Conservation of Nature [IUCN], 2017). There are large numbers of Indo-Pacific humpback dolphins (hereafter, humpback dolphins) distributed in Chinese waters. Indeed, the first and second largest humpback dolphin populations were documented in the northern part of the South China Sea (Chen et al., 2010; Zhou et al., 2010; Xu et al., 2015). These dolphins face a variety of threats from ship noise (Liu et al., 2017a), pollution (Jefferson & Hung, 2008), and fisheries bycatch (Liu et al., 2017b). Humpback dolphins are a first-class national protected species in China; they have the highest level of animal protection by laws. For the purpose of studying them so they can be better protected, researchers have carried out many studies on the ecology and bioacoustics of this species (Jefferson et al., 2006; Chen et al., 2008; Li et al., 2012). However, studies relying on field sampling of specimens (e.g., population genetics, phylogenetic development, reproduction, and effects of contaminants) are lacking because the vast majority of available stranded specimens are heavily decomposed (Jefferson et al., 2006). Hence, sample acquisition from the humpback dolphins using RBS is imperative. Although it is a well-known cetacean sampling approach worldwide, RBS has not been employed in the waters of mainland China yet. In the present study, a RBS system was used to sample the humpback dolphin in Chinese waters, aiming to evaluate the application potential of this system on this species.

Methods

Sampling Tools and Study Area

This study used the Paxarms MK24C Projector (PMP) biopsy sampling system and Paxarms biopsy dart, with a dart tip length of 7 mm and a diameter of 4 mm (www.paxarms.com) to collect samples from free-ranging humpback dolphins. This is a pneumatic gun biopsy system, which is described in detail in Krützen et al. (2002).

The RBS was conducted in Leizhou Bay (20° 40' to 21° 10' N, 110° 20' to 110° 40' E) and Sanniang Bay (21° 30' to 21° 36' N, 108° 45' to 109° 00' E) located in the northern South China Sea (Figure 1). The Leizhou Bay is the second largest habitat of the humpback dolphins worldwide, with a population of roughly 1,485 individuals (Xu et al., 2015), while Sanniang Bay has 398 to 444 individuals (Chen et al., 2016).

Data Collection and Analysis

Field research was conducted on days with good visibility and Beaufort Sea States of ≤ 3. We conducted the study from a 6 m yacht in Leizhou Bay and an 8 m yacht in Sanniang Bay. The vessel speed was controlled at 15 to 25 km/h when searching and 5 to 10 km/h when making biopsy attempts. Dolphins were opportunistically sampled. We recorded dolphin group behaviors and sizes before sampling. Behavior patterns were divided into foraging, travelling, resting, and socializing (Table 1) based on Stensland et al. (2006). Group sizes were classified as 1 to 10, 11 to 20, 21 to 30, and 31 to 40 individuals.

We defined a *biopsy attempt* to include one shot at the targeted dolphin and a *successful attempt* to be the successful collection of a dolphin biopsy sample. The sampling success rate was measured as the number of samples obtained divided by the total number of biopsy attempts. Sampling distance was estimated as the straight-line distance from the shooter to the targeted dolphin and was classified into 1 to 5 m, 6 to 10 m, 11 to 15 m, 16 to 20 m, 21 to 25 m, 26 to 30 m, 31 to 35 m, and 36 to 40 m. Targeted dolphins' reactions to RBS were classified into five grades from low to strong (Table 2), similar to Jefferson & Hung (2008). Experienced shooters made all biopsy attempts. Additionally, during each biopsy attempt, we took photos and recorded sampling distance, Beaufort scales, dolphin group behaviors, dolphin group sizes, and animal reaction grades. Chi-square tests in *SPSS 19.0* (SPSS Inc., Chicago, IL, USA) were used to analyze the correlations of sampling distance, Beaufort scales, dolphin behaviors to the sampling success rate, and animal reactions.

Animal Welfare Implication

The RBS system in this research was previously employed by Krützen et al. (2002) and Tezanos-Pinto & Baker (2010) for collecting samples from *Tursiops* spp. Tezanos-Pinto & Baker (2010) documented that 99% of the biopsy attempts resulted in low reactions, with only two attempts resulting in moderate reactions (reference reaction level in Table 2). Further, analyses of resighting rates and capture probabilities based on individual identification records from 40 of the biopsied dolphins showed no evidence of long-term vessel aversion (Tezanos-Pinto & Baker, 2010). Krützen et al. (2002) reported that biopsied areas healed after approximately 23 days. Preliminary behavior observations in the present study indicated that the PMP caused only short-term reactions in both the targeted individuals and the group, which was similar to previous findings for other sampling systems (Weller et al., 1997; Tezanos-Pinto & Baker, 2010; Noren & Mocklin, 2012).

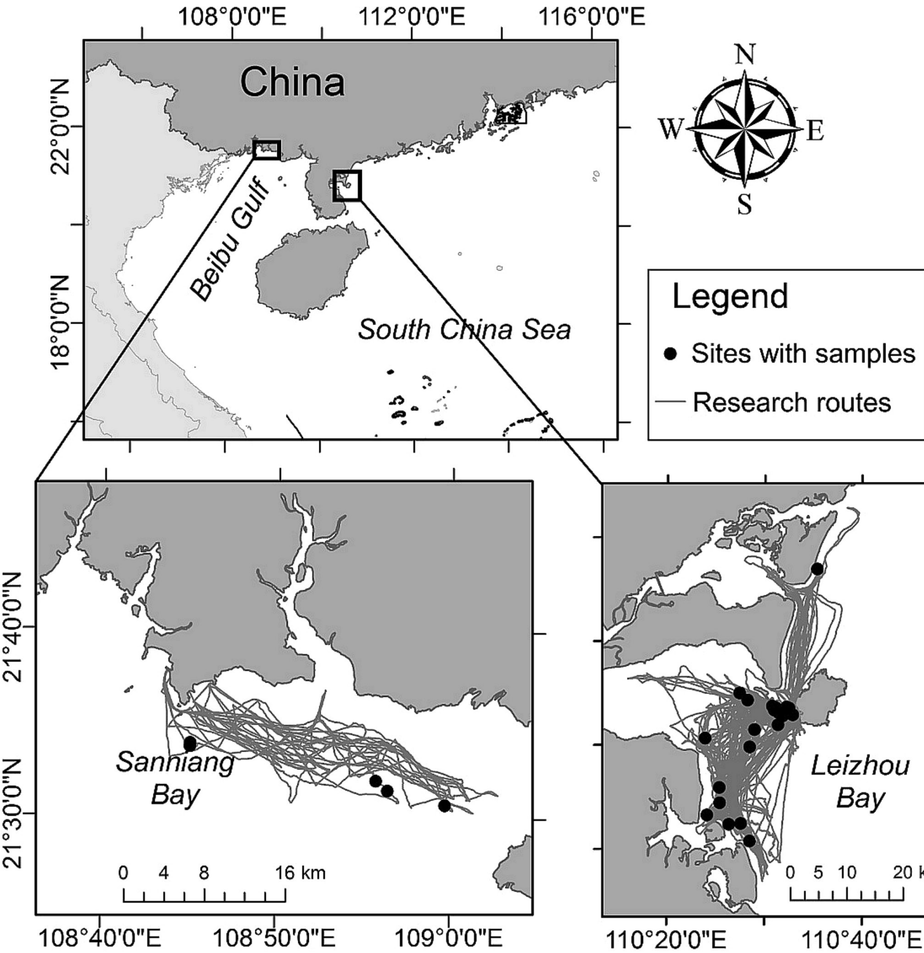

Figure 1. The research routes and locations of sites with successful collection of samples in Sanniang Bay and Leizhou Bay in the northern South China Sea, China

In addition, the dart tip of this system was 7 mm in length and 4 mm in inner diameter; therefore, the size of the epidermal samples would not be large enough to hurt the dolphins. No tissue samples except the epidermis were taken in the whole process. Vessel-related disturbance to dolphin groups were minimized to the extent possible when not sampling. Mother–calf pairs were not targeted for sampling.

Sample collection and use protocols were approved by the Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences, with the ethics approval code SIDSSE-SYLL-MMMBL-01.

Results

Sampling Efforts

From July 2015 to September 2017, out of a total research effort of 92 days, 27 days resulted in successful sample collection. We recorded 96 dolphin sightings and made 193 biopsy attempts. Of the 193 biopsy attempts, 16 occurred in Sanniang Bay and the rest in Leizhou Bay. Research routes in both bays were chosen based on previously reported high-frequency sighting areas (Pan et al., 2006; Xu et al., 2012; Wu et al., 2017). The sites with samples and the research routes are shown in Figure 1.

In total, we had 35 successful biopsy attempts and two extra successful hits with failing in sampling, resulting in a sampling success rate

Table 1. Definition of dolphin behaviors

Behavior	Definition (referring to Stensland et al., 2006)
Travelling	Persistent and directional movement. Dolphins could be meandering but still moving in a general direction.
Foraging	Rapid energetic surfacing, frequent directional changes, fish chases, and observations of dolphins with fish in mouth. Peduncle and tail-out dives common.
Socializing	Fondling, rubbing, mounting, chasing, genital inspections, play and displays, and other physical contact among individuals.
Resting	Low level of activity, moving slowly (speed < 2 kts). Slow surfacing three to four times before diving for an extended period of time.

Table 2. Definition of remote biopsy sampling reaction grades (referring to Jefferson & Hung, 2008)

Level	Reaction grade	Description
Low	1	No obvious reaction
	2	Making a slight jerk of the tail or body, with little or no splash
Moderate	3	Slapping its fluke on water surface with a splash
Strong	4	Leaping out of the water and falling back with a splash
	5	Leaping out of the water several times and falling back with a splash

of 18% overall. The first failure was caused by a broken biopsy dart; and in the second failed event, the dart tip did not penetrate the dolphin's epidermis. Of the 35 samples collected, 30 were obtained in Leizhou Bay and five in Sanniang Bay. Descriptions of samples/attempts with sampling distance, Beaufort scales, group behaviors, and group sizes in Leizhou Bay and Sanniang Bay are shown in Table 3.

Sampling Efficiency

The sampling success rate at different sampling distances varied (Figure 2). Chi-square test analysis showed that the sampling distance significantly influenced the sampling success rate ($p < 0.05$)—that is, shorter sampling distances had higher sampling success rates (Figure 2a).

The sea states also affected the biopsy attempts and successful sampling attempts, regardless of other factors as shown in Figure 2b. A Beaufort scale of 1 resulted in the most successful sampling attempts and the highest sampling success rates. No biopsy samples were obtained in sea states above a Beaufort scale of 3. Significant differences in sampling success rates in different Beaufort scales were detected using the Chi-square test ($p < 0.05$).

For the correlations between dolphin group behaviors and the sampling success rate (Figure 2c), no significant difference in the sampling success rate was found between foraging and resting behaviors ($p > 0.05$) nor between travelling and socializing behaviors ($p > 0.05$). However, the sampling success rate was significantly higher for dolphins showing foraging and resting behaviors than for those showing travelling and socializing behaviors ($p < 0.05$).

Sampling success rates under different group sizes were analyzed. No significant difference in the sampling success rate was detected among the different sized groups ($p > 0.05$; Figure 2d).

Dolphin Reaction

We calculated the proportions of different reaction grades of dolphins towards RBS under each category of sampling distance (Figure 3a), Beaufort scales (Figure 3b), dolphin behaviors (Figure 3c), and group sizes (Figure 3d). As the sampling distance increased, the reaction grade decreased significantly ($p < 0.05$). For the Beaufort scales, as the sea states deteriorated (increasing Beaufort scales), the reaction grade decreased significantly ($p < 0.05$). The reaction grade did not change significantly among dolphins showing foraging, socializing, and travelling behaviors ($p > 0.05$); however, a significantly higher sampling reaction appeared in dolphins displaying resting behavior ($p < 0.05$) compared with those in dolphins showing the other three behaviors. Group size did not significantly influence the reaction grades ($p > 0.05$).

Table 3. Descriptions of samples/attempts with sampling distance, Beaufort scales, group behaviors, and group sizes in Leizhou Bay and Sanniang Bay

		Leizhou Bay (30/177)	Sanniang Bay (5/16)
Sampling distance (m)	0-5	6/18	1/2
	6-10	12/44	3/7
	11-15	6/41	1/3
	16-20	4/35	0/1
	21-25	2/22	0/0
	26-30	0/9	0/0
	31-35	0/7	0/0
	36-40	0/1	0/3
Beaufort scales	1	21/85	5/14
	2	8/70	0/0
	3	1/22	0/2
Group behaviors	Foraging	12/42	3/10
	Resting	2/5	0/0
	Socializing	2/14	2/6
	Travelling	14/116	0/0
Group sizes (number of individuals)	1-10	7/53	3/4
	11-20	16/79	2/7
	21-30	6/36	0/3
	31-40	1/9	0/2

Total dolphin reactions towards RBS were analyzed. Grade 1 accounted for 34%; Grade 2, 50%; and Grade 3, 16% among all attempts. No significant difference in dolphin reaction grades towards RBS was detected between successful and failed sampling attempts ($p > 0.05$). The majority of the biopsy attempts induced low and moderate reactions, while no strong reactions were observed.

Discussion and Conclusions

Biopsy Sampling Efficiency

The present study showed that the sampling efficiency was highly influenced by sampling distance and sea states. As the sampling distance increased, sampling success rate decreased significantly. Despite dolphin avoidance of vessels, opportunistic sampling was still feasible within a certain distance. Barrett-Lennard et al. (1996) showed that vessel aversion of killer whales (*Orcinus orca*) occurred within a distance of 25 m; even so, killer whales can be easily sampled at a distance of 5 to 25 m. Our study results are similar to those obtained for killer whales.

The definition of *sampling success rate* in this research (defined as the number of successful sampling attempts divided by the number of attempts) was the same as that used in some previous studies (Brown et al., 1994; Jefferson & Hung, 2008; Bilgmann et al., 2011), but different from that used in others (Weller et al., 1997; Hooker et al., 2001; Best et al., 2005; Noren & Mocklin, 2012). They used *sampling rate* rather than *sampling success rate* to define the percentage of the number of samples in hit attempts (i.e., biopsy attempts hitting the target no matter whether a sample was obtained or not). Krützen et al. (2002) showed that the Paxarms biopsy system had a high sampling rate when the biopsy dart hit the animal, ranging from 96.6 to 100%. Noren & Mocklin (2012) also showed that the sampling rate is normally high. In the present study, if the results were calculated in the same way, the sampling rate was 95% (35/37), a value consistent with previous reports. Our results confirmed that with the advanced sampling system design, the success rate in retaining a sample was high when the targeted animal was hit, especially using the PMP.

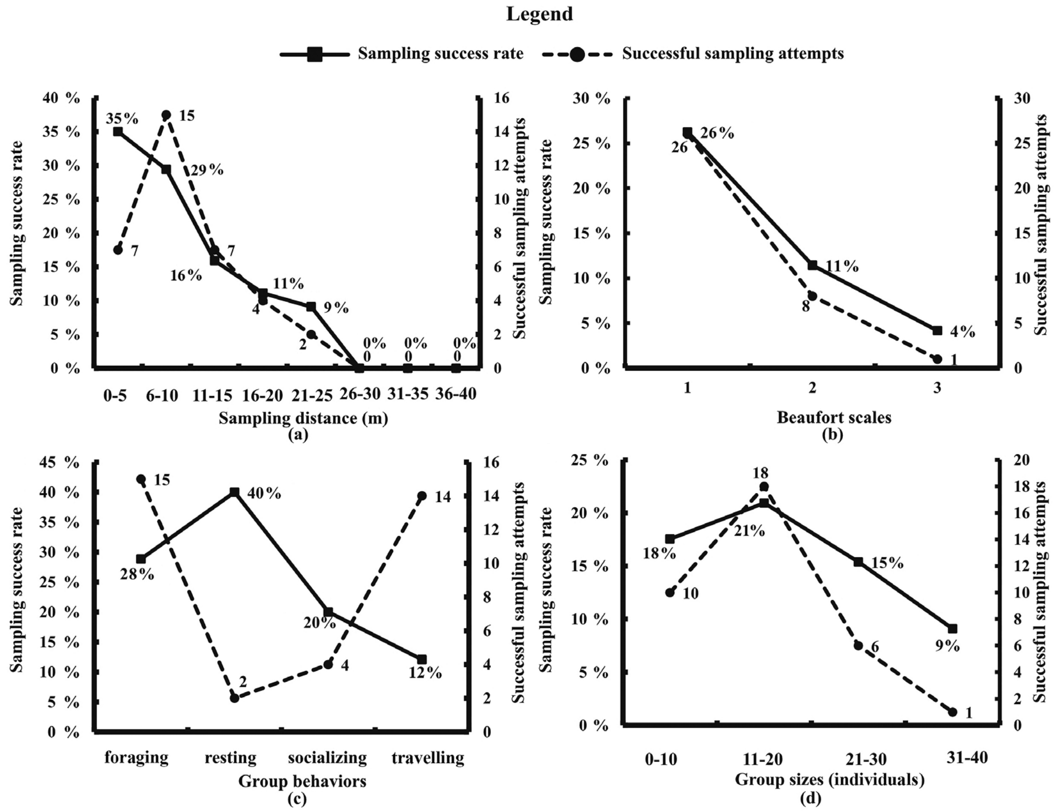

Figure 2. Sampling success rate and successful sampling attempts for sampling distance (a), Beaufort scales (b), dolphin group behaviors (c), and group sizes (d) when conducting RBS on Indo-Pacific humpback dolphins

Sampling Effects on Dolphins

To date, all published studies showed that small dolphins were not influenced by the pneumatic gun biopsy system (Barrett-Lennard et al., 1996; Krützen et al., 2002; Tezanos-Pinto & Baker, 2010). Besides, research employing other sampling systems, such as crossbows (Gauthier & Sears, 1999; Jefferson & Hung, 2008) and compound bows (Whitehead et al., 1990; Brown et al., 1991), indicated limitations in the influence of RBS on cetaceans. The results of the present study verified this conclusion in the humpback dolphin. According to previous research, the reaction of humpback dolphins can best be characterized as a startled response rather than a reaction to any pain or discomfort caused by the impact or penetration of the biopsy dart (Jefferson & Hung, 2008). The resting and socializing groups showed a stronger reaction than milling and travelling groups among spinner dolphins (*Stenella longirostris*) when sampled using a crossbow system (BARNETT Veloci-Speed® Class, 68-kg draw weight; Kiszka et al., 2010), a phenomenon also observed in the present study. The present study also showed that group size did not influence the dolphins' sampling reaction grades, which was also reported earlier (Gauthier & Sears, 1999).

In this research, all the observed reactions of the dolphins during sampling were recorded by following suggestions from previous studies in other cetacean species (Whitehead et al., 1990; Brown et al., 1994; Tezanos-Pinto & Baker, 2010). Only the short-term reaction of the humpback dolphins towards PMP was assessed in the present research. Monitoring the animals' long-term reactions towards PMP should be the subject of future research, including information from photo identification.

Research Significance

RBS is a popular and efficient way to conduct studies on cetaceans worldwide, but there are only a few applications used in the study of humpback dolphins. Testing the application efficiency and effects of RBS is important and essential for humpback dolphins. The present study was a

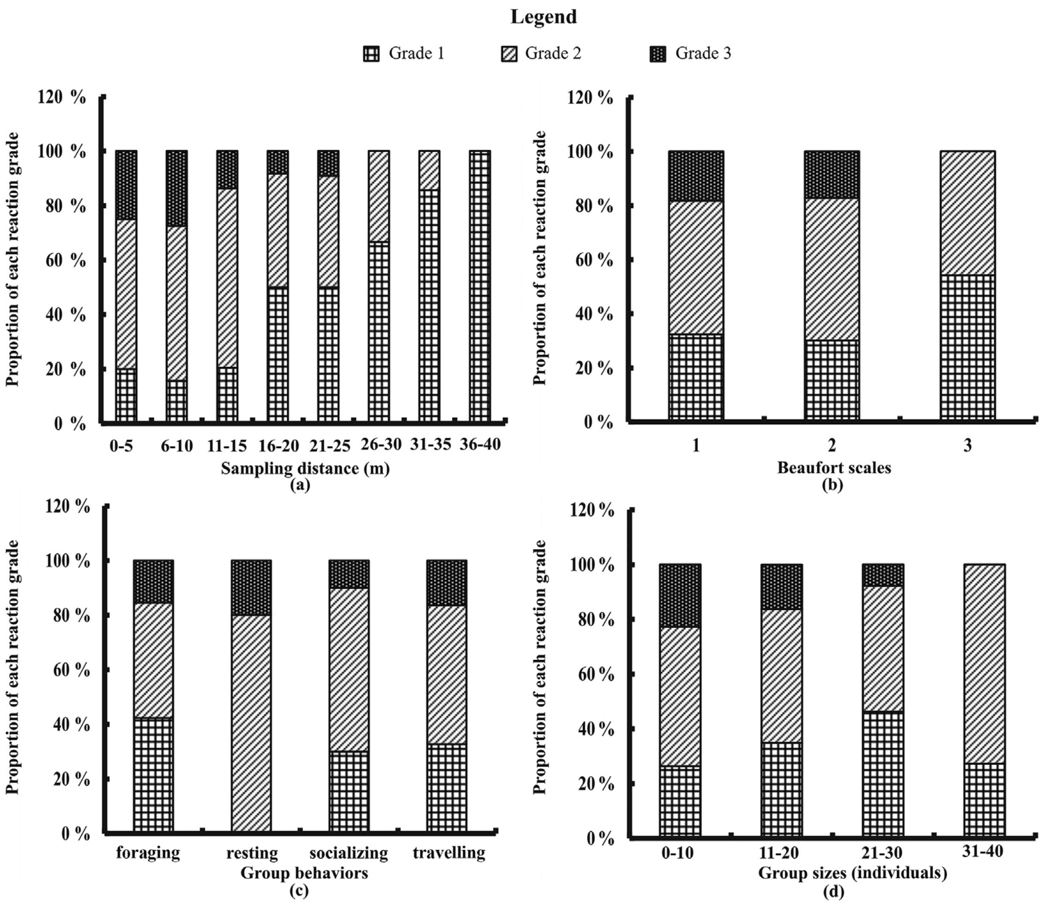

Figure 3. Proportion of each reaction grade under sampling distance (a), Beaufort scales (b), dolphin group behaviors (c), and group sizes (d)

preliminary test of the pneumatic gun system on humpback dolphins in Leizhou Bay and Sanniang Bay in the northern South China Sea. The results of the present study will be useful to guide the design of future RBS studies.

Sample collection is important in cetacean conservation study worldwide. These samples could be used in genetics (Winn et al., 1973; Torres et al., 2003), toxicology (Herman et al., 2005; Jefferson et al., 2006; Krahn et al., 2007), cytobiology (Mathews et al., 1988), endocrinology (Kellar et al., 2006, 2013), and so on. Traditional sample collection methods are mostly focused on the stranded individuals of humpback dolphins (Chen et al., 2008; Chen & Yang, 2009; Chen et al., 2010; Lin et al., 2012). Normally, most of the stranded individuals were dead, sometimes with the carcass seriously decomposed, resulting in quite a low-quality sample. Also, there may be misjudgment as to which population the stranded individual really belongs since the carcasses could be carried elsewhere by ocean currents. Further, study based on a stranded sample collection sometimes leads to a misunderstanding of a population's status. A previous study showed that the sole sampling of carcasses leads to an underestimate of cetacean population genetic differentiation (Bilgmann et al., 2011). Considering these three situations mentioned above, the RBS method is helpful in the collection of samples from humpback dolphins and can provide fresh samples with good quality and accurate population representation. With these advantages, conservation studies on humpback dolphins can be carried out more effectively.

Acknowledgments

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